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The COVID-19 and TB syndemic: Differences and similarities

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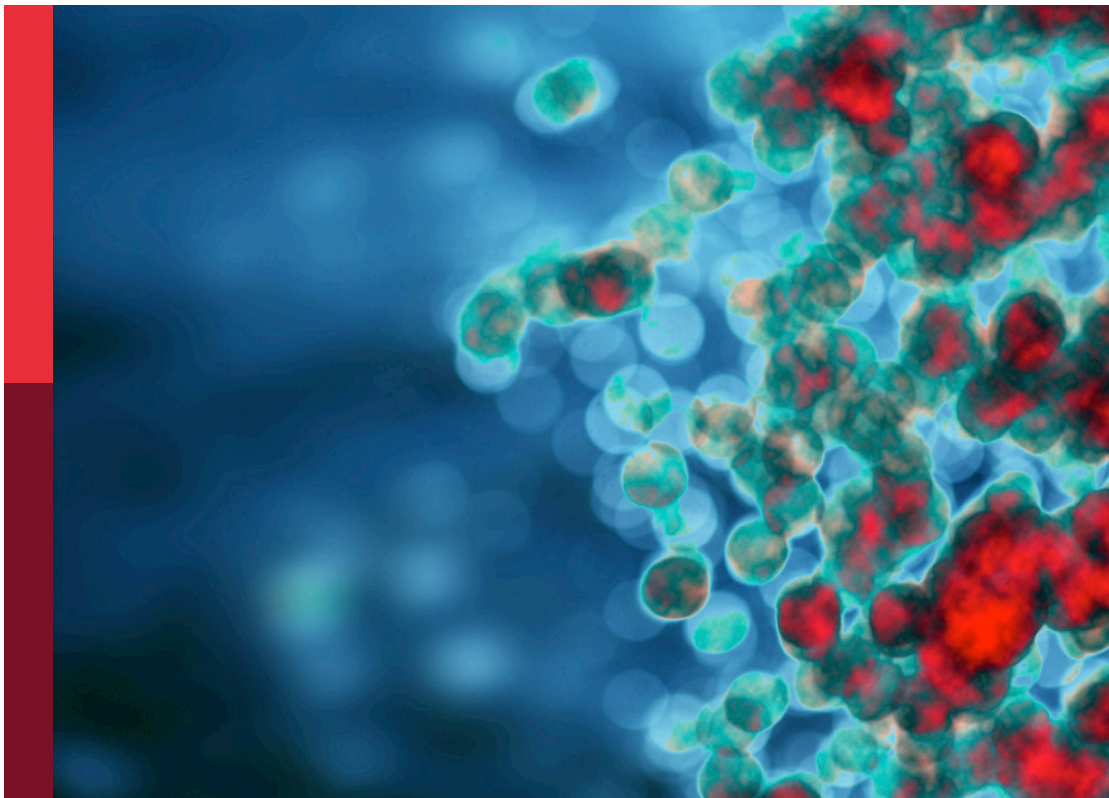

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The COVID-19 and TB syndemic: Differences and similarities

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Editorial: The Covid-19 and TB syndemic: differences and similarities

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tuberculosis, immunity, COVID-19, vaccination, BCG

Editorial on the Research Topic
The Covid-19 and TB syndemic: differences and similarities

Three years ago, in 2020, when the Covid-19 outbreak in China became a pandemic, the World Health Organization (WHO) declared Covid-19 a global health emergency (1). The consequences of this declaration were profound and led to the worldwide mobilization of virtually unlimited funding for needed intervention strategies. As a result, in 2023, the WHO could announce the end of Covid-19 as a global health emergency. Thirty years ago, when tuberculosis (TB) caused 3 million deaths per year, an earlier declaration by WHO came to a similar conclusion, namely that TB was a global health emergency (2). This announcement was more or less ignored and the WHO could never declare the end of TB as a global health emergency. Probably one major reason was that Covid-19 was a pandemic equally affecting countries worldwide while TB is primarily a disease of low-to-middle-income countries.

Consequently, financial support for TB has remained insufficient with support for research and development (R&D) in the order of 1 billion USD per year in the early 2020s. In comparison, the 3 years of the Covid-19 pandemic witnessed financial support for R&D in the order of 100 billion USD. There are other dissimilarities, but also similarities, between TB and Covid-19, and they are discussed in this Frontiers in Immunology Research Topic. The emphasis here is on the lessons learned from the successful handling of the Covid-19 pandemic for mitigating the dire TB crisis. A further issue addressed is whether interactions occur between the two infections, with one pathogen blocking or promoting the disease induced by the other.

In describing epidemiologic aspects of Covid-19 and TB, Falzon et al. emphasize the negative impact of Covid-19 on TB worldwide, which led to a reversal of the slight decline in TB morbidity and mortality before the Covid-19 crisis.

Booysen et al. provide an update on immune interactions between the two causative agents, SARS-CoV-2 and *Mycobacterium tuberculosis* (Mtb). They conclude that

coinfections could impair immunity to SARS-CoV-2 due to elevated inflammation. Furthermore, they find a lack of evidence for a beneficial effect of the TB vaccine, BCG, against Covid-19. These issues have been further analyzed in experimental animal studies.

Hilligan et al. show in an experimental animal model that only intravenous administration of BCG can provide protection against Covid-19 and Baker et al. reveal in an experimental mouse model that prior infection with SARS-CoV-2 did not affect subsequent Mtb infection, whereas prior Mtb infection restricted replication of SARS-CoV-2 after subsequent challenge.

Aiello et al. further elaborate on the immune response against Mtb and SARS-CoV-2 in humans with an emphasis on the initial stage.

Allué-Guardia et al. elaborate on another similarity between TB and Covid-19, namely the increased susceptibility and the heightened burden of disease in the elderly. As they point out, a better understanding of mechanisms underlying TB, Covid-19, and other respiratory infections should be harnessed for the design of future intervention strategies to increase the health span of the elderly.

Shaw et al. discuss one possible mechanism that could operate as a disease magnifier in infectious diseases, such as Covid-19 and TB, namely myeloid-deprived suppressor cells.

Kaufmann provides an overview of the current status of vaccine R&D against TB and Covid-19 and provides possible explanations for the differential speed of R&D for Covid-19 vs. TB vaccine development with an emphasis on the mechanisms underlying immune protection that were mobilized for the development of preventive measures against the two diseases.

Corleis et al. provide an overview of different animal models harnessed for TB and Covid-19 investigation and underline an optimization of models, notably for pulmonary infectious diseases such as Covid-19 and TB. A complementary, rather than alternate approach, would be controlled human challenge studies.

Morrison et al. describe the state-of-the-art and potential future developments for controlled human infection models for SARS-CoV-2 and TB. Such models could also provide models for newly emerging pathogens.

The rapid and highly efficient response against Covid-19 has demonstrated the importance of public awareness of the threat of infectious diseases including not only newly emerging but also current threats. Hopefully, the lessons learned from the response to Covid-19 will also impact efforts toward better control of TB. In September 2023, the United Nations convened a high-level meeting on TB, which resulted in a commitment to provide life-saving treatment for up to 45 million people between 2023 and 2027, including up to 4.5 million children and up to 1.5 million people with drug-resistant TB (3, 4).

Furthermore, they endorsed preventive treatment for up to 45 million people between 2023 and 2027, including 30 million household contacts of TB patients and children, and 15 million people living with HIV. To achieve these goals, an increase in annual global TB funding to 22 billion USD annually by 2027 and to 35 billion USD by 2030 was promised. This also included the mobilization of 5 billion USD per year by 2027 for R&D for TB.

Even though the UN declaration did not include clear accountability of the signatories, it would be important for the whole world to accomplish these goals since, without such measures, an estimated 24 million deaths will be caused by TB by 2050, leading to economic losses on the order of 13 trillion USD. The example of Covid-19 has shown that this level of financial support can make a difference (5). Therefore, while the effects of the Covid-19 pandemic on TB control have been predominantly negative, we learned that high investment, both in labor and funding resources, brings impactful results. We hope this lesson will be transferred from a new pandemic to an old one.

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Conflict of interest

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References

1. World Health Organization. *COVID-19 dashboard* (2020). Available at: https://covid19.who.int/.

2. World Health Organization. *Tuberculosis: a global emergency*. In: *World health 1993*. vol. 46: p. 3–31. Available at: https://iris.who.int/handle/10665/52639.

3. World Health Organization. *UN General Assembly high-level meeting on tuberculosis* (2018). Available at: https://www.who.int/teams/global-tuberculosis-programme/unga-high-level-meeting-on-the-fight-against-tb/unga-high-level-meeting-on-ending-tb.

4. Stop TB Partnership. *United nations high-level meeting on TB* (2023). Available at: https://www.stoptb.org/advocate-to-endtb/united-nations-high-level-meeting-tb.

5. Stop TB Partnership. *The Global Plan to end TB 2023-2030* (2022). Available at: https://www.stoptb.org/file/15583/download.

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Controlled human infection models in COVID-19 and tuberculosis: current progress and future challenges

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Controlled Human Infection Models (CHIMs) involve deliberately exposing healthy human volunteers to a known pathogen, to allow the detailed study of disease processes and evaluate methods of treatment and prevention, including next generation vaccines. CHIMs are in development for both tuberculosis (TB) and Covid-19, but challenges remain in their ongoing optimisation and refinement. It would be unethical to deliberately infect humans with virulent *Mycobacteria tuberculosis* (*M.tb*), however surrogate models involving other mycobacteria, *M.tb* Purified Protein Derivative or genetically modified forms of *M.tb* either exist or are under development. These utilise varying routes of administration, including *via* aerosol, per bronchoscope or intradermal injection, each with their own advantages and disadvantages. Intranasal CHIMs with SARS-CoV-2 were developed against the backdrop of the evolving Covid-19 pandemic and are currently being utilised to both assess viral kinetics, interrogate the local and systemic immunological responses post exposure, and identify immune correlates of protection. In future it is hoped they can be used to assess new treatments and vaccines. The changing face of the pandemic, including the emergence of new virus variants and increasing levels of vaccination and natural immunity within populations, has provided a unique and complex environment within which to develop a SARS-CoV-2 CHIM. This article will discuss current progress and potential future developments in CHIMs for these two globally significant pathogens.

KEYWORDS

tuberculosis, COVID-19, CHIM, controlled human infection, challenge models

Introduction

Controlled human infection models (CHIMs) involve the deliberate inoculation of volunteers with a pathogen under carefully controlled conditions, facilitating detailed study of host-pathogen immunobiology. Validated models can then be used to expedite the development of novel vaccines and therapeutics by allowing efficacy testing in small scale

clinical trials, prior to field efficacy studies. Dating back to Edward Jenner's 18th century smallpox experiments, historically, the ethical conduct of CHIMs has been controversial. With the implementation of modern ethical frameworks and considered study design (Figure 1), they have proven to be a safe and efficacious tool, particularly in the field of vaccinology, contributing to the development of vaccines for malaria, influenza, typhoid and cholera (1–4).

Tuberculosis (TB) remains a major global health issue, second only to COVID-19 as the leading cause of death from a single infectious pathogen (5). The COVID pandemic has itself reversed decades of progress towards meeting global TB reduction targets and new tools to combat TB are urgently needed (6, 7). Whilst astonishing research efforts worldwide have rapidly led to multiple licensed COVID-19 vaccines and therapeutics (8, 9), the ongoing potential of the virus to mutate, coupled with changing population immunity, means we cannot be complacent in our quest to develop new scientific tools and evaluate next generation vaccines and treatments. CHIMs against these two different, but both highly consequential, respiratory pathogens could be harnessed to help accelerate progress.

Tuberculosis controlled human infection models

Background and need for a TB CHIM

The only licenced vaccine against TB, Bacillus-Calmette Guérin (BCG), provides good protection against severe forms of infant TB, but highly variable efficacy against pulmonary TB and therefore limited impact on disease transmission. Ongoing challenges also exist in the accurate diagnosis of both TB infection and active disease, increasing drug resistance and treatment burden even for fully sensitive disease (10). Despite huge research efforts, developments in all of these areas are hampered by gaps in our understanding of intricate host-pathogen interactions, the complex spectrum of disease states that cannot be replicated fully in animal models and lack of defined immune correlates of protection (CoP). Judicious use of a mycobacterial CHIM could help facilitate advances in many of these domains, as a complement to animal and field studies (11). For example, a mycobacterial CHIM could enable the prioritisation of vaccine candidates that most effectively control mycobacterial growth, prior to larger, more costly field

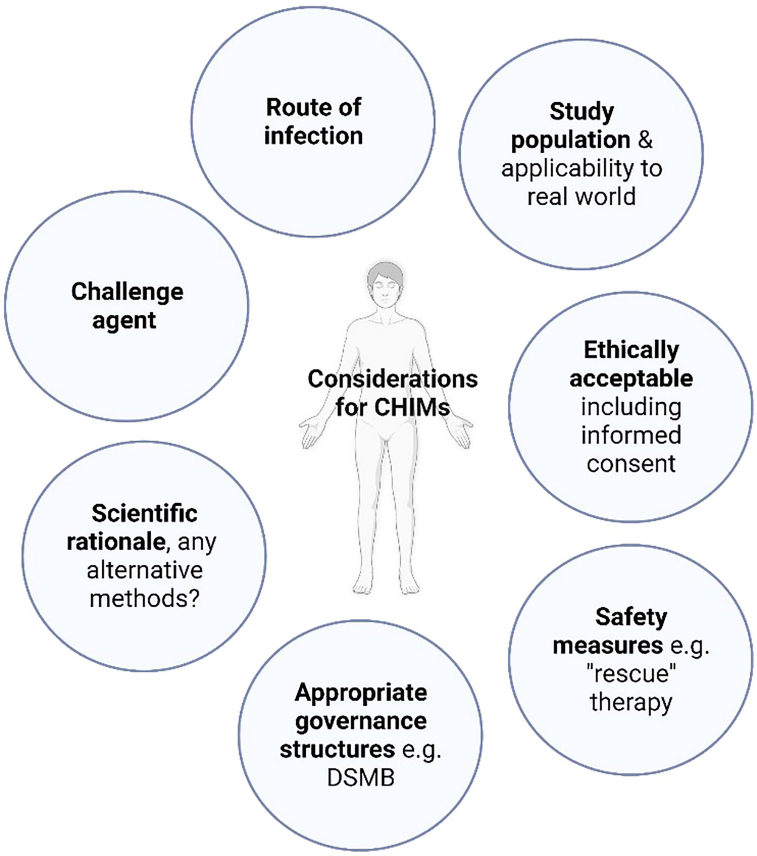

FIGURE 1
Controlled human infection model design. A common framework of considerations for CHIM design should be employed. "Rescue" therapy: treatment employed in CHIMs either to prevent the progression of volunteer symptoms experienced beyond mild disease or to abrogate infection, DSMB: Data safety monitoring board.

efficacy studies. Samples from such a CHIM could also be used to interrogate immune parameters that correlate with control after a defined timepoint infection, with any positive steps towards finding a validated TB immune CoP proving potentially transformative.

Current and future approaches to developing a TB CHIM

Intentionally infecting humans with virulent *Mycobacterium tuberculosis* (*M.tb*) would not be ethical, with the potential for significant morbidity and mortality. Even if these are avoided, long treatment duration with the risk of significant drug side effects, risk of *M.tb* transmission to others, inability to prove cure at the end of treatment and possibility of disease recurrence are all substantial arguments against a CHIM with wild-type *M.tb*. Therefore, researchers must pursue the use of alternative challenge agents (Summarised in Table 1), aiming to address key scientific questions with an acceptable risk profile to both volunteers and the wider community.

The tuberculin skin test (TST), where tuberculin Purified Protein Derivative (PPD) is injected intradermally, has traditionally been used as a diagnostic test for latent TB infection (LTBI). It has been employed as a challenge agent to investigate immunological responses to mycobacterial antigens at the site of skin challenge, for example identifying exaggerated Th17 responses in those with active TB disease as a potential target for host directed therapies (12, 13, 24). PPD has also been used to assess local respiratory mucosal responses following intrabronchial instillation (14, 15, 21). Whilst these methods may contribute to our knowledge of mycobacterial immunopathogenesis they cannot be utilised directly to assess efficacy of vaccines or therapeutics.

A CHIM that is to be used to evaluate vaccine efficacy requires a live replicating organism, for example an attenuated strain of mycobacteria. BCG itself is such a live attenuated mycobacteria, initially derived *via* passage from *Mycobacterium bovis (M. Bovis)*, that does not cause disease or latency in healthy humans (25). The loss of key virulence genes encoded in the Region of Difference 1 (RD1) during this process confers the advantageous safety profile of BCG but means the full immunopathogenic pathways of *M.tb* are not entirely replicated and it could not be used to evaluate vaccines which incorporate RD1-encoded antigens, such as ESAT-6 and CFP-10. However, BCG has been shown to induce similar canonical $CD4^+$ T cell-mediated immune responses to *M. tb* in humans (26) and assessment of vaccine efficacy using a BCG challenge in animal models are comparable to results obtained using *M.tb* as the challenge agent (27, 28). BCG manufactured under good manufacturing practice (GMP) conditions for human use is readily available and this therefore represents the only live replicating TB CHIM agent currently available (11).

CHIMs using intradermal (ID) BCG as a mycobacterial challenge agent have been developed and are able to detect a known BCG vaccine effect in animals and humans (17, 18, 27–29). The ID route allows straightforward quantification of mycobacteria from an easily accessible site, for example via minimally invasive punch skin biopsies (17). However, the natural route of *M.tb* infection is via the respiratory tract and initial pathogen interactions with the specialised host respiratory mucosal system cannot be evaluated using an ID CHIM.

Efforts are ongoing to develop pulmonary CHIMs that more closely mimic the natural route of *M.tb* infection. BCG delivered via aerosol (Clinicatrials.gov NCT02709278, NCT03912207, NCT04777721) or instilled directly into the lungs per bronchoscope (21) are both being evaluated and have been shown to be safe and well tolerated. A defined timepoint pulmonary mycobacterial infection would allow examination of localised mucosal immunology and the relationship to induced system responses, which are key areas of research interest. Vaccines or therapeutics tested using these CHIMs would have the advantage of accounting for the contribution of the specialised respiratory mucosa in conferring protective immunity. However, sampling of the respiratory mucosa for immunological interrogation and quantification of recoverable BCG in pulmonary models are both more complex and invasive than in skin models (30).

Following on from initial studies using BCG, live mycobacterial CHIMs could be enhanced by the use of rationally attenuated genetically modified organisms. BCG which has been modified, for example to include a fluorophore reporter gene or exhaled volatile compound detectable by mass spectrometry could reduce the need for invasive sampling for mycobacterial recovery and quantification (31, 32). Use of current live vaccine candidates such as MTBVAC, a rationally attenuated form of *M.tb* (33, 34) or VPM1002, a recombinant BCG (35, 36), could allow investigation of the antigens or immunological pathways missing from BCG.

Whilst it is some way off from clinical evaluation, efforts are underway to develop a conditionally replicating *M.tb* strain with a genetically inserted suicide mechanism. This would aim to recapitulate the initial immunological mechanisms of *M.tb*, whilst ensuring complete eradication at a predefined timepoint and, if successful, could hugely advance the field of human TB study (22, 31).

Finally, for a TB CHIM to be truly useful, it should be safe, acceptable and deliverable in TB endemic populations and settings. Different environmental exposures, level of nutrition, microbiome composition, prior exposure to mycobacteria and prevalence of co-infections are just some of the known factors impacting vaccine efficacy. Utilising an ethically appropriate CHIM in endemic settings would ensure vaccines are tested in relevant populations (11, 37).

SARS-CoV-2 controlled human infection models

Background and need for a SARS-CoV-2 CHIM

Early in the COVID-19 pandemic, the World Health Organisation (WHO) acknowledged the potential benefits of a

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TABLE 1 Controlled Human Challenge Studies in Tuberculosis and Covid-19.

Studies, country	Challenge agent, *route*	Challenge overview	Key findings	Challenge method comments
Tuberculosis				
Tomlinson et al. 2011. UK, South Africa (12).	PPD 5 TU *ID*	Volunteers with a spectrum of mycobacterial exposure underwent concurrent TSTs in each arm, with skin biopsies at 6 and 48 hours	• Recruitment of TH1-polarised responses and cytotoxic T-cells at TST site • Immune responses predominantly due to cell recruitment, not proliferation	**ID PPD** ✓ Minimally invasive ✓ Allows dissection of immune responses and interactions *in vivo* ✗ Not at site of natural infection ✗ Unable to assess vaccine/ therapeutic efficacy as no replication
Pollara et al. 2017. South Africa, UK, Peru (13).	PPD 5 TU *ID*	PPD or saline control injection in individuals with active TB, LTBI or cured disease, followed by skin biopsy of TST site at 48 hours	• Elevated levels of IL17A/F and enrichment of Th17 cells in active TB compared to LTBI • Associated with increased neutrophils and MMP-1 • Changes reversed in cured group	
Silver et al. 2003. USA (14).	PPD 0.01-0.5 TU *Intrabronchial*	Dose escalation study of PPD instillation per bronchoscope followed by bronchoalveolar lavage (BAL) at 48 hours in TST positive and negative individuals	• Local inflammatory response at 0.5 TU, with increased mobilisation of CD4+ T-cells and antigen-specific IFNγ producing cells in the lungs of TST positive volunteers	**Intrabronchial PPD** ✓ Allows dissection of immune responses and interactions *in vivo* ✓ At mucosal site of natural infection ✗ Invasive instillation and sampling ✗ Unable to assess vaccine/ therapeutic efficacy as no replication
Walrath et al. 2005. USA (15).	PPD 0.5 TU *Intrabronchial*	Follow on PPD per bronchoscope study to examine mucosal immune responses by BAL 48 hours after installation in TST positive and negative individuals	• IFN-γ-inducible chemokines including CXCR3 ligands increased in TST individuals • Evidence of compartmentalised resident memory cell induction	
Schreiber et al. 2010. UK (16).	BCG Moreau 10^7 viable bacilli *Oral*	Repeated oral challenge days 0, 28, 49 in historically BCG vaccinated volunteers with subsequent peripheral blood sampling	• Increase in PPD-specific IFNγ seen 6 months after 1st challenge • Increase in IL6-enriched pathways at day 7, no changes after repeat challenge	**Oral BCG** ✓ Non-invasive ✓ Live, replicating organism ✓ Challenge involves mucosal site ✗ Not natural site of TB infection ✗ Study designed as surrogate CHIM for gastrointestinal infections not TB ✗ Mucosal sampling difficult ✗ Minimally immunogenic ✗ Difficult/unable to quantify viable BCG
Minassian et al. 2012. UK (17).	BCG Danish 1331 1-4×10^5 CFU *ID*	Feasibility study of ID BCG challenge in BCG-naïve and historically vaccinated. Skin biopsies and suction blisters used to quantify BCG recovery and examine cellular infiltrate	• Peak BCG recovery in challenge site at 2 weeks (detectable up to 4 weeks) • CD15+ neutrophilic infiltration at blister site • Prior BCG vaccination lead to reduction in recoverable BCG by PCR	**ID BCG** ✓ Same route as vaccination, same safety profile ✓ Live, replicating organism ✓ Minimally-invasive (skin biopsies) ✓ Easily controllable and quantifiable ✓ Proven to detect a BCG vaccine effect ✗ Cannot be used to study vaccines based on RD1 deleted antigens ✗ Not at natural site of infection ✗ Unable to assess involvement of respiratory mucosal immunity in control
Harris et al. 2013. UK (18).	BCG Danish 1331, 2-8x10^5 CFU *ID*	Use of ID BCG CHIM to asses vaccine candidate MVA85A prime or as a booster following historical BCG-vaccination, with skin biopsies taken 2 weeks following BCG challenge	• Protective BCG vaccine effect again detectable by PCR • No added benefit of MVA85A over BCG (in keeping with field trials)	
Minhinnick et al. 2016. UK (19).	BCG Danish 1331 or BCG TICE, standard (2-8 x10^5 CFU) or high (3 x standard) *ID*	Optimisation of ID BCG challenge model by BCG strain and dose in BCG-naïve volunteers, with skin biopsies taken 2 weeks following BCG challenge	• No significant difference in BCG recovery by strain • High dose ID BCG was well tolerated with improved BCG recovery • High-dose BCG Danish 1331 identified as optimal agent for future studies	
Blazevic et al. 2017. USA (20).	BCG TICE 2x10^6 *ID*	ID BCG challenge to asses use of skin swabs to detect BCG	• BCG detection possible *via* swabs, but less reproducible and consistent	

(Continued)

TABLE 1 Continued

Studies, country	Challenge agent, *route*	Challenge overview	Key findings	Challenge method comments
			than biopsy • Later recovery of BCG (3-4 weeks)	
Davids et al. 2020. South Africa (21).	BCG Danish 1331 $1x10^3$-$1x10^5$ CFU *Intrabronchial* Also PPD 0.2 TU and 0.5 TU *Intrabronchial*	Safety and feasibility study with per bronchoscope instillation of BCG and PPD (different lung segments) in volunteers with a broad range of prior mycobacterial sensitisation	• Highly compartmentalised immune responses demonstrated, localised to the challenged lung segments • Frequency of Th17 homing cells unexpectedly seen to decrease after PPD or BCG challenge	**Intrabronchial BCG** ✓ At mucosal site of natural infection ✓ Allows dissection of pulmonary and systemic immune responses *in vivo* ✓ Live, replicating organism ✓ Safety shown in sensitised individuals ✗ Cannot be used to study vaccines based on RD1 deleted antigens ✗ Invasive challenge ✗ Invasive sampling ✗ Accurate quantification of BCG recovery from pulmonary samples challenging **Intrabronchial PPD** See comments on previous studies
TB041, UK. Clinicaltrials.gov NCT02709278 Completed, manuscript under review (11)	BCG Danish 1331 /BCG Bulgaria $1×10^3$ - $1×10^7$ CFU *Aerosol inhaled*	Dose escalation study of aerosol BCG in BCG-naïve volunteers, with comparison ID BCG am	• Aerosol BCG is safe, and immunogenic in BCG naïve volunteers • Live BCG can be detected from BAL samples	**Aerosol BCG** ✓ At mucosal site of natural infection ✓ Non-invasive challenge, most closely mimics natural inoculation ✓ Allows dissection of immune responses and interactions *in vivo* ✓ Live, replicating ✗ Cannot be used to study vaccines based on RD1 deleted antigens ✗ Invasive sampling ✗ Accurate quantification of BCG recovery from pulmonary samples challenging
TB043, UK. Clinicaltrials.gov NCT03912207 Ongoing (22)	BCG Danish 1331 $1X10^7$ CFU *Aerosol inhaled*	Exploratory study into innate and adaptive immune response to aerosol mycobacterial challenge	Trial protocol only, results awaited	
TB044, UK. Clinicaltrials.gov NCT04777721, Ongoing (22)	BCG Danish 1331 $1x10^4$ - $1×10^7$ *Aerosol inhaled*	Dose escalation study of aerosol BCG in historically BCG vaccinated volunteers	Trial protocol only, results awaited	
COVID-19/ SARS-CoV-2				
Killingley et al. 2022. UK (23).	Wild-type SARS-CoV-2 virus (SARS-CoV-2 /human/GBR/ 484861/2020) 10 $TCID_{50}$ *Intranasal*	Dose finding study, healthy 18-30-year olds, seronegative with no prior SARS-CoV-2 infection or vaccination	• 18/34 (53%) of volunteers developed productive infection at 10 TCID50 • Accurate description of 1° infection viral kinetics with pre-Alpha strain • Virus detectable in throat significantly earlier than the nose, but reaching higher titres in nose • Viral shedding started at 2 days post inoculation and peaked at 5 days at 8.87log10 copies per millilitre • Viable virus was detected on FFA for an average of 10 days (up to 12 days) • Challenge was safe and well tolerated. No evidence of lower respiratory tract infection but 83% of volunteers demonstrated measurable smell disturbance. • Strong correlation with lateral flow positivity and viable virus on FFA	**Wild type, seronegative** ✓ Proof of concept, ethical acceptability and safety ✓ Able to establish productive infection ✓ Able to dissect primary infection kinetics and immune response under standardised conditions ✗ No longer dominant variant ✗ Seronegative model cannot be used for vaccine/ therapeutic development given global seroprevalence *via* vaccination/ infection

(Continued)

TABLE 1 Continued

Studies, country	Challenge agent, *route*	Challenge overview	Key findings	Challenge method comments
COV-CHIM01, UK. Clinicaltrials.gov NCT04864548, Ongoing	Wild-type SARS-CoV-2 virus (SARS-CoV-2 /human/GBR/ 484861/2020) 10-1x10^{5}TCID$_{50}$ *Intranasal*	Dose finding study, healthy 18-30-year olds, prior SARS-CoV-2 infection +/- vaccination. Utilising same pre-Alpha SARS-CoV-2 virus as seronegative CHIM. Starting at dose of 1x10^1 up to 1x10^{5}TCID50.	Trial protocol only, results awaited	**Wild type, seropositive** ✓ Seropositive studies needed for real world utility ✗ Potent protection against re-infection demonstrated in field studies prior to emergence of Delta & Omicron variants ? feasibility (results awaited)
COVHIC002, UK. ISRCTN94747181, Ongoing	Delta SARS-CoV-2 virus Starting dose 1x10^2 TCID$_{50}$ *Intranasal*	Dose finding study, healthy 18-30-year olds, SARS-CoV-2 vaccinated (+/- prior infection).	Trial protocol only, results awaited	**Viral variants, seropositive** ✓ Seropositive studies needed for real world utility ✓ Use of variants allows dissection of heterologous immunity ✓ More reflective of real world ✓ Proof of concept with viral variants could allow selection of optimum challenge strain for future use ✗ Viral mutation likely to outpace GMP manufacture of challenge strains ✗ Potent protection against re-infection demonstrated in field studies prior to emergence of Omicron variant ?feasibility

✓ Positive aspect of model ✗ Drawback to model.
BAL, bronchoalveolar lavage; BCG, Bacillus-Calmette Guérin; ID, intradermal; FFA, focus forming assay; IL6, interleukin 6; IL17, interleukin 17; LTBI, latent TB infection; MMP-1, matrix metalloproteinase-1; PPD, tuberculin purified protein derivative, TCID$_{50}$, median tissue culture infectious dose; Th17, T-helper 17; TST, tuberculin skin test; TU, tuberculin Unit.

SARS-CoV-2 CHIM, for example to allow rapid prioritisation of vaccine candidates. A working group was promptly established to consider the practicalities, feasibility and ethics (38). Initial expert consensus was divided with concern about the lack of a suitable "rescue" therapy, potential for severe illness and high transmissibility, as well as the benefit and applicability of such a model over field studies (39).

Accruing data suggested that infection of young, healthy adults in whom disease was generally much milder could be justifiable. This prompted UK manufacture of a challenge virus under GMP conditions and development and rigorous ethical review of study protocols for both a UK SARS-CoV-2 naïve CHIM (NCT04865237) and one in previously infected volunteers (NCT04864548) (40). GMP manufacture of challenge viruses is a time-consuming process and enrolment did not commence in these studies until March (NCT04865237) and May, 2021 (NCT04864548) respectively, by which point several highly efficacious vaccine candidates were being deployed in the UK population (41, 42).

Despite the widespread availability of highly effective vaccines against SARS-CoV-2, there remains a justifiable role for SARS CoV-2 CHIMs. A clear advantage of a CHIM over natural infection field studies is the known-timepoint of infection; allowing the detailed characterisation of both viral kinetics and the host immune response post-exposure. The dose of virus can also be carefully controlled and adjusted, providing crucial information about how the infectious dose affects the clinical and immunological response to the virus. Importantly, CHIMs also allow the collection of pre-exposure samples. These baseline samples can be assessed against clinical outcomes to identify immune correlates of protection (CoP).

Whilst current literature clearly defines the role of neutralising antibodies (nABs) as a correlate for sterilising immunity against SARS-CoV-2 (43–46), emerging evidence, particularly with the evolution of Variants of Concern (VoC) that escape nABs, is that the immune response to SARS-CoV-2 is more complex. Cell-mediated immunity, memory B cells and non-neutralising Fc-mediated effector functions may all play a role (47–53). Local mucosal immune responses have demonstrably protected against infection from other respiratory pathogens (54, 55) but mucosal immunity against SARS-CoV-2 remains poorly described in the literature. A CHIM with infection at a controlled timepoint allows the detailed interrogation of all aspects of the protective immune response, particularly the early host mucosal responses that are often missed in natural field infection studies.

Furthermore, the ability to control confounders such as inoculum strain, route of exposure, viral load and patient heterogeneity in a CHIM allows direct comparison of vaccine and therapeutic candidates as well as dosing regimens. With the roll-out of successful vaccines, it is unfeasible and unethical to maintain an unvaccinated placebo group for the testing of new vaccine candidates. Non-inferiority trials require large sample sizes and sufficient naturally acquired infection which can be time consuming and expensive. A CHIM could be of particular use in assessing novel vaccines, including those developed to be mucosally-delivered, which may have differing end-points (such as prevention of infection or viral shedding) that would be extremely difficult to study

without a defined timepoint of infection. Whilst field studies are considered gold standard for vaccine licensure, there are instances where CHIMs have been used directly as proof of efficacy (4).

Current and future approaches to developing a SARS-CoV-2 CHIM

To date there are three registered SARS-CoV-2 CHIMs (Summarised in Table 1). The wild-type (pre-Alpha) SARS-CoV-2 CHIM in healthy, seronegative, UK 18-29-year olds demonstrated infection in 53% (18/34) of volunteers using a low inoculum dose of 10TCID50 (50% tissue culture infectious dose). Challenge was safe and well-tolerated with no evidence of lower respiratory tract involvement, although smell disturbance was common and prolonged in a small number of volunteers (23). Killingley et al. were able to accurately delineate the viral kinetics of primary infection and identified differences in viral dynamics depending on swab site. Viable virus measured by focus forming assay (FFA) persisted for on average 10 (maximum of 12) days, consistent with pre-Alpha isolation guidance (23). FFA was shown to closely correlate with lateral flow antigen (LFA) tests performed on the same swab samples. This first in human SARS-CoV-2 CHIM has demonstrated the broad utility of CHIMs, strengthening confidence in the public health measures (such as isolation periods and use of LFA tests) employed in the UK. Exploration of immune correlates of protection in this seronegative cohort, such as cross-reactive responses from seasonal coronaviruses, is ongoing.

With increasing global seroprevalence to SARS-CoV-2 from vaccination and/or infection (56), a seropositive SARS-CoV-2 CHIM is needed in order to facilitate future vaccine and therapeutic development in volunteers that reflects real world immunity. Successfully establishing a re-infection model additionally allows the identification of both local and systemic immune markers attained via the infection or vaccination process that are protective against re-infection, which could inform future public health strategies as well as design of therapeutics and vaccines.

Ongoing use of a pre-Alpha strain for a seropositive CHIM has several potential issues. Field data suggests that acquired immunity (either by vaccination, natural infection or both – hybrid immunity) offers strong resistance to homologous re-infection (57, 58). Achieving consistent infection rates may therefore prove more difficult than in a study of naïve participants.

Much of knowledge of re-infection rates was obtained prior to the emergence of variants such as Delta and subsequently Omicron, which are known to escape immunity. Both variants have antigenic divergence due to mutations in the spike protein and have been shown to demonstrate reduced neutralisation titres compared to pre-Alpha strains in vaccinated and hybrid cohorts (59–62). One approach which may circumvent any difficulty achieving infection in seropositive volunteers is to use variants more likely to cause breakthrough infections as the challenge agent, such as the Delta variant (isrctn.com ISRCTN94747181). Manufacture of an Omicron challenge agent is also being pursued (63).

There are pros and cons to the use of Delta or Omicron in a CHIM. Neutralisation against the Omicron variant is more markedly reduced than delta and associated with a higher rate of breakthrough infections (47, 61, 64) making it plausible that it would be easier to achieve infection in a CHIM. Omicron may also be a safer challenge agent demonstrating milder disease severity and reduced lower respiratory tract disease (65–68). However, the shorter infection course seen with the Omicron variant may also make it difficult to assess post-infection therapeutics (69).

Studies using currently prevalent variants are arguably more relevant both for the development pipeline of vaccines and therapeutics and understanding CoP. Limitations to this approach are that manufacturing a new challenge strain under GMP conditions takes at least 6 months (70). Furthermore, any specific clinical risks of that variant need to be understood from real world data prior to use in an ethically sound CHIM. The high incidence of SARS-CoV-2 and associated viral replication globally has resulted in the relatively rapid acquisition of mutations and development of new VoCs, meaning that by the time an inoculum strain is ready for use in a CHIM it may no longer be the dominant variant in the real world. However, developing several CHIMs that use variants derived from different lineages will enable broad assessment of different therapeutics and vaccines.

Discussion

Tuberculosis and Covid-19 represent two deadly, but distinct, respiratory diseases. Whilst highly efficacious vaccines against Covid-19 were developed at unprecedented speed against the backdrop of the evolving pandemic, progress in improving on the limited overall efficacy of the BCG vaccine against TB has been much slower. All possible research approaches that can be utilised to expedite progress should be harnessed to improve this situation. We must also remain vigilant against the potential for further SARS-CoV-2 mutations and need to have methods available to be able to rapidly assess new vaccines and therapeutics.

CHIMs may prove to be useful tools in our armoury against both of these pandemic pathogens, despite their unique situations and challenges. There are no validated CoP in TB and use of CHIMs to interrogate human immunological responses following a defined timepoint infection could increase our understanding in this area. Whilst validated CoP, for example in the form of nABs, do exist for Covid-19, these are clearly not the only factor contributing to immunity, particularly against initial infection and transmissibility. The early host mucosal immune response to *M.tb* and SARS-CoV-2 represent an important knowledge gap for both pathogens. The ability to abort infection at its point of entry could prevent LTBI and provide epidemic control by blocking onward transmission of SARS-CoV-2. These initial mucosal responses can only really be studied in an experimental setting with a known timepoint of infection.

Identification of the ideal challenge agent for a CHIM remains an issue for both of these diseases. Use of virulent *M.tb* is unethical and therefore any deployable TB CHIM will only provide partial information about the true protective efficacy of a tested vaccine or therapeutic against *M.tb*. Progress is underway to identify surrogate agents which could be utilised and, given the differing advantages and disadvantages of various agents and routes of challenge (see Table 1),

it may be that a combination of the available options will need to be employed depending on the exact question to be answered or until new modified organisms are available (11). In Covid-19, viral mutations mean that manufacture of a challenge agent may lag behind currently circulating variants. Utilisation of a variant with optimal challenge properties (for example, high levels of infectivity with low potential to cause severe disease), such as those seen in the Omicron variant may be one approach. Or it may be that, similarly to TB, a range of challenge agents could be developed and utilised depending on the specific question to be answered.

A CHIM for the purpose of novel vaccine and therapeutic evaluation needs to be able to accurately quantify pathogen load. This is undertaken with quantitative PCR (qPCR) on minimally invasive samples from the oral or nasal mucosa for SARS-CoV-2. The sampling and quantification of mycobacteria, particularly from the respiratory tract, for a TB CHIM remains much less straightforward, for example due to the fastidious and slow growing nature of mycobacteria and colonisation of the respiratory tract with organisms including non-tuberculous mycobacteria. One potential entirely non-invasive solution under development is the use of specially adapted face masks, containing a collection matrix to sample exhaled pathogens, which are then detected *via* qPCR. Initially developed as a potential diagnostic tool for TB (71), these are currently being evaluated in both TB (Clinicaltrials.gov NCT03912207) and COVID (Clinicaltrials.gov NCT04864548), highlighting how solutions initially designed for one pathogen can be utilised in another.

Applicability of CHIMs utilised in young, healthy adults to real world populations of interest is an area of consideration for both pathogens. In TB, there is a drive to deliver CHIMs in TB endemic settings, to ensure information derived and interventions tested are relevant to eventual target populations (11). In Covid-19, applicability of results obtained in a CHIM to those most at risk of disease, including the elderly and immunocompromised, is not yet known. There may be fundamental differences in the way these populations respond to the virus that limit the generalisability of a CHIM conducted purely in young, immunocompetent adults. Interestingly, in more established respiratory pathogens, efforts are underway to develop safe CHIMs in older adults (72), but it is not at all clear that this would be ethical or feasible with SARS-CoV-2

With multiple studies ongoing to develop and optimise CHIMs within both TB and Covid-19, this is an area of considerable scientific interest and promise. Momentum gained in research during the Covid-19 pandemic should be harnessed to ensure CHIMs for these, and other, pathogens continue to be developed and to exploit their full potential, in particular the fields of vaccine development and to further our understanding of host-pathogen immunobiology.

Author contributions

HMo and SJ contirubuted equally to this work and share first authorship. HMo and SJ wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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References

1. Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, et al. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. *J Infect Dis* (2009) 200(3):337–46. doi: 10.1086/600120

2. Clements ML, Betts RF, Murphy BR. Advantage of live attenuated cold-adapted influenza a virus over inactivated vaccine for A/Washington/80 (H3N2) wild-type virus infection. *Lancet* (1984) 1(8379):705–8. doi: 10.1016/S0140-6736(84)92222-0

3. Jin C, Gibani MM, Moore M, Juel HB, Jones E, Meiring J, et al. Efficacy and immunogenicity of a vi-tetanus toxoid conjugate vaccine in the prevention of typhoid fever using a controlled human infection model of salmonella typhi: a randomised controlled, phase 2b trial. *Lancet* (2017) 390(10111):2472–80. doi: 10.1016/S0140-6736(17)32149-9

4. Chen WH, Cohen MB, Kirkpatrick BD, Brady RC, Galloway D, Gurwith M, et al. Single-dose live oral cholera vaccine CVD 103-HgR protects against human

experimental infection with vibrio cholerae O1 El tor. *Clin Infect Dis* (2016) 62 (11):1329–35. doi: 10.1093/cid/ciw145

5. WHO. *World health organisation: global tuberculosis report 2022* (2022). Available at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022.

6. Kirby T. Global tuberculosis progress reversed by COVID-19 pandemic. *Lancet Respir Med* (2021) 9(12):e118–9. doi: 10.1016/S2213-2600(21)00496-3

7. Giovanni Battista M, Pei Min T, Jan-Willem A, Justin D, Marina T, Fatma A, et al. Gauging the impact of the COVID-19 pandemic on tuberculosis services: a global study. *Eur Respir J* (2021) 58(5):2101786. doi: 10.1136/bmj.m3379

8. Lamontagne F, Agarwal A, Rochwerg B, Siemieniuk RA, Agoritsas T, Askie L, et al. A living WHO guideline on drugs for covid-19. *Bmj* (2020) 370:m3379. doi: 10.1136/bmj.m3379

9. Ramchandani R, Kazatchkine M, Liu J, Sudan P, Dybul M, Matsoso P, et al. Vaccines, therapeutics, and diagnostics for covid-19: redesigning systems to improve pandemic response. *Bmj* (2021) 375:e067488. doi: 10.1136/bmj-2021-067488

10. Sakamoto H, Lee S, Ishizuka A, Hinoshita E, Hori H, Ishibashi N, et al. Challenges and opportunities for eliminating tuberculosis – leveraging political momentum of the UN high-level meeting on tuberculosis. *BMC Public Health* (2019) 19(1):76. doi: 10.1186/s12889-019-6399-8

11. Gordon S, Sichone S, Chirwa A, Hazenberg P, Kafuko Z, Ferreira D, et al. Practical considerations for a TB controlled human infection model (TB-CHIM); the case for TB-CHIM in Africa, a systematic review of the literature and report of 2 workshop discussions in UK and Malawi [version 1; peer review: 2 approved, 1 approved with reservations]. *Wellcome Open Res* (2023) 8(71). doi: 10.12688/wellcomeopenres.18767.1

12. Tomlinson GS, Cashmore TJ, Elkington PT, Yates J, Lehloenya RJ, Tsang J, et al. Transcriptional profiling of innate and adaptive human immune responses to mycobacteria in the tuberculin skin test. *Eur J Immunol* (2011) 41(11):3253–60. doi: 10.1002/eji.201141841

13. Pollara G, Turner CT, Rosenheim J, Chandran A, Bell LCK, Khan A, et al. Exaggerated IL-17A activity in human in vivo recall responses discriminates active tuberculosis from latent infection and cured disease. *Sci Transl Med* (2021) 13(592). doi: 10.1126/scitranslmed.abg7673

14. Silver RF, Zukowski L, Kotake S, Li Q, Pozuelo F, Krywiak A, et al. Recruitment of antigen-specific Th1-like responses to the human lung following bronchoscopic segmental challenge with purified protein derivative of mycobacterium tuberculosis. *Am J Respir Cell Mol Biol* (2003) 29(1):117–23. doi: 10.1165/rcmb.4840

15. Walrath J, Zukowski Krywiak L, Silver A, RF. Resident Th1-like effector memory cells in pulmonary recall responses to mycobacterium tuberculosis. *Am J Respir Cell Mol Biol* (2005) 33(1):48–55. doi: 10.1165/rcmb.2005-0060OC

16. Schreiber F, Huo Z, Giemza R, Woodrow M, Fenner N, Stephens Z, et al. An investigation of clinical and immunological events following repeated aerodigestive tract challenge infections with live mycobacterium bovis bacille calmette guérin. *Vaccine* (2010) 28(33):5427–31. doi: 10.1016/j.vaccine.2010.06.005

17. Minassian AM, Satti I, Poulton ID, Meyer J, Hill AV, McShane H. A human challenge model for mycobacterium tuberculosis using mycobacterium bovis bacille calmette-guerin. *J Infect Dis* (2012) 205(7):1035–42. doi: 10.1093/infdis/jis012

18. Harris SA, Meyer J, Satti I, Marsay L, Poulton ID, Tanner R, et al. Evaluation of a human BCG challenge model to assess antimycobacterial immunity induced by BCG and a candidate tuberculosis vaccine, MVA85A, alone and in combination. *J Infect Dis* (2014) 209(8):1259–68. doi: 10.1093/infdis/jit647

19. Minhinnick A, Harris S, Wilkie M, Peter J, Stockdale L, Manjaly-Thomas ZR, et al. Optimization of a human bacille calmette-guérin challenge model: a tool to evaluate antimycobacterial immunity. *J Infect Dis* (2016) 213(5):824–30. doi: 10.1093/infdis/jiv482

20. Blazevic A, Xia M, Turan A, Tennant J, Hoft DF. Pilot studies of a human BCG challenge model. *Tuberculosis (Edinb)* (2017) 105:108–12. doi: 10.1016/j.tube.2017.05.001

21. Davids M, Pooran A, Hermann C, Mottay L, Thompson F, Cardenas J, et al. A human lung challenge model to evaluate the safety and immunogenicity of PPD and live bacillus calmette-guérin. *Am J Respir Crit Care Med* (2020) 201(10):1277–91. doi: 10.1164/rccm.201908-1580OC

22. Jackson S, McShane H. *Challenges in developing a controlled human tuberculosis challenge model*. (2022) Springer Berlin Heidelberg: Berlin, Heidelberg p. 1–27.

23. Killingley B, Mann AJ, Kalinova M, Boyers A, Goonawardane N, Zhou J, et al. Safety, tolerability and viral kinetics during SARS-CoV-2 human challenge in young adults. *Nat Med* (2022) 28(5):1031–41. doi: 10.1038/s41591-022-01780-9

24. Bell LC, Pollara G, Pascoe M, Tomlinson GS, Lehloenya RJ, Roe J, et al. In vivo molecular dissection of the effects of HIV-1 in active tuberculosis. *PloS Pathog* (2016) 12(3):e1005469. doi: 10.1371/journal.ppat.1005469

25. Calmette. A. *L'infection bacillaire et la tuberculose chez l'homme et chez les animaux* (1920). Paris: Masson.

26. Hanekom WA. The immune response to BCG vaccination of newborns. *Ann N Y Acad Sci 2005* (1062) p:69–78. doi: 10.1196/annals.1358.010

27. Minassian AM, Ronan EO, Poyntz H, Hill AV, McShane H. Preclinical development of an in vivo BCG challenge model for testing candidate TB vaccine efficacy. *PloS One* (2011) 6(5):e19840. doi: 10.1371/journal.pone.0019840

28. Harris SA, White A, Stockdale L, Tanner R, Sibley L, Sarfas C, et al. Development of a non-human primate BCG infection model for the evaluation of candidate tuberculosis vaccines. *Tuberculosis* (2018) 108:99–105. doi: 10.1016/j.tube.2017.11.006

29. Villarreal-Ramos B, Berg S, Chamberlain L, McShane H, Hewinson RG, Clifford D, et al. Development of a BCG challenge model for the testing of vaccine candidates against tuberculosis in cattle. *Vaccine* (2014) 32(43):5645–9. doi: 10.1016/j.vaccine.2014.08.009

30. Morrison H, McShane H. Local pulmonary immunological biomarkers in tuberculosis. *Front Immunol* (2021) 12:640916. doi: 10.3389/fimmu.2021.640916

31. Bekeredjian-Ding I, Van Molle W, Baay M, Neels P, Berthels N, Conrad C, et al. Human challenge trial workshop: focus on quality requirements for challenge agents, langen, Germany, October 22, 2019. *Biologicals* (2020) 66:53–61. doi: 10.1016/j.biologicals.2020.04.005

32. Basu Roy R, Sambou B, Uhía I, Roetynck S, Robertson BD, Kampmann B, et al. An auto-luminescent fluorescent BCG whole blood assay to enable evaluation of paediatric mycobacterial responses using minimal blood volumes. *Front Pediatr* (2019) 7:151. doi: 10.3389/fped.2019.00151

33. Dijkman K, Aguilo N, Boot C, Hofman SO, Sombroek CC, Vervenne RAW, et al. Pulmonary MTBVAC vaccination induces immune signatures previously correlated with prevention of tuberculosis infection. *Cell Rep Med* (2021) 2 (1):100187. doi: 10.1016/j.xcrm.2020.100187

34. Tameris M, Mearns H, Penn-Nicholson A, Gregg Y, Bilek N, Mabwe S, et al. Live-attenuated mycobacterium tuberculosis vaccine MTBVAC versus BCG in adults and neonates: a randomised controlled, double-blind dose-escalation trial. *Lancet Respir Med* (2019) 7(9):757–70. doi: 10.1016/S2213-2600(19)30251-6

35. Loxton AG, Knaul JK, Grode L, Gutschmidt A, Meller C, Eisele B, et al. Safety and immunogenicity of the recombinant mycobacterium bovis BCG vaccine VPM1002 in HIV-unexposed newborn infants in south Africa. *Clin Vaccine Immunol* (2017) 24 (2). doi: 10.1128/CVI.00439-16

36. Nieuwenhuizen NE, Kulkarni PS, Shaligram U, Cotton MF, Rentsch CA, Eisele B, et al. The recombinant bacille calmette-guérin vaccine VPM1002: ready for clinical efficacy testing. *Front Immunol* (2017) 8:1147. doi: 10.3389/fimmu.2017.01147

37. Gordon SB, Rylance J, Luck A, Jambo K, Ferreira DM, Manda-Taylor L, et al. A framework for controlled human infection model (CHIM) studies in Malawi: report of a wellcome trust workshop on CHIM in low income countries held in Blantyre, Malawi. *Wellcome Open Res* (2017) 2:70. doi: 10.12688/wellcomeopenres.12256.1

38. Jamrozik E, Littler K, Bull S, Emerson C, Kang G, Kapulu M, et al. Key criteria for the ethical acceptability of COVID-19 human challenge studies: report of a WHO working group. *Vaccine* (2021) 39(4):633–40. doi: 10.1016/j.vaccine.2020.10.075

39. Levine MM, Abdullah S, Arabi YM, Darko DM, Durbin AP, Estrada V, et al. Viewpoint of a WHO advisory group tasked to consider establishing a closely-monitored challenge model of coronavirus disease 2019 (COVID-19) in healthy volunteers. *Clin Infect Dis* (2021) 72(11):2035–41. doi: 10.1093/cid/ciaa1290

40. Rapeport G, Smith E, Gilbert A, Catchpole A, McShane H, Chiu C. SARS-CoV-2 human challenge studies - establishing the model during an evolving pandemic. *N Engl J Med* (2021) 385(11):961–4. doi: 10.1056/NEJMp2106970

41. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, south Africa, and the UK. *Lancet* (2021) 397(10269):99–111. doi: 10.1016/S0140-6736(20)32661-1

42. Kleinwaks G, Schmit V, Morrison J. Considering human challenge trials for tuberculosis vaccine development. *Vaccine* (2021) 2:173–4. doi: 10.1016/j.vaccine.2021.11.024

43. Garcia-Beltran WF, Lam EC, Astudillo MG, Yang D, Miller TE, Feldman J, et al. COVID-19-neutralizing antibodies predict disease severity and survival. *Cell* (2021) 184(2):476–488.e11. doi: 10.1016/j.cell.2020.12.015

44. Addetia A, Crawford KHD, Dingens A, Zhu H, Roychoudhury P, Huang ML, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. *J Clin Microbiol* (2020) 58(11). doi: 10.1128/JCM.02107-20

45. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 breakthrough infections in vaccinated health care workers. *N Engl J Med* (2021) 385 (16):1474–84. doi: 10.1056/NEJMoa2109072

46. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* (2021) 27(7):1205–11. doi: 10.1038/s41591-021-01377-8

47. Garcia-Beltran WF, St Denis KJ, Hoelzemer A, Lam EC, Nitido AD, Sheehan ML, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 omicron variant. *Cell* (2022) 185(3):457–466.e4. doi: 10.1016/j.cell.2021.12.033

48. Tarke A, Coelho CH, Zhang Z, Dan JM, Yu ED, Methot N, et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from alpha to omicron. *Cell* (2022) 185:847–59.e11. doi: 10.1101/2021.12.28.474333

49. Keeton R, Tincho MB, Ngomti A, Baguma R, Benede N, Suzuki A, et al. T Cell responses to SARS-CoV-2 spike cross-recognize omicron. *Nature* (2022) 603 (7901):488–92. doi: 10.1038/s41586-022-04460-3

50. Liu J, Chandrashekar A, Sellers D, Barrett J, Jacob-Dolan C, Lifton M, et al. Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 omicron. *Nature* (2022) 603(7901):493–6. doi: 10.1038/s41586-022-04465-y

51. Kaplonek P, Fischinger S, Cizmeci D, Bartsch YC, Kang J, Burke JS, et al. mRNA-1273 vaccine-induced antibodies maintain fc effector functions across SARS-CoV-2 variants of concern. *Immunity* (2022) 55(2):355–365.e4. doi: 10.1016/j.immuni.2022.01.001

52. Wang Z, Muecksch F, Schaefer-Babajew D, Finkin S, Viant C, Gaebler C, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* (2021) 595(7867):426–31. doi: 10.1038/s41586-021-03696-9

53. Muecksch F, Wang Z, Cho A, Gaebler C, Ben Tanfous T, DaSilva J, et al. Increased memory b cell potency and breadth after a SARS-CoV-2 mRNA boost. *Nature* (2022) 607(7917):128–34. doi: 10.1038/s41586-022-04778-y

54. Bagga B, Cehelsky JE, Vaishnaw A, Wilkinson T, Meyers R, Harrison LM, et al. Effect of preexisting serum and mucosal antibody on experimental respiratory syncytial virus (RSV) challenge and infection of adults. *J Infect Dis* (2015) 212(11):1719–25. doi: 10.1093/infdis/jiv281

55. Renegar KB, Small PA Jr, Boykins LG, Wright PF. Role of IgA versus IgG in the control of influenza viral infection in the murine respiratory tract. *J Immunol* (2004) 173(3):1978–86. doi: 10.4049/jimmunol.173.3.1978

56. Bergeri I, Whelan MG, Ware H, Subissi L, Nardone A, Lewis HC, et al. Global SARS-CoV-2 seroprevalence from January 2020 to April 2022: a systematic review and meta-analysis of standardized population-based studies. *PloS Med* (2022) 19(11): e1004107. doi: 10.1371/journal.pmed.1004107

57. Hall V, Foulkes S, Insalata F, Kirwan P, Saei A, Atti A, et al. Protection against SARS-CoV-2 after covid-19 vaccination and previous infection. *New Engl J Med* (2022) 386(13):1207–20. doi: 10.1056/NEJMoa2118691

58. Abu-Raddad LJ, Chemaitelly H, Coyle P, Malek JA, Ahmed AA, Mohamoud YA, et al. SARS-CoV-2 antibody-positivity protects against reinfection for at least seven months with 95% efficacy. *EClinicalMedicine* (2021) 35:100861. doi: 10.1016/j.eclinm.2021.100861

59. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira I, et al. SARS-CoV-2 B.1.617.2 delta variant replication and immune evasion. *Nature* (2021) 599 (7883):114–9. doi: 10.1038/s41586-021-03944-y

60. Planas D, Veyer D, Baidaliuk A, Staropoli I, Guivel-Benhassine F, Rajah MM, et al. Reduced sensitivity of SARS-CoV-2 variant delta to antibody neutralization. *Nature* (2021) 596(7871):276–80. doi: 10.1038/s41586-021-03777-9

61. Rössler A, Riepler L, Bante D, von Laer D, Kimpel J. SARS-CoV-2 omicron variant neutralization in serum from vaccinated and convalescent persons. *New Engl J Med* (2022) 386(7):698–700. doi: 10.1056/NEJMc2119236

62. Cele S, Jackson L, Khoury DS, Khan K, Moyo-Gwete T, Tegally H, et al. Omicron extensively but incompletely escapes pfizer BNT162b2 neutralization. *Nature* (2022) 602(7898):654–6. doi: 10.1038/s41586-021-04387-1

63. hVIVO. *Covid-19 challenge models* (2023). Available at: https://hvivo.com/human-challenge-models/covid-19/.

64. Goga A, Bekker L-G, Garrett N, Reddy T, Yende-Zuma N, Fairall L, et al. Breakthrough SARS-CoV-2 infections during periods of delta and omicron predominance, south Africa. *Lancet* (2022) 400(10348):269–71. doi: 10.1016/S0140-6736(22)01190-4

65. Wolter N, Jassat W, Walaza S, Welch R, Moultrie H, Groome M, et al. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in south Africa: a data linkage study. *Lancet* (2022) 399(10323):437–46. doi: 10.1016/S0140-6736(22)00017-4

66. Christensen PA, Olsen RJ, Long SW, Snehal R, Davis JJ, Ojeda Saavedra M, et al. Signals of significantly increased vaccine breakthrough, decreased hospitalization rates, and less severe disease in patients with coronavirus disease 2019 caused by the omicron variant of severe acute respiratory syndrome coronavirus 2 in Houston, Texas. *Am J Pathol* (2022) 192(4):642–52. doi: 10.1016/j.ajpath.2022.01.007

67. Hui KPY, Ho JCW, Cheung MC, Ng KC, Ching RHH, Lai KL, et al. SARS-CoV-2 omicron variant replication in human bronchus and lung ex vivo. *Nature* (2022) 603:715–20. doi: 10.1038/s41586-022-04479-6

68. Tsakok MT, Watson RA, Saujani SJ, Kong M, Xie C, Peschl H, et al. Reduction in chest CT severity and improved hospital outcomes in SARS-CoV-2 omicron compared with delta variant infection. *Radiology* (2023) 306(1):261–9. doi: 10.1148/radiol.220533

69. Wu Y, Guo Z, Yuan J, Cao G, Wang Y, Gao P, et al. Duration of viable virus shedding and polymerase chain reaction positivity of the SARS-CoV-2 omicron variant in the upper respiratory tract: a systematic review and meta-analysis. *Int J Infect Dis* (2023) 129:228–35. doi: 10.1016/j.ijid.2023.02.011

70. Catchpole AP, Fullen DJ, Noulin N, Mann A, Gilbert AS, Lambkin-Williams R. The manufacturing of human viral challenge agents for use in clinical studies to accelerate the drug development process. *BMC Res Notes* (2018) 11(1):620. doi: 10.1186/s13104-018-3636-7

71. Williams CM, Abdulwhhab M, Birring SS, De Kock E, Garton NJ, Townsend E, et al. Exhaled mycobacterium tuberculosis output and detection of subclinical disease by face-mask sampling: prospective observational studies. *Lancet Infect Dis* (2020) 20 (5):607–17. doi: 10.1016/S1473-3099(19)30707-8

72. Ascough S, Dayananda P, Kalyan M, Kuong SU, Gardener Z, Bergstrom E, et al. Divergent age-related humoral correlates of protection against respiratory syncytial virus infection in older and young adults: a pilot, controlled, human infection challenge model. *Lancet Healthy Longev* (2022) 3(6):e405–16. doi: 10.1016/S2666-7568(22)00103-9

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Suppressive myeloid cells in SARS-CoV-2 and *Mycobacterium tuberculosis* co-infection

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Epidemiologic data show that both current and previous tuberculosis (TB) increase the risk of in-hospital mortality from coronavirus disease-2019 (COVID-19), and there is a similar trend for poor outcomes from *Mycobacterium tuberculosis* (Mtb) infection after recent SARS-CoV-2. A shared dysregulation of immunity explains the dual risk posed by co-infection, but the specific mechanisms are being explored. While initial attention focused on T cell immunity, more comprehensive analyses revealed a dysfunctional innate immune response in COVID-19, characterized by reduced numbers of dendritic cells, NK cells and a redistribution of mononuclear phagocytes towards intermediate myeloid subsets. During hyper- or chronic inflammatory processes, activation signals from molecules such as growth factors and alarmins lead to the expansion of an immature population of myeloid cells called myeloid-deprived suppressor cells (MDSC). These cells enter a state of pathological activation, lose their ability to rapidly clear pathogens, and instead become broadly immunosuppressive. MDSC are enriched in the peripheral blood of patients with severe COVID-19; associated with mortality; and with higher levels of inflammatory cytokines. In TB, MDSC have been implicated in loss of control of Mtb in the granuloma and ineffective innate and T cell immunity to the pathogen. Considering that innate immune sensing serves as first line of both anti-bacterial and anti-viral defence mechanisms, we propose MDSC as a crucial mechanism for the adverse clinical trajectories of TB-COVID-19 coinfection.

KEYWORDS

myeloid derived suppressor cells, tuberculosis, SARS-CoV-2, COVID-19, coinfection

1 Introduction

There is now ample evidence that regions with a high prevalence of tuberculosis (TB) disease and latent TB infection (LTBI, where an asymptomatic person has a positive interferon-γ release assay or skin test), also have a high prevalence of recent SARS-CoV-2 infection (1–4). As a result, acute or chronic coinfection, or acute sequential infection with *Mycobacterium tuberculosis* (Mtb) and SARS-CoV-2 has become inevitable.

We know from epidemiologic reports during the pandemic that coinfection with Mtb and SARS-CoV-2 worsens patient outcomes. Both current active TB disease (defined as culture, molecular test, or other Mtb test positive, with symptoms or imaging changes that justify the initiation of full TB treatment) and previous TB, increase the risk of in-hospital mortality from coronavirus disease-2019 (COVID-19), and the case fatality rate for coinfection is higher than for COVID-19 alone (5, 6). The lymphopaenia which characterizes COVID-19 is exaggerated in coinfection, and markers of inflammation such as D-dimer and ferritin are increased over and above the COVID-19 levels (7, 8). Transcriptomics and RNAseq data from whole blood, peripheral blood mononuclear cells (PBMC) and bronchoalveolar lavage fluid (BALF) of patients with COVID-19, and those with TB across the clinical spectrum, has shown that there is similarity in the immunopathogenesis of the two diseases through commonly enriched genes in 12-gene disease-exacerbation hot spots (9). Moreover, the inflammatory milieu from Mtb-infected human macrophages increased SARS-CoV-2 infection *in vitro*, which correlated with IFNA1, IFNB1, IFNG, TNF, and other inflammatory gene induction. Interestingly, in Mtb-infected mice, superinfection with SARS-CoV-2 resulted in increased Mtb dissemination, but a lower SARS-CoV-2 viral load in the tissues (10). In a different murine study, the protective effect of pre-existing Mtb infection on the pathological consequences of SARS-CoV-2 occurred without adversely affecting TB outcomes (11). This collection of findings shows that Mtb infection increases the risk of severe COVID-19 in humans, and suggests the possibility that SARS-CoV-2 coinfection may also trigger the progression of subclinical TB to TB disease.

Patients with active TB disease and SARS-CoV-2 coinfection have lower numbers of Mtb-specific T cells (12). They also produce less IFN-γ and other proinflammatory cytokines, chemokines and growth factors on SARS-CoV-2 stimulation; produce less interferon-γ (IFN-γ) and several other cytokines on Mtb stimulation (though to a lesser extent than the reduction on SARS-CoV-2 stimulation); and have different overall cytokine signatures compared to infection with each pathogen alone (12–14). One possible mechanism underlying this observed immune suppression in the presence of chronic stimulation by Mtb or SARS-CoV-2 antigens, is the presence of suppressive myeloid cells such as myeloid-derived suppressor cells (MDSC). MDSC are known to inhibit many immune pathways, particularly T cell responses. They have now been both directly and indirectly implicated in the pathogenesis of both COVID-19 and TB (15–17). However, their role in coinfection is yet to be explored. In this article we will introduce the reader to MDSC, briefly review the evidence for their involvement in TB disease and COVID-19, and then discuss the potential role of these cells in determining the outcome of Mtb/SARS-CoV-2 coinfection.

2 Suppressive myeloid cells

Suppressive myeloid cells are considered critical in immune regulation and tolerance, maintaining the delicate balance between healing and harm during the immune response. They limit excessive inflammation and prevent immune-mediated tissue damage in the early response to a tissue insult, promote immune tolerance during tissue repair or pregnancy, and augment protective anti-pathogen responses in acute infection (18–22). However, in pathological conditions such as chronic inflammation, cancer, or extensive tissue trauma, the scales tip toward more harm than help. The function of MDSC in the pathophysiology of cancer is well described (23–25), but in chronic infection and respiratory disease is still in the early stages of investigation. Our understanding of their role in these conditions is hampered by a few ongoing issues. Firstly, the cell type terminology is not globally accepted. Secondly, the nature of MDSC remains poorly defined, partly because these cells likely differentiate into suppressive macrophage subsets upon entering tissue sites. However, most agree that MDSC are cells of myeloid origin which acquire a state of pathological (or alternative) activation in response to the prolonged weak pro-inflammatory signals that are present in chronic infection or cancer (25–27). As a result, they lose their ability to rapidly and effectively clear pathogens, instead becoming immunosuppressive by inhibiting natural killer (NK) cell, B cell, and T cell responses, amongst others.

MDSC are generally divided into two main subtypes (Figure 1). They are named for their cell lineage of origin as polymorphonuclear or granulocytic MDSC (PMN-MDSC) and monocytic MDSC (M-MDSC). A third group which comprises only a small proportion of the total MDSC population is known as 'early MDSC', and consists of potent immunosuppressive myeloid progenitors (28). Other subtypes such as eosinophilic MDSC have been proposed, but are not well characterized as yet. In circulation, both PMN- and M-MDSC have a short lifespan of a few days, though the latter survive longer *in vitro* (29) and the half-life may well be prolonged in inflammatory states (30). Their continuous recruitment to tissues is what results in long term effects (31). In tumors, M-MDSC rapidly differentiate into tumor-associated macrophages (TAMs) which are associated with tumor progression, and inflammatory dendritic cells (32, 33). Tumor associated neutrophils (TANs) are a heterogeneous population of cells which, in mice, includes both neutrophils with anti-tumor (N1) and suppressive/pro-tumor (N2) properties, the latter sharing some cell surface markers and biochemical properties with PMN-MDSC (34, 35).

Identifying these cells is complex, and not always consistent between studies. Cell surface markers which identify MDSC differ between mice and humans. For the purpose of this review we will focus on human-relevant markers only. PMN-MDSC are identified as $CD11b^+CD14^-CD15^+/CD66b^+$ cells in the low density Ficoll gradient fraction of PBMC. Other marker combinations have been proposed which do not need a Ficoll gradient, such as $CD15^+/CD66b^+CD14^-LOX1^+$ and $CD15^+/CD66b^+CD14^-CD84^+$. All human MDSC are $HLA\text{-}DR^{lo/-}$. M-MDSC are identified as $CD11b^+CD14^+CD15^-HLA\text{-}DR^{lo/-}$ cells in the low density Ficoll fraction of PBMC, or alternatively $CD14^+/CD66b^-CXCR1^+$ or $CD14^+/CD66b^-CD84^+$ (28, 31, 36). Early MDSC are identified as $Lin^-HLA\text{-}DR^-CD33^{+/hi}$ (where Lin is CD3, CD14, CD15, CD19 and CD56) (25, 28). Some of the newer markers such as LOX-1 have yet to be validated in infection-induced MDSC.

Classical neutrophils and monocytes are activated by pathogen- and damage-associated molecular patterns (such as lipopolysaccharide and heat shock proteins respectively) binding

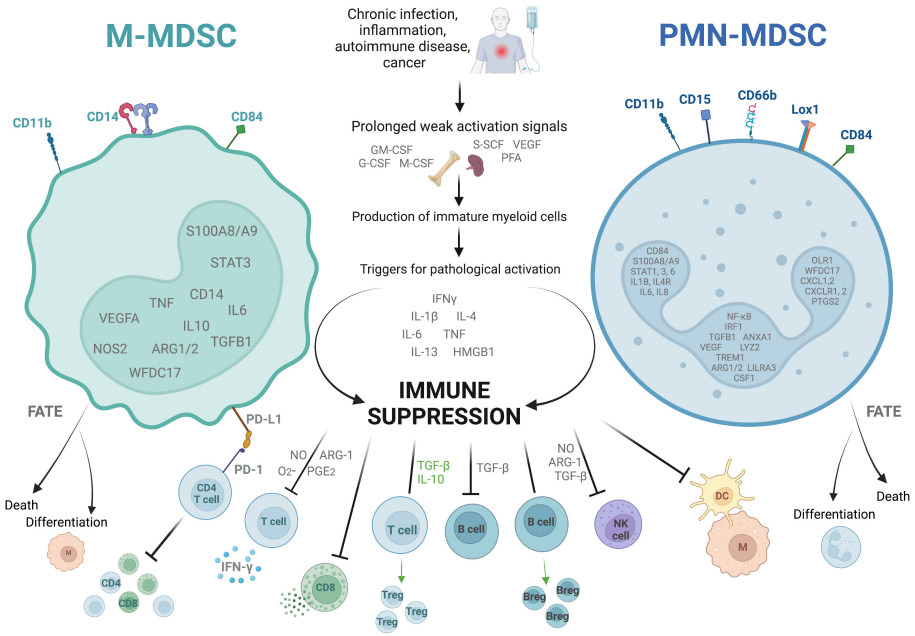

FIGURE 1
Characteristics of the two dominant subtypes of myeloid derived suppressor cells. The figure shows the two main types of myeloid derived suppressor cells (MDSC), monocytic (M)- and polymorphonuclear (PMN)-MDSC. They are identified as cells in the low density portion of a Ficoll gradient with a low expression of HLA-DR, as well as specific combinations of cell surface markers, and the upregulation of specific genes (shown inside the nucleus). They arise in situations of prolonged weak activation signals, by which they are pathologically activated to become immunosuppressive. They exert their immunosuppressive effects through several different mechanisms including suppression of $CD4^+$ and $CD8^+$ T cell differentiation, cytokine release, and cytotoxic degranulation; promotion of T regulatory cell (Treg) and B regulatory cell (Breg) differentiation and function; and inhibition of B cell, natural killer cell (NK), and antigen presenting cell (M, macrophage; DC, dendritic cell) functions. They are short lived, either dying after a few days in circulation or differentiating into suppressive macrophages or suppressive neutrophils. ARG-1, Arginase-1; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HMGB-1, high mobility box group-1; IFNγ, interferon-γ; IL, interleukin; Lox-1, lectin- type oxidized LDL receptor 1; M-CSF, macrophage colony- stimulating factor; NO, nitric oxide; PD-1, programmed cell death protein-1; PD-L1, programmed death ligand-1; PFA, polyunsaturated fatty acids; $PG\text{-}E_2$, prostaglandin-E_2; SCF, stem cell factor; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. Created with BioRender.com.

to pattern recognition receptors such as Toll-like receptors (TLRs). This interaction triggers the innate immune mechanisms for the rapid clearance of microbes and infected cells, such as phagocytosis, the respiratory burst, the production of proinflammatory cytokines and upregulation of co-stimulatory molecules and MHC-II (25). In contrast, MDSC are the result of prolonged but weak stimulation with growth factors, cytokines and other stimuli (such as GM-CSF, VEGF, NLRP3, and the S100A8/A9 alarmins), and activation is triggered by further stimulation with inflammatory cytokines and pathogen- or damage-associated molecular patterns including iIFN-γ, interleukin (IL)-1β, IL-4, IL-6, IL-13, tumor necrosis factor (TNF), high mobility group box (HMGB)-1 (37–41). The transcription factor STAT3 is also invariably upregulated in MDSC, as the main regulator of genes controlling the expansion of MDSC (31, 42).

Overall, the most important characteristic of these cells which distinguishes them from classical neutrophils and monocytes is their ability to inhibit immune responses, specifically T cell activation and function (28, 31). They use multiple mechanisms to achieve their suppressive effect. Depletion of L-arginine from the microenvironment through upregulation of arginase-1 (ARG1) and inducible nitric oxide synthase (iNOS), inhibits the key human T cell receptor (TCR) signaling molecule CD3ζ *in vitro* (43, 44). Nitric oxide from the iNOS also interferes with JAK/STAT signaling in the T cells of mice *in vitro* (45, 46). In addition to this, increased reactive oxygen species (ROS) in murine immature myeloid cells and human low density granulocytes reduces the expression of CD3ζ on T cells, and reactive nitrogen species (RNS) block T cell activation *ex vivo* by nitrating the TCR and CD8 molecule (47–49). MDSC of both humans and mice express programmed death-ligand-1 (PD-L1) which causes T cell dysfunction, exhaustion and IL-10 secretion when it interacts with programmed death protein-1 (PD-1) on the T cell surface *ex vivo* (50–52). Lastly, in murine models MDSC exert their immunosuppressive effects by the secretion of TGF-β and IL-10, which directly suppress T cells, induce differentiation into T regs, and suppress macrophage IL-12 production. Expression of membrane-bound TGF-β also suppresses NK cells (53–55).

Several host-directed therapies which target MDSC have shown efficacy in cancer therapy, through several mechanisms. MDSC expansion and recruitment can be inhibited with, for example, 5-Fluorouracil or tyrosine kinase inhibitors (56, 57). MDSC function can be inhibited through, for example, phosphodiesterase-5 inhibitors or PD-L1 inhibitors (58, 59). Lastly, agents such as All-trans retinoic acid (ATRA) promote the differentiation of MDSC into mature leukocytes or tumor-specific cells (60). Several of these

MDSC targeting agents are in the experimental stages of investigation for use in TB (61, 62). Tasquinimod causes exhaustion of MDSC, and has been shown to enhance mycobacterial clearance in mice (63). Both sildenafil and ATRA initially showed promise as TB host directed therapies, but recent data using human MDSC have been disappointing (64, 65). Many of these MDSC-targeting therapies have been identified as possible treatments for COVID-19, but little data is available on their efficacy (66). The studies in cancer and TB demonstrate the complexity of MDSC. It is likely that particular MDSC subsets predominate in particular conditions, and that they employ different suppressive mechanisms in different disease microenvironments (64). This means that even if an agent is effective in treating one condition, it may not be equally effective in treating a second condition, even if the two conditions have a similar immunopathogenesis. This must be kept in mind for SARS-CoV-2 infection as well.

3 MDSC in tuberculosis

Classically activated myeloid cells are the initial effectors of antimycobacterial responses. They sense Mtb through multiple PRRs, phagocytose the bacteria, contain them, limit replication, kill them, release cytokines and chemokines, and activate T cells which in turn increase the activation state of the myeloid cells to enable then to kill Mtb more effectively. However, while alternatively activated myeloid cell subsets – initially labelled natural suppressor cells but later renamed MDSC – also phagocytose Mtb, they have less effective mycobactericidal activity, low expression of MHC class II, secrete immune mediators which suppress T cell responses, and promote lung damage (17, 62, 67, 68).

Mtb contains structural moieties such as glycolipids, that are known to induce MDSC generation (69). MDSC have been detected in the blood of BCG vaccinated mice, where they were found to reduce T cell priming through an IL-1R-dependent pathway (70). Where mice are in the advanced clinical stages of TB disease, MDSC accumulate in the lungs, bone marrow, spleen, and blood, and suppress T cell proliferation and IFN-γ production *in vitro* through NO-dependent mechanisms (71). In humans, MDSC are enriched in the peripheral blood, bronchoalveolar lavage fluid (BALF), and pleural fluid of patients with active pulmonary and pleural TB disease, to levels and phenotype comparable to lung cancer (17, 72). The predominant subset seems to depend on the anatomical site, as PMN-MDSC were preferentially found in BALF, but M-MDSC were the main subset in the pleural fluid (17, 72). Moreover, after successful TB treatment, not only do peripheral blood levels of MDSC decline (particularly PMN-MDSC), but the MDSC express more maturation surface markers (17, 72). During active TB disease, MDSC inhibit T cell proliferation (possibly through a NO-dependent mechanism), suppress $CD4^+$ T-cell production of IL-2, TNF-α, IFN-γ *in vitro* (17). MDSC also inhibit IL-10 production by $CD4^+$ T cells, thereby inhibiting the regulation of IL-2, TNF and IFNs, and perhaps demonstrating the global shutdown of T cell activation regardless of which cytokines they produce. Furthermore, MDSC suppress $CD8^+$ T cell production of TNF-α, IL-2, IFN-γ, and IL-10 in active TB disease, and subvert effector T-cell-mediated containment of Mtb in monocyte-derived macrophages (17, 72, 73). MDSC impair T cell-mediated killing of Mtb infected cells through down-regulation of Th1 cytokines (17, 70). In addition, $CD8^+$ T cell mediated killing of infected cells using cytotoxic granules such as perforin and granulysin, is critical for Mtb control (74). Through skewing of the immune response toward a regulatory phenotype, MDSC likely suppress granule-associated effector molecules, and thereby impair killing of infected cells (74–76).

Some of the most compelling evidence for MDSC's role in TB comes from granuloma research. In mouse models, MDSC accumulate at the edges of necrotic granulomas in the lung parenchyma of infected Mtb-susceptible mice, and this finding has been associated with TB disease progression and uncontrolled bacterial replication (62, 68). Conversely, mice that are Mtb-resistant, with no necrotic granulomas, have very low levels of MDSC in their lungs (68, 77). Suppressive neutrophils which exhibit immunoregulatory functions resembling MDSC subsets have also been identified in TB granulomas from non-human primates (NHPs) (78). Another recent report in NHPs suggested that PMN-MDSC in the periphery of TB granulomas may restrict T cell access to the granuloma core and Mtb-infected cells (79). In an *in vitro* human granuloma model, *ex vivo* generated human M-MDSC promoted mycobacterial replication, changing the structure of the granuloma and adversely affecting bacterial containment (80). In lymph node granulomas from TB/HIV coinfected people and TB-only controls, MDSC expressing Arg-1 were highly expressed in TB/HIV granulomas (81), and the proportion of $CD15^+$ MDSC correlated with plasma HIV viral load and Mtb antigen load in tissue, but was negatively correlated with peripheral $CD4^+$ T cell numbers. In the same study, PMN-MDSC were also elevated in blood samples from TB/HIV coinfected patients (81). Recently, multiplexed ion beam imaging by time of flight (MIBI-TOF) was used to generate a comprehensive spatial map of 19 cell subsets across 8 spatial microenvironments within TB granulomas from multiple human tissues, including the lung (82). The myeloid core of the granulomas was characterized by expression of the tolerogenic proteins IDO1 and PD-L1, which was highest in $CD11b^+CD11c^+$ macrophages (identical to immunosuppressive TAMs). This expression was also associated with downregulation of HLA-DR in the 'intermediate monocyte' subset. These data support the existing evidence for a highly localized, myeloid-mediated immune suppression in the granuloma (82). These findings also seem to support the hypothesis that MDSC which enter a granuloma differentiate into suppressive macrophages which are permissive to Mtb growth, similar to their activity in solid tumors (74, 83).

The role of MDSC in events early in the TB disease spectrum is less clear. Recent attempts to define more clearly the spectrum and pathogenesis of TB before clinical disease highlight the gaps in knowledge of factors promoting progression from infection to disease or to cure (84). Household contacts and people with presumed LTBI have far lower median frequencies of MDSC in peripheral blood than those with active TB disease, comparable to

healthy donors (72, 73, 85). Higher frequencies of peripheral blood M-MDSC have been correlated with more severe TB disease (based on time to positivity of Mtb culture, cavitary disease on chest radiography, symptoms score, ESR and monocyte/lymphocyte ratio), but higher frequencies of PMN-MDSC have been associated with a lower radiological TB severity score (85, 86). This suggests that when considering the early TB spectrum, which includes such entities as subclinical TB – where there are no symptoms but may be radiographic changes, and which may or may not progress to active TB disease – the function of the subsets of MDSC need to be examined separately.

4 MDSC in COVID-19

As with TB and other viral infections, myeloid cells are the first responders to infection with SARS-CoV-2. After navigating the upper airways, the virus is taken up by alveolar macrophages, without active viral replication, which become activated, and are subsequently responsible for the proinflammatory anti-viral immune response (87). Other innate cells are recruited to the site of infection, and in most people the result is mild disease with eventual eradication of the virus. Yet, a proportion of people infected with SARS-CoV-2 will suffer a marked dysregulation of the innate immune response, especially the myeloid cell compartment, as a result of emergency myelopoiesis (88). This dysregulated state is characterized by the emergence of immature neutrophils and monocytes with suppressive features, including MDSC, which have been directly implicated in the pathogenesis of this dysregulated response, as well as shown to be predictive of severe or fatal disease (88–91).

As we have come to expect from the pandemic literature, there is an abundance of evidence on the topic of MDSC in COVID-19. Several studies in humans have now provided direct evidence of high peripheral blood frequencies of both subsets of MDSC in COVID-19, across all levels of COVID-19 severity but particularly in severe disease, fatal disease, and acute respiratory distress syndrome (ARDS) (91–101). Again, the different roles of the MDSC subsets are evident. Early M-MDSC frequency predicted subsequent COVID-19 severity and mortality, but transient early expansion of PMN-MDSC was associated with survivors of severe COVID-19 (91, 99, 102–104). Studies using single cell RNA sequencing (scRNAseq) in combination with flow cytometry, CyTOF and other assays, have found that populations of immature neutrophils with features of PMN-MDSC and, to a lesser extent, monocytes with features of M-MDSC, emerge in the blood and BALF of patients with severe COVID-19. These cells differentiate them from patients with mild COVID-19, in whom frequencies are still higher than healthy donors (15, 88, 105–107). M-MDSC are not increased in airway samples from nasopharyngeal and endotracheal aspirates, but large numbers of $CD66b^+$ cells with a high expression of intracytoplasmic Arg1, in line with PMN-MDSC, were found in lung tissue from patients who died of COVID-19 (91, 97).

The immunosuppressive abilities of these SARS-CoV-2-induced MDSC have been demonstrated. Both PMN-MDSC and M-MDSC from the peripheral blood of patients with moderate and severe COVID-19 inhibit T cell proliferation and IFN-γ production *in vitro* (91, 94–96, 108). In bacterial sepsis, MDSC expansion, IFN-γ production, and TNF-α production reduced over time from admission, but in COVID-19 these responses accelerated over time, despite initial lesser physiological derangement (109). The presence of PMN-MDSC increased the expression of Arg1 and iNOS mRNA compared to PMN-MDSC-depleted PBMC, and plasma levels of TGF-β directly correlated with PMN-MDSC frequency. Moreover, PMN-MDSC depletion significantly improved the SARS-CoV-2 specific T cell response of PBMC (95). Similarly, PD-L1, ILT-3 and IDO-1-expressing M-MDSC were the dominant producers of IL-10 and IL-6 in severe COVID-19 patients, and this correlated with increased inflammatory markers, as well as accumulation of regulatory T and B cells (110). Cocultures with M-MDSC had high levels of Arg1, the suppressive effect of M-MDSC on T cell proliferation was reduced by the addition of L-Arginine, and plasma levels of Arg-1 and IL-6 were elevated in COVID-19 patients, which increased with increasing severity of disease (91). Several other papers have also reported elevated levels of Arg1, with low plasma levels of L-Arginine in association with MDSC in COVID-19, which may not only have implications for immune function but also for increased platelet aggregation (97, 111–113).

Another consideration is the influence of MDSC on the genesis of lung fibrosis in COVID-19 patients. MDSC can transdifferentiate into extracellular matrix (collagen type I)-producing fibrocytes, which interact with activated T-cells, resulting in the production of IDO and leading to Treg expansion (114). Murine models have suggested that MDSC promote lung fibrogenesis by inhibiting collagen degradation through TGF-β production (115). Elevated serum levels of TGF-β correlated with lung fibrosis in a cohort of severe COVID-19 patients (108).This study also reported increased serum levels of TGF-β and of MDSC in COVID-19 patients, as well as a significant correlation between COVID-19 severity and serum TGF-β levels; and showed that isolated M-MDSC from these patients produced higher levels of intracellular TGF-β than non-M-MDSC (108).

These effects do not appear to be short-lived. Elevations in PMN-MDSC persist from hospital admission to convalescence, and longer than three months after acute COVID-19 – across all severities, but at higher levels in those with severe compared to mild disease (94, 116, 117). Elevated levels of circulating M-MDSC were found up to seven months after moderate to severe COVID-19, along with elevated levels of the immune checkpoint marker CD86 (characteristic of ongoing immune activation and chronic inflammation) (118). In another report, MDSC numbers had normalized by three months after acute COVID-19, but the immune dysfunction persisted (119). When monocytes from COVID-19 patients at three months after hospital discharge were stimulated with LPS and R848, both TNF and IL-6 production was impaired, and levels of other cytokines in plasma were also lower, in both moderate and severe COVID-19 (119). At five months after SARS-CoV-2 infection, levels of M-MDSC remained elevated compared to healthy controls, and continued to suppress SARS-CoV-2-specific T cell cytokine production through arginase, ROS

and TGF-β dependent pathways (120). These data suggest a long-lasting impairment in the immune response after COVID-19, attributable to the suppressive effects of MDSC.

The evidence for MDSC involvement in COVID-19 and TB are summarized in Table 1.

5 MDSC in SARS-CoV-2 and Mtb coinfection

It is thought that up to a quarter of the world's population have been latently infected with Mtb, with at most 15% progressing to active disease in their lifetime, often with a long latency period between initial infection and active TB disease (121). This means that a coinfection sequence of pre-existing LTBI followed by SARS-CoV-2 infection is a highly likely scenario. However, the fact that many patients present with a long history of symptoms and cavitary lung lesions at TB diagnosis, implying chronicity of disease, suggests that a scenario of active TB disease followed by SARS-CoV-2 infection is also likely to be a common occurrence (Figure 2).

The number of Mtb-specific CD4 T cells is lower in coinfected patients who have active TB disease/SARS-CoV-2, than in those with TB alone (12). Also, COVID-19 itself is characterized by reduced T cell numbers and T cell exhaustion (12, 122). Therefore, SARS-CoV-2 infection could hypothetically trigger progression from LTBI to active TB disease. As we have shown in the sections above, MDSC may be responsible for the lower T cell

TABLE 1 Evidence for the role of myeloid derived suppressor cells in the pathogenesis of Tuberculosis compared to COVID-19.

MDSC in Mtb infection and disease	MDSC in SARS-CoV-2 infection and disease
Peripheral blood *In mice* • Raised levels of MDSC in blood of BCG vaccinated mice, and Mtb susceptible mice with advanced TB disease *In humans* • Raised levels of MDSC in blood of humans with active TB disease • High frequencies of M-MDSC correlate with more severe clinical and radiological active TB disease • High frequencies of PMN-MDSC associated with lower radiological severity • Levels decline after successful TB treatment, cells express more maturation markers • Levels in LTBI comparable to healthy donors	**Peripheral blood** • High frequencies of both M-MDSC and PMN-MDSC, highest in severe and fatal COVID-19 • Early M-MDSC levels predict COVID-19 severity and mortality • Transient early expansion of PMN-MDSC associated with surviving severe COVID-19 • High frequencies persist up to 7 months after acute COVID-19 • Convalescent MDSC levels are higher in those recovering from severe COVID-19 than mild COVID-19
Lungs *In mice* • Accumulate in lungs (and bone marrow, spleen) in Mtb susceptible mice with active TB disease *In humans* • Accumulate in BALF and pleural fluid of humans with active TB disease • PMN-MDSC dominant in BALF of humans with pulmonary TB • M-MDSC dominant in the pleural fluid of humans with pleural TB disease **Granulomas** *In mice* • Accumulate at the edge of necrotic granulomas • Associated with TB progression and uncontrolled Mtb replication *In humans* • Found in the myeloid core • Promote mycobacterial replication • Impair Mtb containment	**Lungs** • Not increased in nasopharyngeal and endotracheal aspirates • Found in BALF of patients with COVID-19, higher in severe disease • Likely PMN-MDSC found in lung tissue from deceased COVID-19 patients • TGF-β levels correlates with lung fibrosis and COVID-19 severity
Immunosuppressive mechanisms *In mice* • Reduce T cell priming through an IL-1R-dependent pathway • Inhibit T cell proliferation and IFN-γ production *in vitro* through NO-dependent mechanisms *In humans* • Upregulated ARG1 and PD-L1 expression • Inhibit T cell proliferation (possibly through a NO-dependent mechanism) • Suppress $CD4^+$ T-cell > $CD8^+$ T cell production of IL-2, TNF-α, IFN-γ • Suppress $CD4^+$ T cell > $CD8^+$ T cell production of IL-10 • Subvert effector T-cell-mediated containment of Mtb in monocyte-derived macrophages	**Immunosuppressive mechanisms** • M-MDSC and PMN-MDSC inhibit T cell proliferation and IFN-γ production • Associated with increased expression of ARG1 and iNOS mRNA • Levels of PMN-MDSC correlate with plasma levels of TGF-β • M-MDSC are dominant producers of IL-10 and IL-6 in severe COVID-19 • M-MDSC produce higher levels of TGF-β than other MDSC • M-MDSC correlated with increased inflammatory markers, B cell and Treg accumulation • Associated with high levels of ARG1, low plasma L-Arginine • Addition of L-Arginine partially inhibits suppressive effect of M-MDSC on T cell proliferation • Suppress T cell cytokine production at 5 months after recovery from COVID-19 through ARG1, ROS, and TGF-β dependent pathways

ARG1, arginase-1; BALF, bronchoalveolar lavage fluid; BCG, Bacille Calmette Guerin intradermal vaccine; IFN, interferon; IL-1R, interleukin 1 receptor; iNOS, inducible nitric oxide synthase; LTBI, latent tuberculosis infection; mRNA, messenger ribonucleic acid; Mtb, Mycobacterium tuberculosis; MDSC, myeloid-derived suppressor cells; M-MDSC, monocytic MDSC; NO, nitric oxide; PMN-MDSC, polymorphonuclear MDSC; ROS, reactive oxygen species; TB, tuberculosis; TGF, transforming growth factor; TNF, tumor necrosis factor; Treg, regulatory T cell.

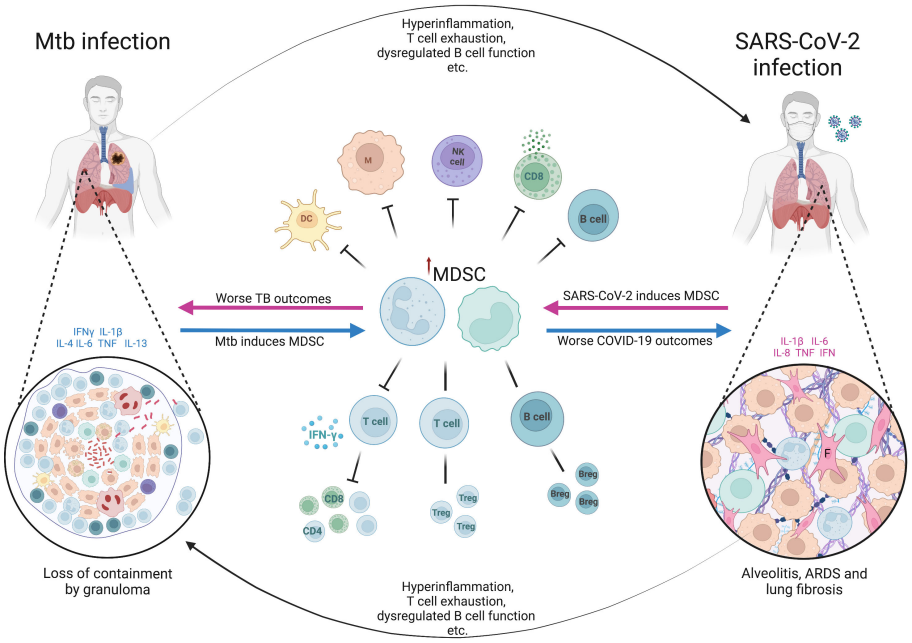

FIGURE 2
The hypothetical role of myeloid derived suppressor cells in Mtb/SARS-CoV-2 coinfection. The figure shows the bidirectional effects of myeloid derived suppressor cells (MDSC) on the clinical outcomes in patients coinfected with *Mycobacterium tuberculosis* (Mtb) and SARS-CoV-2. In latent infection with Mtb or active TB disease, the presence of cytokines such as IFN-γ, IL-1β, IL-4, IL-6, TNF and IL-13, triggers the pathological activation of MDSC (blue arrows), which become immune suppressive. The effects of MDSC, along with other effects of Mtb infection and disease (hyperinflammation, T cell exhaustion etc.), predispose to worse clinical outcomes from SARS-CoV-2 infection including acute respiratory distress syndrome (ARDS) and lung fibrosis. MDSC accumulate in the lungs of severe SARS-CoV-2 cases (shown in the cut-out on the right), further exacerbating the immune suppression and profibrotic effects. Similarly, in current or recent SARS-CoV-2 infection, there is induction of proinflammatory cytokines, often pathologically high (termed hyperinflammation), along with other aspects of SARS-CoV-2-related immune dysfunction and dysregulation, and pathological activation of MDSC (pink arrows). These factors combined result in worse clinical outcomes from active tuberculosis disease, as well as the potential loss of containment of Mtb within granulomas in latent infection. Suppressive neutrophils and macrophages within the myeloid core of the granuloma (shown in the cut-out on the left) further contribute to the loss of Mtb control. Breg, regulatory B cell; COVID-19, coronavirus disease-2019; DC, dendritic cell; F, fibroblast; IFN, interferon; IL, interleukin; M, macrophage; NK, natural killer cell; TB, tuberculosis; Treg, regulatory T cell. Created with BioRender.com

numbers in COVID-19, and for reduced expression of key cytokines required to maintain the immune response to Mtb.

The levels of IFN-γ produced by whole blood on stimulation by the SARS-CoV-2 spike protein-derived peptide CD4S were lower in patients with active TB disease/COVID-19 coinfection than in those with LTBI/COVID-19 and COVID-19 alone (13). In a more comprehensive analysis by the same group, an immune signature consisting of TNF-α, macrophage inflammatory protein-1β and IL-9 associated with active TB disease/COVID-19 compared to COVID-19 alone, and another signature including TNF-α, IL-1β, IL-17A, IL-5, fibroblast growth factor-basic, and GM-CSF, associated with active TB disease/COVID-19 compared to TB alone (14). In addition to this they confirmed the reduced SARS-CoV-2 specific response in coinfected patients for IFN-γ, as well as IL-10, IP-10, and other key cytokines (14). This suggests the possibility that the pre-existing immune milieu of active TB disease impaired the critical T cell response to SARS-CoV-2 infection. Hypothetically, MDSC induced by Mtb infection may be responsible for this, through the expression of PD-L1 which directly inhibits further T cell proliferation and induces both dysregulation and an exhausted immune profile in T cells. If this is the case, then the clinical implication may be a higher viral load, more severe virus-related lung damage, and an increased immune dysregulation which results in more severe clinical manifestations.

The impact of MDSC on initial or recent SARS-CoV-2 infection with secondary Mtb infection must also be considered. As described in detail above, on infection with SARS-CoV-2, emergency myelopoiesis is stimulated and MDSC released into the peripheral blood, accumulating in the lungs in severe COVID-19. Those who die from COVID-19 will most likely have early and persistently elevated M-MDSC, whereas survivors are more likely to have an early peak of PMN-MDSC which reduces as they improve. Nonetheless, the immune suppression mediated by these cells persists for many months irrespective of severity. In theory, Mtb infection during the acute phase of SARS-CoV-2 illness or in the months thereafter, may therefore have a higher likelihood of a poor outcome, as the key T cell responses to Mtb are impaired. This is supported by reports of deficient IFN-γ release assay (IGRA) responses to Mtb antigens (and mitogen) in patients with severe COVID-19, which suggest a generalized unresponsiveness of T cells to all antigens, and specifically to Mtb (123, 124). Production of IFN-γ, IP-10, and IL-1β, amongst others, in response to Mtb-antigen stimulation was impaired in the whole blood from coinfected active TB disease/COVID-19 patients (14). On the

other hand, some data implies that the Mtb-antigen cytokine responses are augmented by recent SARS-CoV-2 infection, and immunosuppressive responses are reduced (125). The latter study examined the baseline, Mtb antigen-, and mitogen-stimulated levels of key cytokines and chemokines in elderly patients with positive and negative SARS-CoV-2 serology, both with and without LTBI (based on IGRA). They found higher baseline/unstimulated and Mtb antigen-stimulated levels of IFN-γ, IL-2, TNF-α, and others, in patients with LTBI who were seropositive for SARS-CoV-2 compared to those with LTBI who were seronegative for SARS-CoV-2. Moreover, the levels of immunosuppressive IL-10 were lower in LTBI/seropositive SARS-CoV-2 individuals. There were no differences in response to mitogen between groups in this study, and the LTBI negative control group did not show any enhanced cytokine response to Mtb antigen (125). These data show that if SARS-CoV-2-induced MDSC are the mechanism underlying the poor response of T cells to Mtb antigen, then their effects are likely Mtb antigen-specific, rather than part of a non-antigen-specific response. These observations also suggest that SARS-CoV-2-induced MDSC may have a different effect on the outcome of Mtb infection, in different COVID-19 severities.

Whilst it is not direct evidence for the effect of MDSC, the fact that Mtb-specific T cell numbers are reduced in SARS-CoV-2 coinfection suggests that if MDSC are involved, then it is in an antigen-specific manner (12). In other words, MDSC induced by SARS-CoV-2 may well suppress Mtb-specific T cell responses, as well as suppressing global T cell proliferation and cytokine production in an antigen non-specific way. Similarly, evidence that there is a reduced T cell response to stimulation with SARS-CoV-2 antigen in patients with preexisting Mtb infection might imply that Mtb-induced MDSC also suppress SARS-CoV-2 T cells in both an antigen specific and non-specific way (14). This is not unknown, as MDSC induced by HIV infection also suppress T cell function *in vitro* by both antigen-specific and non-specific mechanisms (126). However, this antigen-specific effect may be limited to $CD8^+$ T cells because of the low expression of MHC Class II by MDSC – a theory supported by the inhibitory effects of tumor-associated MDSC on $CD8^+$ T cells demonstrated in human cells *in vitro* and mouse models, which can be reversed by anti-MDSC host directed therapies (127, 128).

Whether any of the MDSC-targeting host directed therapies with efficacy in TB will also prove effective in Mtb/SARS-CoV-2 co-infection remains to be seen. Because of the high prevalence of both infections, it is likely that by default coinfected participants will be included in any human trials in the future. An agent such as Imatinib (a tyrosine kinase inhibitor which targets the ABL kinase domain) which has experimental evidence in both diseases – despite disappointing clinical disease outcomes in COVID-19 – is an attractive option for future investigation in coinfection (129–132).

Lastly, we must consider if preexisting Mtb infection (LTBI or active TB disease) with MDSC induction might affect the efficacy of a subsequent SARS-CoV-2 vaccination, and if recent COVID-19 might adversely affect a subsequent Mtb vaccine response. MDSC impair both T and B cell responses, even inducing regulatory and suppressive B cells in the tumor microenvironment (133). Theoretically, the presence of MDSC from Mtb or SARS-CoV-2 infection might impair adaptive and cell-mediated responses to a vaccine administered in their presence, resulting in reduced immune memory to the vaccine (133). Adults with TB have lower levels of anti-SARS-CoV-2 antibodies after three doses of inactivated vaccine (134). Assuming antibody levels are a correlate of protection, this would mean that a SARS-CoV-2 vaccinated person with preexisting Mtb infection would lose the vaccine-mediated protection for severe COVID-19, and lead to worse clinical outcomes. MDSC are known to be part of the response to Bacillus Calmette-Guérin vaccination (BCG), possibly contributing to incomplete protection against Mtb by restraining T cell priming (70). BCG has immunomodulatory effects on myeloid cells, including epigenetic reprogramming, which affect these cells' ability to respond to pathogens (including Mtb) (135, 136). A person receiving BCG or another Mtb vaccine candidate in a clinical trial may not develop the desired immune response if they have had recent COVID-19, and importantly, the SARS-CoV-2-induced MDSCs continue to exert their effects months after the acute infection. This would need to be adjusted for in the analysis.

Overall, there is evidence supporting a potential role for MDSC in determining the outcomes of Mtb/SARS-CoV-2 coinfection, in all disease severities and iterations of the coinfection sequence. More evidence is needed to find out if MDSC impact the outcome of SARS-CoV-2 infection in early TB or LTBI; if there are any vaccine-relevant interactions; and if a host directed therapy aimed at modulating the effect of MDSC might improve the outcome of active TB disease/COVID-19 coinfection.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

NdP, GW, SM and JS conceived of the article, JS wrote the first draft of the manuscript and all authors edited and approved the final version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Nasimiyu C, Ngere I, Dawa J, Amoth P, Oluga O, Ngunu C, et al. Near-Complete SARS-CoV-2 Seroprevalence among Rural and Urban Kenyans despite Significant Vaccine Hesitancy and Refusal. *Vaccines (Basel).* (2022) 11(1):68. doi: 10.3390/vaccines11010068

2. Madhi SA, Kwatra G, Myers JE, Jassat W, Dhar N, Mukendi CK, et al. Population immunity and covid-19 severity with omicron variant in South Africa. *N Engl J Med* (2022) 386(14):1314–26. doi: 10.1056/NEJMoa2119658

3. Jahan N, Brahma A, Kumar MS, Bagepally BS, Ponnaiah M, Bhatnagar T, et al. Seroprevalence of IgG antibodies against SARS-CoV-2 in India, March 2020 to August 2021: a systematic review and meta-analysis. *Int J Infect Dis* (2022) 116:59–67. doi: 10.1016/j.ijid.2021.12.353

4. World Health Organization. *Global Tuberculosis Report 2022* (2022). Geneva. Available at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022 (Accessed 20 Jan 2023).

5. Jassat W, Cohen C, Tempia S, Masha M, Goldstein S, Kufa T, et al. Risk factors for COVID-19-related in-hospital mortality in a high HIV and tuberculosis prevalence setting in South Africa: a cohort study. *Lancet HIV.* (2021) 8(9):e554–67. doi: 10.1016/S2352-3018(21)00151-X

6. Sy KTL, Haw NJL, Uy J. Previous and active tuberculosis increases risk of death and prolongs recovery in patients with COVID-19. *Infect Dis (London England)* (2020) 52(12):902–7. doi: 10.1080/23744235.2020.1806353

7. Wang Y, Chen Y, Gu L, Lou L, Zhang J, Zhang K. The clinical characteristics and risk factors for severe COVID-19 in patients with COVID-19 and tuberculosis coinfection. *Front Microbiol* (2022) 13:1061879. doi: 10.3389/fmicb.2022.1061879

8. du Bruyn E, Stek C, Daroowala R, Said-Hartley Q, Hsiao M, Schafer G, et al. Effects of tuberculosis and/or HIV-1 infection on COVID-19 presentation and immune response in Africa. *Nat Commun* (2023) 14(1):188. doi: 10.1038/s41467-022-35689-1

9. Sheerin D, Abhimanyu, Peton N, Vo W, Allison CC, Wang X, et al. Immunopathogenic overlap between COVID-19 and tuberculosis identified from transcriptomic meta-analysis and human macrophage infection. *iScience* (2022) 25(6):104464. doi: 10.1016/j.isci.2022.104464

10. Hildebrand RE, Chandrasekar SS, Riel M, Touray BJB, Aschenbroich SA, Talaat AM. Superinfection with SARS-CoV-2 Has Deleterious Effects on Mycobacterium bovis BCG Immunity and Promotes Dissemination of Mycobacterium tuberculosis. *Microbiol Spectr.* (2022) 10(5):e0307522. doi: 10.1128/spectrum.03075-22

11. Rosas Mejia O, Gloag ES, Li J, Ruane-Foster M, Claeys TA, Farkas D, et al. Mice infected with Mycobacterium tuberculosis are resistant to acute disease caused by secondary infection with SARS-CoV-2. *PloS Pathog* (2022) 18(3):e1010093. doi: 10.1371/journal.ppat.1010093

12. Riou C, du Bruyn E, Stek C, Daroowala R, Goliath RT, Abrahams F, et al. Relationship of SARS-CoV-2–specific CD4 response to COVID-19 severity and impact of HIV-1 and tuberculosis coinfection. *J Clin Invest.* (2021) 131(12):e149125. doi: 10.1172/JCI149125

13. Petrone L, Petruccioli E, Vanini V, Cuzzi G, Gualano G, Vittozzi P, et al. Coinfection of tuberculosis and COVID-19 limits the ability to in *vitro* respond to SARS-CoV-2. *Int J Infect Dis* (2021) 113 Suppl 1:S82–7. doi: 10.1016/j.ijid.2021.02.090

14. Najafi-Fard S, Aiello A, Navarra A, Cuzzi G, Vanini V, Migliori GB, et al. Characterization of the immune impairment of tuberculosis and COVID-19 coinfected patients. *Int J Infect Dis* (2023) 130 Suppl 1:S34–42. doi: 10.1016/j.ijid.2023.03.021

15. Silvin A, Chapuis N, Dunsmore G, Goubet A-G, Dubuisson A, Derosa L, et al. Elevated calprotectin and abnormal myeloid cell subsets discriminate severe from mild COVID-19. *Cell* (2020) 182(6):1401–1418.e18. doi: 10.1016/j.cell.2020.08.002

16. Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* (2020) 584(7821):463–9. doi: 10.1038/s41586-020-2588-y

17. du Plessis N, Loebenberg L, Kriel M, von Groote-Bidlingmaier F, Ribechini E, Loxton AG, et al. Increased frequency of myeloid-derived suppressor cells during active tuberculosis and after recent mycobacterium tuberculosis infection suppresses T-cell function. *Am J Respir Crit Care Med* (2013) 188(6):724–32. doi: 10.1164/rccm.201302-0249OC

18. Ou L, Shi Y, Dong W, Liu C, Schmidt TJ, Nagarkatti P, et al. Kruppel-like factor KLF4 facilitates cutaneous wound healing by promoting fibrocyte generation from myeloid-derived suppressor cells. *J Invest Dermatol* (2015) 135(5):1425–34. doi: 10.1038/jid.2015.3

19. Mahdipour E, Charnock JC, Mace KA. Hoxa3 promotes the differentiation of hematopoietic progenitor cells into proangiogenic Gr-1+CD11b+ myeloid cells. *Blood* (2011) 117(3):815–26. doi: 10.1182/blood-2009-12-259549

20. Köstlin N, Kugel H, Spring B, Leiber A, Marmé A, Henes M, et al. Granulocytic myeloid derived suppressor cells expand in human pregnancy and modulate T-cell responses. *Eur J Immunol* (2014) 44(9):2582–91. doi: 10.1002/eji.201344200

21. Kang X, Zhang X, Liu Z, Xu H, Wang T, He L, et al. Granulocytic myeloid-derived suppressor cells maintain feto-maternal tolerance by inducing Foxp3 expression in CD4+CD25–T cells by activation of the TGF-β/β-catenin pathway. *Mol Hum Reprod* (2016) 22(7):499–511. doi: 10.1093/molehr/gaw026

22. Poe SL, Arora M, Oriss TB, Yarlagadda M, Isse K, Khare A, et al. STAT1-regulated lung MDSC-like cells produce IL-10 and efferocytose apoptotic neutrophils with relevance in resolution of bacterial pneumonia. *Mucosal Immunol* (2013) 6(1):189–99. doi: 10.1038/mi.2012.62

23. Zhang S, Ma X, Zhu C, Liu L, Wang G, Yuan X. The role of myeloid-derived suppressor cells in patients with solid tumors: A meta-analysis. *PloS One* (2016) 11(10):e0164514. doi: 10.1371/journal.pone.0164514

24. Highfill SL, Cui Y, Giles AJ, Smith JP, Zhang H, Morse E, et al. Disruption of CXCR2-mediated MDSC tumor trafficking enhances anti-PD1 efficacy. *Sci Transl Med* (2014) 6(237):237ra67. doi: 10.1126/scitranslmed.3007974

25. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol* (2018) 19(2):108–19. doi: 10.1038/s41590-017-0022-x

26. Landskron G, de la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic inflammation and cytokines in the tumor microenvironment. *J Immunol Res* (2014) 2014:149185. doi: 10.1155/2014/149185

27. Youn J-I, Collazo M, Shalova IN, Biswas SK, Gabrilovich DI. Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *J Leukoc Biol* (2012) 91(1):167–81. doi: 10.1189/jlb.0311177

28. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun* (2016) 7:12150. doi: 10.1038/ncomms12150

29. Ostrand-Rosenberg S, Fenselau C. Myeloid-derived suppressor cells: immune-suppressive cells that impair antitumor immunity and are sculpted by their environment. *J Immunol* (2018) 200(2):422–31. doi: 10.4049/jimmunol.1701019

30. Bunt SK, Yang L, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. *Cancer Res* (2007) 67(20):10019–26. doi: 10.1158/0008-5472.CAN-07-2354

31. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. *Nat Rev Immunol* (2021) 21(8):485–98. doi: 10.1038/s41577-020-00490-y

32. Qian B-Z, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell* (2010) 141(1):39–51. doi: 10.1016/j.cell.2010.03.014

33. Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, Liu K, et al. The cellular and molecular origin of tumor-associated macrophages. *Science* (2014) 344(6186):921–5. doi: 10.1126/science.1252510

34. Shen M, Hu P, Donskov F, Wang G, Liu Q, Du J. Tumor-associated neutrophils as a new prognostic factor in cancer: A systematic review and meta-analysis. *PloS One* (2014) 9(6):e98259. doi: 10.1371/journal.pone.0098259

35. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* (2009) 16(3):183–94. doi: 10.1016/j.ccr.2009.06.017

36. Condamine T, Dominguez GA, Youn J-I, Kossenkov AV, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol* (2016) 1(2):aaf8943. doi: 10.1126/sciimmunol.aaf8943

37. Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in *vivo*. *Blood* (1998) 92 (11):4150–66. doi: 10.1182/blood.V92.11.4150

38. Serafini P, Carbley R, Noonan KA, Tan G, Bronte V, Borrello I. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Res* (2004) 64(17):6337–43. doi: 10.1158/0008-5472.CAN-04-0757

39. Wu C-T, Hsieh C-C, Lin C-C, Chen W-C, Hong J-H, Chen M-F. Significance of IL-6 in the transition of hormone-resistant prostate cancer and the induction of myeloid-derived suppressor cells. *J Mol Med (Berl).* (2012) 90(11):1343–55. doi: 10.1007/s00109-012-0916-x

40. Gallina G, Dolcetti L, Serafini P, De Santo C, Marigo I, Colombo MP, et al. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T cells. *J Clin Invest.* (2006) 116(10):2777–90. doi: 10.1172/JCI28828

41. Sade-Feldman M, Kanterman J, Ish-Shalom E, Elnekave M, Horwitz E, Baniyash M. Tumor necrosis factor-α blocks differentiation and enhances suppressive activity of immature myeloid cells during chronic inflammation. *Immunity* (2013) 38(3):541–54. doi: 10.1016/j.immuni.2013.02.007

42. Nefedova Y, Nagaraj S, Rosenbauer A, Muro-Cacho C, Sebti SM, Gabrilovich DI. Regulation of dendritic cell differentiation and antitumor immune response in cancer by pharmacologic-selective inhibition of the janus-activated kinase 2/signal transducers and activators of transcription 3 pathway. *Cancer Res* (2005) 65(20):9525–35. doi: 10.1158/0008-5472.CAN-05-0529

43. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res* (2004) 64 (16):5839–49. doi: 10.1158/0008-5472.CAN-04-0465

44. Rodriguez PC, Zea AH, DeSalvo J, Culotta KS, Zabaleta J, Quiceno DG, et al. L-arginine consumption by macrophages modulates the expression of CD3 zeta chain in T lymphocytes. *J Immunol* (2003) 171(3):1232–9. doi: 10.4049/jimmunol.171.3.1232

45. Bingisser RM, Tilbrook PA, Holt PG, Kees UR. Macrophage-derived nitric oxide regulates T cell activation *via* reversible disruption of the Jak3/STAT5 signaling pathway. *J Immunol* (1998) 160(12):5729–34. doi: 10.4049/jimmunol.160.12.5729

46. Rodriguez PC, Quiceno DG, Ochoa AC. L-arginine availability regulates T-lymphocyte cell-cycle progression. *Blood* (2007) 109(4):1568–73. doi: 10.1182/blood-2006-06-031856

47. Kusmartsev S, Nefedova Y, Yoder D, Gabrilovich DI. Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. *J Immunol* (2004) 172(2):989–99. doi: 10.4049/jimmunol.172.2.989

48. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, et al. Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nat Med* (2007) 13(7):828–35. doi: 10.1038/nm1609

49. Schmielau J, Finn OJ. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Res* (2001) 61(12):4756–60.

50. Sun Z, Fourcade J, Pagliano O, Chauvin J-M, Sander C, Kirkwood JM, et al. IL10 and PD-1 cooperate to limit the activity of tumor-specific CD8+ T cells. *Cancer Res* (2015) 75(8):1635–44. doi: 10.1158/0008-5472.CAN-14-3016

51. Lu C, Redd PS, Lee JR, Savage N, Liu K. The expression profiles and regulation of PD-L1 in tumor-induced myeloid-derived suppressor cells. *Oncoimmunology* (2016) 5 (12):e1247135. doi: 10.1080/2162402X.2016.1247135

52. Yamauchi Y, Safi S, Blattner C, Rathinasamy A, Umansky L, Juenger S, et al. Circulating and tumor myeloid-derived suppressor cells in resectable non-small cell lung cancer. *Am J Respir Crit Care Med* (2018) 198(6):777–87. doi: 10.1164/rccm.201708-1707OC

53. Huang B, Pan P-Y, Li Q, Sato AI, Levy DE, Bromberg J, et al. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Cancer Res* (2006) 66 (2):1123–31. doi: 10.1158/0008-5472.CAN-05-1299

54. Li H, Han Y, Guo Q, Zhang M, Cao X. Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1. *J Immunol* (2009) 182(1):240–9. doi: 10.4049/jimmunol.182.1.240

55. Sinha P, Clements VK, Bunt SK, Albelda SM, Ostrand-Rosenberg S. Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. *J Immunol* (2007) 179(2):977–83. doi: 10.4049/jimmunol.179.2.977

56. Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res* (2010) 70 (8):3052–61. doi: 10.1158/0008-5472.CAN-09-3690

57. du Plessis N, Kotze LA, Leukes V, Walzl G. Translational potential of therapeutics targeting regulatory myeloid cells in tuberculosis. *Front Cell Infect Microbiol* (2018) 8:332. doi: 10.3389/fcimb.2018.00332

58. Serafini P, Meckel K, Kelso M, Noonan K, Califano J, Koch W, et al. Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. *J Exp Med* (2006) 203(12):2691–702. doi: 10.1084/jem.20061104

59. Kleinovink JW, Marijt KA, Schoonderwoerd MJA, van Hall T, Ossendorp F, Fransen MF. PD-L1 expression on malignant cells is no prerequisite for checkpoint therapy. *Oncoimmunology* (2017) 6(4):e1294299. doi: 10.1080/2162402X.2017.1294299

60. Nefedova Y, Fishman M, Sherman S, Wang X, Beg AA, Gabrilovich DI. Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells. *Cancer Res* (2007) 67(22):11021–8. doi: 10.1158/0008-5472.CAN-07-2593

61. Singh V, Brecik M, Mukherjee R, Evans JC, Svetlíková Z, Blaško J, et al. The complex mechanism of antimycobacterial action of 5-fluorouracil. *Chem Biol* (2015) 22 (1):63–75. doi: 10.1016/j.chembiol.2014.11.006

62. Knaul JK, Jörg S, Oberbeck-Mueller D, Heinemann E, Scheuermann L, Brinkmann V, et al. Lung-residing myeloid-derived suppressors display dual functionality in murine pulmonary tuberculosis. *Am J Respir Crit Care Med* (2014) 190(9):1053–66. doi: 10.1164/rccm.201405-0828OC

63. Gupta S, Krug S, Pokkali S, Leanderson T, Isaacs JT, Srikrishna G, et al. Pharmacologic exhaustion of suppressor cells with tasquinimod enhances bacterial clearance during tuberculosis. *Am J Respir Crit Care Med* (2019) 199(3):386–9. doi: 10.1164/rccm.201805-0820LE

64. Leukes VN, Dorhoi A, Malherbe ST, Maasdorp E, Khoury J, McAnda S, et al. Targeting of myeloid-derived suppressor cells by all-trans retinoic acid as host-directed therapy for human tuberculosis. *Cell Immunol* (2021) 364:104359. doi: 10.1016/j.cellimm.2021.104359

65. Leukes VN, Malherbe ST, Hiemstra A, Kotze LA, Roos K, Keyser A, et al. Sildenafil, a type-5 phosphodiesterase inhibitor, fails to reverse myeloid-derived suppressor cell-mediated T cell suppression in cells isolated from tuberculosis patients. *Front Immunol* (2022) 13:883886. doi: 10.3389/fimmu.2022.883886

66. Park S-J, Nam D-E, Seong HC, Hahn YS. New discovery of myeloid-derived suppressor cell's tale on viral infection and COVID-19. *Front Immunol* (2022) 13:842535. doi: 10.3389/fimmu.2022.842535

67. Dorhoi A, Kaufmann SHE. Versatile myeloid cell subsets contribute to tuberculosis-associated inflammation. *Eur J Immunol* (2015) 45(8):2191–202. doi: 10.1002/eji.201545493

68. Obregón-Henao A, Henao-Tamayo M, Orme IM, Ordway DJ. Gr1intCD11b+ Myeloid-derived suppressor cells in mycobacterium tuberculosis infection. *PloS One* (2013) 8(11):e80669. doi: 10.1371/journal.pone.0080669

69. Wang Z, Jiang J, Li Z, Zhang J, Wang H, Qin Z. A myeloid cell population induced by Freund adjuvant suppresses T-cell-mediated antitumor immunity. *J Immunother.* (2010) 33(2):167–77. doi: 10.1097/CJI.0b013e3181bed2ba

70. Martino A, Badell E, Abadie V, Balloy V, Chignard M, Mistou M-Y, et al. Mycobacterium bovis bacillus Calmette-Guérin vaccination mobilizes innate myeloid-derived suppressor cells restraining in *vivo* T cell priming *via* IL-1R-dependent nitric oxide production. *J Immunol* (2010) 184(4):2038–47. doi: 10.4049/jimmunol.0903348

71. Tsiganov EN, Verbina EM, Radaeva TV, Sosunov VV, Kosmiadi GA, Nikitina IY, et al. Gr-1dimCD11b+ immature myeloid-derived suppressor cells but not neutrophils are markers of lethal tuberculosis infection in mice. *J Immunol* (2014) 192(10):4718–27. doi: 10.4049/jimmunol.1301365

72. El Daker S, Sacchi A, Tempestilli M, Carducci C, Goletti D, Vanini V, et al. Granulocytic myeloid derived suppressor cells expansion during active pulmonary tuberculosis is associated with high nitric oxide plasma level. *PloS One* (2015) 10(4): e0123772. doi: 10.1371/journal.pone.0123772

73. Davids M, Pooran A, Smith L, Tomasicchio M, Dheda K. The frequency and effect of granulocytic myeloid-derived suppressor cells on mycobacterial survival in patients with tuberculosis: A preliminary report. *Front Immunol* (2021) 12:676679. doi: 10.3389/fimmu.2021.676679

74. Ashenafi S, Brighenti S. Reinventing the human tuberculosis (TB) granuloma: Learning from the cancer field. *Front Immunol* (2022) 13:1059725. doi: 10.3389/fimmu.2022.1059725

75. Jiang H, Gong H, Zhang Q, Gu J, Liang L, Zhang J. Decreased expression of perforin in CD8(+) T lymphocytes in patients with Mycobacterium tuberculosis infection and its potential value as a marker for efficacy of treatment. *J Thorac Dis* (2017) 9(5):1353–60. doi: 10.21037/jtd.2017.05.74

76. Schmidt A, Oberle N, Krammer PH. Molecular mechanisms of treg-mediated T cell suppression. *Front Immunol* (2012) 3:51. doi: 10.3389/fimmu.2012.00051

77. Gopal R, Monin L, Torres D, Slight S, Mehra S, McKenna KC, et al. S100A8/A9 proteins mediate neutrophilic inflammation and lung pathology during tuberculosis. *Am J Respir Crit Care Med* (2013) 188(9):1137–46. doi: 10.1164/rccm.201304-0803OC

78. Gideon HP, Phuah J, Junecko BA, Mattila JT. Neutrophils express pro- and anti-inflammatory cytokines in granulomas from Mycobacterium tuberculosis-infected cynomolgus macaques. *Mucosal Immunol* (2019) 12(6):1370–81. doi: 10.1038/s41385-019-0195-8

79. Singh B, Singh DK, Ganatra SR, Escobedo RA, Khader S, Schlesinger LS, et al. Myeloid-derived suppressor cells mediate T cell dysfunction in nonhuman primate TB granulomas. *MBio* (2021) 12(6):e0318921. doi: 10.1128/mbio.03189-21

80. Agrawal N, Streata I, Pei G, Weiner J, Kotze L, Bandermann S, et al. Human Monocytic Suppressive Cells Promote Replication of Mycobacterium tuberculosis and Alter Stability of in *vitro* Generated Granulomas. *Front Immunol* (2018) 9:2417. doi: 10.3389/fimmu.2018.02417

81. Ashenafi S, Muvva JR, Mily A, Snäll J, Zewdie M, Chanyalew M, et al. Immunosuppressive features of the microenvironment in lymph nodes granulomas from tuberculosis and HIV-co-infected patients. *Am J Pathol* (2022) 192(4):653–70. doi: 10.1016/j.ajpath.2021.12.013

82. McCaffrey EF, Donato M, Keren L, Chen Z, Delmastro A, Fitzpatrick MB, et al. The immunoregulatory landscape of human tuberculosis granulomas. *Nat Immunol* (2022) 23(2):318–29. doi: 10.1038/s41590-021-01121-x

83. Dorhoi A, Kotzé LA, Berzofsky JA, Sui Y, Gabrilovich DI, Garg A, et al. Therapies for tuberculosis and AIDS: myeloid-derived suppressor cells in focus. *J Clin Invest.* (2020) 130(6):2789–99. doi: 10.1172/JCI136288

84. Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, et al. Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. *Clin Microbiol Rev* (2018) 31(4):e00021–18. doi: 10.1128/CMR.00021-18

85. Grassi G, Vanini V, De Santis F, Romagnoli A, Aiello A, Casetti R, et al. PMN-MDSC frequency discriminates active versus latent tuberculosis and could play a role in counteracting the immune-mediated lung damage in active disease. *Front Immunol* (2021) 12:594376. doi: 10.3389/fimmu.2021.594376

86. Jøntvedt Jørgensen M, Jenum S, Tonby K, Mortensen R, Walzl G, Du Plessis N, et al. Monocytic myeloid-derived suppressor cells reflect tuberculosis severity and are influenced by cyclooxygenase-2 inhibitors. *J Leukoc Biol* (2021) 110(1):177–86. doi: 10.1002/JLB.4A0720-409RR

87. Hönzke K, Obermayer B, Mache C, Fathykova D, Kessler M, Dökel S, et al. Human lungs show limited permissiveness for SARS-CoV-2 due to scarce ACE2 levels but virus-induced expansion of inflammatory macrophages. *Eur Respir J* (2022) 60 (6):2102725. doi: 10.1183/13993003.02725-2021

88. Schulte-Schrepping J, Reusch N, Paclik D, Baßler K, Schlickeiser S, Zhang B, et al. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. *Cell* (2020) 182(6):1419–1440.e23. doi: 10.1016/j.cell.2020.08.001

89. Wilk AJ, Rustagi A, Zhao NQ, Roque J, Martínez-Colón GJ, McKechnie JL, et al. A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nat Med* (2020) 26(7):1070–6. doi: 10.1038/s41591-020-0944-y

90. Laing AG, Lorenc A, del Molino del Barrio I, Das A, Fish M, Monin L, et al. A dynamic COVID-19 immune signature includes associations with poor prognosis. *Nat Med* (2020) 26(10):1623–35. doi: 10.1038/s41591-020-1038-6

91. Falck-Jones S, Vangeti S, Yu M, Falck-Jones R, Cagigi A, Badolati I, et al. Functional monocytic myeloid-derived suppressor cells increase in blood but not airways and predict COVID-19 severity. *J Clin Invest.* (2021) 131(6):e144734. doi: 10.1172/JCI144734

92. Bordoni V, Sacchi A, Cimini E, Notari S, Grassi G, Tartaglia E, et al. An inflammatory profile correlates with decreased frequency of cytotoxic cells in coronavirus disease 2019. *Clin Infect Dis* (2020) 71(16):2272–5. doi: 10.1093/cid/ciaa577

93. Coudereau R, Waeckel L, Cour M, Rimmele T, Pescarmona R, Fabri A, et al. Emergence of immunosuppressive LOX-1+ PMN-MDSC in septic shock and severe COVID-19 patients with acute respiratory distress syndrome. *J Leukoc Biol* (2022) 111 (2):489–96. doi: 10.1002/JLB.4COVBCR0321-129R

94. Agrati C, Sacchi A, Bordoni V, Cimini E, Notari S, Grassi G, et al. Expansion of myeloid-derived suppressor cells in patients with severe coronavirus disease (COVID-19). *Cell Death Differ* (2020) 27(11):3196–207. doi: 10.1038/s41418-020-0572-6

95. Sacchi A, Grassi G, Bordoni V, Lorenzini P, Cimini E, Casetti R, et al. Early expansion of myeloid-derived suppressor cells inhibits SARS-CoV-2 specific T-cell response and may predict fatal COVID-19 outcome. *Cell Death Dis* (2020) 11(10):921. doi: 10.1038/s41419-020-03125-1

96. Cabrera LE, Pekkarinen PT, Alander M, Nowlan KHA, Nguyen NA, Jokiranta S, et al. Characterization of low-density granulocytes in COVID-19. *PloS Pathog* (2021) 17(7):e1009721. doi: 10.1371/journal.ppat.1009721

97. Dean MJ, Ochoa JB, Sanchez-Pino MD, Zabaleta J, Garai J, Del Valle L, et al. Severe COVID-19 is characterized by an impaired type I interferon response and elevated levels of arginase producing granulocytic myeloid derived suppressor cells. *Front Immunol* (2021) 12:695972. doi: 10.3389/fimmu.2021.695972

98. Jiménez-Cortegana C, Liró J, Palazón-Carrión N, Salamanca E, Sojo-Dorado J, de la Cruz-Merino L, et al. Increased blood monocytic myeloid derived suppressor cells but low regulatory T lymphocytes in patients with mild COVID-19. *Viral Immunol* (2021) 34(9):639–45. doi: 10.1089/vim.2021.0044

99. Thompson EA, Cascino K, Ordonez AA, Zhou W, Vaghasia A, Hamacher-Brady A, et al. Metabolic programs define dysfunctional immune responses in severe COVID-19 patients. *Cell Rep* (2021) 34(11):108863. doi: 10.1016/j.celrep.2021.108863

100. Rendeiro AF, Casano J, Vorkas CK, Singh H, Morales A, DeSimone RA, et al. Profiling of immune dysfunction in COVID-19 patients allows early prediction of disease progression. *Life Sci Alliance.* (2021) 4(2):e202000955. doi: 10.26508/lsa.202000955

101. Mortaz E, Movassaghi M, Bassir A, Dezfuli NK, Roofchayee ND, Jamaati H, et al. Evaluation of myeloid-derived suppressor cells in the blood of iranian COVID-19 patients. *Iran J Allergy Asthma Immunol* (2022) 21(4):467–77. doi: 10.18502/ijaai.v21i4.10294

102. Takano T, Matsumura T, Adachi Y, Terahara K, Moriyama S, Onodera T, et al. Myeloid cell dynamics correlating with clinical outcomes of severe COVID-19 in Japan. *Int Immunol* (2021) 33(4):241–7. doi: 10.1093/intimm/dxab005

103. Jiménez-Cortegana C, Sánchez-Jiménez F, Pérez-Pérez A, Álvarez N, Sousa A, Cantón-Bulnes L, et al. Low levels of granulocytic myeloid-derived suppressor cells may be a good marker of survival in the follow-up of patients with severe COVID-19. *Front Immunol* (2022) 12:801410. doi: 10.3389/fimmu.2021.801410

104. Marais C, Claude C, Semaan N, Charbel R, Barreault S, Travert B, et al. Myeloid phenotypes in severe COVID-19 predict secondary infection and mortality: a pilot study. *Ann Intensive Care* (2021) 11(1):111. doi: 10.1186/s13613-021-00896-4

105. Grassi G, Notari S, Gili S, Bordoni V, Casetti R, Cimini E, et al. Myeloid-derived suppressor cells in COVID-19: the paradox of good. *Front Immunol* (2022) 13:842949. doi: 10.3389/fimmu.2022.842949

106. Kvedaraite E, Hertwig L, Sinha I, Ponzetta A, Hed Myrberg I, Lourda M, et al. Major alterations in the mononuclear phagocyte landscape associated with COVID-19 severity. *Proc Natl Acad Sci* (2021) 118(6):e2018587118. doi: 10.1073/pnas.2018587118

107. Xu G, Qi F, Li H, Yang Q, Wang H, Wang X, et al. The differential immune responses to COVID-19 in peripheral and lung revealed by single-cell RNA sequencing. *Cell Discovery* (2020) 6(1):73. doi: 10.1038/s41421-020-00225-2

108. Kiaee F, Jamaati H, Shahi H, Roofchayee ND, Varahram M, Folkerts G, et al. Immunophenotype and function of circulating myeloid derived suppressor cells in COVID-19 patients. *Sci Rep* (2022) 12(1):22570. doi: 10.1038/s41598-022-26943-z

109. Loftus TJ, Ungaro R, Dirain M, Efron PA, Mazer MB, Remy KE, et al. Overlapping but disparate inflammatory and immunosuppressive responses to SARS-coV-2 and bacterial sepsis: an immunological time course analysis. *Front Immunol* (2021) 12:792448. doi: 10.3389/fimmu.2021.792448

110. Tomić S, Đokić J, Stevanović D, Ilić N, Gruden-Movsesijan A, Dinić M, et al. Reduced expression of autophagy markers and expansion of myeloid-derived suppressor cells correlate with poor T cell response in severe COVID-19 patients. *Front Immunol* (2021) 12:614599. doi: 10.3389/fimmu.2021.614599

111. Reizine F, Lesouhaitier M, Gregoire M, Pinceaux K, Gacouin A, Maamar A, et al. SARS-coV-2-induced ARDS associates with MDSC expansion, lymphocyte dysfunction, and arginine shortage. *J Clin Immunol* (2021) 41(3):515–25. doi: 10.1007/s10875-020-00920-5

112. Sacchi A, Grassi G, Notari S, Gili S, Bordoni V, Tartaglia E, et al. Expansion of myeloid derived suppressor cells contributes to platelet activation by L-arginine deprivation during SARS-coV-2 infection. *Cells* (2021) 10(8):2111. doi: 10.3390/cells10082111

113. Bost P, De Sanctis F, Canè S, Ugel S, Donadello K, Castellucci M, et al. Deciphering the state of immune silence in fatal COVID-19 patients. *Nat Commun* (2021) 12(1):1428. doi: 10.1038/s41467-021-21702-6

114. Zoso A, Mazza EMC, Bicciato S, Mandruzzato S, Bronte V, Serafini P, et al. Human fibrocytic myeloid-derived suppressor cells express IDO and promote tolerance *via* Treg-cell expansion. *Eur J Immunol* (2014) 44(11):3307–19. doi: 10.1002/eji.201444522

115. Lebrun A, Lo Re S, Chantry M, Izquierdo Carerra X, Uwambayinema F, Ricci D, et al. CCR2(+) monocytic myeloid-derived suppressor cells (M-MDSCs) inhibit collagen degradation and promote lung fibrosis by producing transforming growth factor-β1. *J Pathol* (2017) 243(3):320–30. doi: 10.1002/path.4956

116. Ruenjaiman V, Sodsai P, Kueanjinda P, Bunrasmee W, Klinchanhom S, Reantragoon R, et al. Impact of SARS-CoV-2 infection on the profiles and responses of innate immune cells after recovery. *J Microbiol Immunol Infect* (2022) 55(6 Pt 1):993–1004. doi: 10.1016/j.jmii.2022.09.001

117. Siemińska I, Węglarczyk K, Surmiak M, Kurowska-Baran D, Sanak M, Siedlar M, et al. Mild and asymptomatic COVID-19 convalescents present long-term endotype of immunosuppression associated with neutrophil subsets possessing regulatory functions. *Front Immunol* (2021) 12:748097. doi: 10.3389/fimmu.2021.748097

118. Hopkins FR, Govender M, Svanberg C, Nordgren J, Waller H, Nilsdotter-Augustinsson Å, et al. Major alterations to monocyte and dendritic cell subsets lasting more than 6 months after hospitalization for COVID-19. *Front Immunol* (2023) 13:1082912. doi: 10.3389/fimmu.2022.1082912

119. Schrijver IT, Théroude C, Antonakos N, Regina J, Le Roy D, Bart P-A, et al. COVID-19 rapidly increases MDSCs and prolongs innate immune dysfunctions. *Eur J Immunol* (2022) 52(10):1676–9. doi: 10.1002/eji.202249827

120. Beliakova-Bethell N, Maruthai K, Xu R, Salvador LCM, Garg A. Monocytic-myeloid derived suppressor cells suppress T-cell responses in recovered SARS CoV2-infected individuals. *Front Immunol* (2022) 13:894543. doi: 10.3389/fimmu.2022.894543

121. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA* (1999) 282 (7):677–86. doi: 10.1001/jama.282.7.677

122. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Front Immunol* (2020) 11:827. doi: 10.3389/fimmu.2020.00827

123. Ward JD, Cornaby C, Schmitz JL. Indeterminate quantiFERON gold plus results reveal deficient interferon gamma responses in severely ill COVID-19 patients. *J Clin Microbiol* (2021) 59(10):e0081121. doi: 10.1128/JCM.00811-21

124. Gupta A, Sural S, Gupta A, Rousa S, Koner BC, Bhalotra A, et al. Positive QuantiFERON test and the severity of COVID-19 disease: A prospective study. *Indian J Tuberc.* (2021) 68(4):474–80. doi: 10.1016/j.ijtb.2020.12.013

125. Rajamanickam A, Pavan Kumar N, Chandrasekaran P, Nancy A, Bhavani PK, Selvaraj N, et al. Effect of SARS-CoV-2 seropositivity on antigen – specific cytokine and chemokine responses in latent tuberculosis. *Cytokine* (2022) 150:155785. doi: 10.1016/j.cyto.2021.155785

126. Qin A, Cai W, Pan T, Wu K, Yang Q, Wang N, et al. Expansion of monocytic myeloid-derived suppressor cells dampens T cell function in HIV-1-seropositive individuals. *J Virol* (2013) 87(3):1477–90. doi: 10.1128/JVI.01759-12

127. Steggerda SM, Bennett MK, Chen J, Emberley E, Huang T, Janes JR, et al. Inhibition of arginase by CB-1158 blocks myeloid cell-mediated immune suppression in the tumor microenvironment. *J Immunother cancer.* (2017) 5(1):101. doi: 10.1186/s40425-017-0308-4

128. Iannone R, Miele L, Maiolino P, Pinto A, Morello S. Blockade of A2b adenosine receptor reduces tumor growth and immune suppression mediated by myeloid-derived suppressor cells in a mouse model of melanoma. *Neoplasia* (2013) 15(12):1400–9. doi: 10.1593/neo.131748

129. Cleverley TL, Peddineni S, Guarner J, Cingolani F, Garcia PK, Koehler H, et al. The host-directed therapeutic imatinib mesylate accelerates immune responses to Mycobacterium marinum infection and limits pathology associated with granulomas. *PloS Pathog* (2023) 19(5):e1011387. doi: 10.1371/journal.ppat.1011387

130. Napier RJ, Rafi W, Cheruvu M, Powell KR, Zaunbrecher MA, Bornmann W, et al. Imatinib-sensitive tyrosine kinases regulate mycobacterial pathogenesis and represent therapeutic targets against tuberculosis. *Cell Host Microbe* (2011) 10(5):475–85. doi: 10.1016/j.chom.2011.09.010

131. Xia L, Yuan L-Z, Hu Y-H, Liu J-Y, Hu G-S, Qi R-Y, et al. A SARS-CoV-2-specific CAR-T-cell model identifies felodipine, fasudil, imatinib, and caspofungin as potential treatments for lethal COVID-19. *Cell Mol Immunol* (2023) 20(4):351–64. doi: 10.1038/s41423-023-00985-3

132. Aman J, Duijvelaar E, Botros L, Kianzad A, Schippers JR, Smeele PJ, et al. Imatinib in patients with severe COVID-19: a randomised, double-blind, placebo-controlled, clinical trial. *Lancet Respir Med* (2021) 9(9):957–68. doi: 10.1016/S2213-2600(21)00237-X

133. Shen M, Wang J, Yu W, Zhang C, Liu M, Wang K, et al. A novel MDSC-induced PD-1(-)PD-L1(+) B-cell subset in breast tumor microenvironment possesses immuno-suppressive properties. *Oncoimmunology* (2018) 7(4):e1413520. doi: 10.1080/2162402X.2017.1413520

134. Zhan H, Yang L, Liu Y, Li H, Li X, Wang H, et al. Acceptance, safety and immunogenicity of a booster dose of inactivated SARS-CoV-2 vaccine in adults with tuberculosis. *J Infect* (2023) 86(5):e138–41. doi: 10.1016/j.jinf.2023.01.030

135. Verma D, Parasa VR, Raffetseder J, Martis M, Mehta RB, Netea M, et al. Anti-mycobacterial activity correlates with altered DNA methylation pattern in immune cells from BCG-vaccinated subjects. *Sci Rep* (2017) 7(1):12305. doi: 10.1038/s41598-017-12110-2

136. Koeken VACM, van der Pasch ES, Leijte GP, Mourits VP, de Bree LCJ, Moorlag SJCFM, et al. The effect of BCG vaccination on alveolar macrophages obtained from induced sputum from healthy volunteers. *Cytokine* (2020) 133:155135. doi: 10.1016/j.cyto.2020.155135

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Animal models for COVID-19 and tuberculosis

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Respiratory infections cause tremendous morbidity and mortality worldwide. Amongst these diseases, tuberculosis (TB), a bacterial illness caused by *Mycobacterium tuberculosis* which often affects the lung, and coronavirus disease 2019 (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2), stand out as major drivers of epidemics of global concern. Despite their unrelated etiology and distinct pathology, these infections affect the same vital organ and share immunopathogenesis traits and an imperative demand to model the diseases at their various progression stages and localizations. Due to the clinical spectrum and heterogeneity of both diseases experimental infections were pursued in a variety of animal models. We summarize mammalian models employed in TB and COVID-19 experimental investigations, highlighting the diversity of rodent models and species peculiarities for each infection. We discuss the utility of non-human primates for translational research and emphasize on the benefits of non-conventional experimental models such as livestock. We epitomize advances facilitated by animal models with regard to understanding disease pathophysiology and immune responses. Finally, we highlight research areas necessitating optimized models and advocate that research of pulmonary infectious diseases could benefit from cross-fertilization between studies of apparently unrelated diseases, such as TB and COVID-19.

KEYWORDS

animal model, mycobacteria, tuberculosis, SARS-CoV-2, COVID-19, immunology, pathology, respiratory infection

1 Introduction

Animal models are essential for understanding disease pathophysiology in its complexity. Pinning down coordinated immune processes as well as the continuous host reaction to pathogen assault can only be achieved by investigating the infected host. Although controlled human infection models and human challenge trials have been advanced for flu (1), malaria (2), coronavirus disease 2019 (COVID-19) (3), and tuberculosis (TB) (4, 5), and studying

infection in natural hosts in most circumstances is feasible for livestock, disease pathogenesis is studied in great detail in surrogate animals in experimental animal models. In such controlled settings pathogen entry, replication and transmission, immune responses, and pathology are elucidated unambiguously. Importantly, causality can be established in animal models and thereby such experimental approaches are instrumental for devising measures limiting pathogen transmission and for developing vaccines and therapies. The importance of animal models for vaccine testing should be emphasized. Here, animal models are without alternative (6) and should mimic the pathogenesis known in humans as closely as possible to increase transfer of the results to the human host (7). Standard laboratory animal models have been established to enable applications in many laboratories worldwide. Such models have been indispensable for the understanding, prevention and cure of two major respiratory infectious diseases: TB and COVID-19. We critically discuss experimental models in a comparative manner and highlight commonalities and differences in the context of these lung infections.

TB and COVID-19 are acquired respiratory infections which primarily affect the respiratory tract and are usually transmitted via aerosol droplets. TB represents one of the most ancient infectious diseases, a continuous threat to public health and currently among the top 10 causes of death worldwide (8). It was declared as a global emergency by the WHO in 1993 (9). TB is caused by genetically related microorganisms of the *Mycobacterium tuberculosis* complex (MTBC), with the human-adapted *M. tuberculosis* (Mtb) affecting mankind worldwide. COVID-19 represents the 21st century pandemic event and was declared as a global emergency by the WHO in 2020 (10). The global emergency phase was ended in May 2023, yet the WHO emphasizes that COVID-19 still remains a significant threat for human health[1]. It is caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). Both infections are dynamic and provoke a spectrum of diseases and pathologies. Their causative agents, although taxonomically unrelated, undergo continuous adaptation to the human host. The potential to evade immunity has been observed promptly during the COVID-19 pandemic, for instance by the emergence of virus variants, whereas for TB resistance to available therapies is on the rise, as illustrated by heightened incidences of disease caused by drug-resistant mycobacteria. Mtb enters alveolar macrophages, rarely pneumocytes, and spreads to lung-resident and recruited macrophages, whereas SARS-CoV-2 primarily infects ciliated and alveolar epithelia (11). Although variable pathology is observed in TB and COVID-19, recent systems analysis of human cohorts revealed commonalities in immunopathogenesis (12). TB is characterized by unique lesions termed granulomas, whereas severe COVID-19 manifests as pneumonia. Given the preference for respiratory tissue, mammalian animal models have been developed for both infections. We discuss the experimental models employed for the study of each disease and emphasize advantages and limitations these models bring regarding disease pathophysiology and immune responses. Considering spectra of TB and COVID-19, we identify challenges related to improving or developing new animal models and propose purpose-oriented approaches which extend beyond conventional animal models. Finally, we elaborate on multi-species approaches and co-infections, as these are currently feasible and inspired by recent advances in high-resolution technologies.

2 Small animal models

Historically, small animal models, including rodents and leporids, were paramount for the identification of Mtb as the causative agent of TB and for the elucidation of TB pathogenesis (13). They continue to be implemented in preclinical TB research and have been equally instrumental for the accelerated progress achieved for COVID-19 vaccines. Although murine infection models are by far the most frequently used for TB and COVID-19 research, they reproduce some, but not all aspects of the human disease. Other rodent species, including rats, hamsters and guinea pigs, provide important insights into pathophysiological aspects of the two respiratory diseases that are not sufficiently covered by murine models. Each model organism offers particular advantages and bears certain limitations, which are detailed in the following sections.

2.1 Mouse models

Mice are easy to handle, accessible, inexpensive, and the broad availability of immunological and genetic tools makes them very attractive for preclinical investigations. Laboratory mice provide the most established and implemented animal model in SARS-CoV-2 as well as in TB studies (14, 15). Advantages and limitations of murine models in the two respiratory infections are presented in Table 1.

2.1.1 Murine TB models

The experimental murine TB model has elucidated host fate upon natural infection which is achieved by aerosol exposure. It has unveiled the complexity of the kinetics of the infectious process in great detail. This model has also enabled mutual integration of host and pathogen traits in experimental studies. However, mice do not fully recapitulate TB pathology. Granuloma liquefaction, cavitation and fibrosis remain undetected in Mtb-infected mice, and hence murine TB is an imperfect disease model. This model allows to comprehensively study the immune responses to Mtb. Pulmonary anatomy and immune mechanisms in mice have a great degree of similarity to humans (16) which make them ideally suited for studying immune dynamics within tissues and for testing vaccine efficacy. Although mice are not natural hosts for Mtb and are generally tolerant to TB (17), they have proven instrumental for understanding some disease mechanisms.

Susceptibility of laboratory mice to Mtb depends on both host and bacterial features. Among the host factors, mouse genetics, age, sex, and immune status control TB outcome (18–21). The route of infection (22), inoculum size and bacterial genetics, e.g. Mtb lineages and virulence factors, impact as well on the course of TB.

1 WHO. WHO Director-General's opening remarks at the media briefing, Vol. 2023. (2023).

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TABLE 1 Murine models for tuberculosis (TB) and coronavirus disease 2019 (COVID-19).

Mouse models	Tuberculosis		COVID-19	
	Advantage	Disadvantage	Advantage	Disadvantage
Inbred "resistant" (C57BL/6/ Balb/c)	High reproducibility, availability of gene knock-out mice, long-term studies and kinetics, correlates of protection, immunological tools	Lack of human-like pathology (e.g. liquefaction, fibrosis), lack of relevant Mtb-induced cell types (e.g. multinucleated giant cells)	Availability of gene knock-out mice, long-term studies and kinetics, correlates of protection, immunological tools	Only mouse-adapted virus strains or specific SARS-CoV-2 variants (e.g. B.1.351), limited pathology
Inbred "susceptible" (C3HeB/FeJ, 129Sv, I/St, DBA/2)	Investigation of pathology, necrotic granuloma, correlates of susceptibility, drug testing	Limited number of knock-out mice available, no chronic or latent stage of disease	More severe pathology (129Sv) compared to C57BL/6, lung pathology (compared to K18-hACE2)	Only mouse-adapted virus strains or specific SARS-CoV-2 variants (e.g. B.1.351)
Outbred	Genetic diversity, microbiome diversity	Housing with inbred and pathogen-free mice difficult, reproducibility	N/A	N/A
Collaborative cross (CC) lines	Genetic diversity, gene association studies	Low reproducibility, expensive, resource intense	Genetic diversity, gene association studies	Require further genetic manipulation for usage as a model for COVID-19 (e.g. cross-breeding with K18-hACE2)
Humanized mouse	Reflection of human specific cell types or effector functions	Expensive, high variability, technology intensive, highly susceptible to attenuated strains (e.g. BCG)	Better reflect COVID-19 pathology, severe lung pathology, study of drug or antibody therapy	Expensive, high variability, technology intensive, cross-reactivity of human-mouse immune networks
Transgenic (K18-hACE2)	N/A	N/A	Robust, highly permissive model, suitable for all SARS-CoV-2 variants, excellent vaccine model	Limited lung pathology, brain pathology, some SARS-CoV-2 strains interact with human and mouse ACE2, expensive
Vector hACE2 delivery (AAV, Ad, LV)	N/A	N/A	Mild lung pathology (reflects COVID-19 in the majority of patients), suitable for vaccine studies, amenable for different mouse strains and genetic manipulations	Transient, low pathology (possible disadvantage for vaccine studies), immune response against vector
SARS-CoV-2 Mouse adaptation	N/A	N/A	Lung pathology (compared to K18-hACE2), can be used in combination with different mouse strains and genetic manipulations	Mouse adaptation might not reflect human isolates, new variants underrepresented

The various mouse models employed for the study of each disease are included, emphasizing on key advantages and limitations of each model. AAV, adeno-associated virus; Ad, adenovirus; COVID-19, coronavirus disease 2019; hACE2, human angiotensin-converting enzyme 2; K18, keratin 18 promoter; LV, lentivirus; N/A, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus type 2.

For instance, aerogenic exposure to the East/Asian Beijing strain HN878, in contrast to infection with the reference Euro-American strain H37Rv, triggers heightened susceptibility in C57BL/6 mice (23) and granulomatous lesions resembling human-like pathology (24). Various Mtb strains differ in propensity to infect myeloid cells (25), disseminate (26) or trigger inflammation (27). Mtb attenuation by deletion of the PhoP regulon (28), or deletion of entire virulence coding genomic regions, e.g. region of difference 1 (RD1) (29), or pathogenicity factors such as the early secreted antigenic target 6-kDa protein (ESAT-6) (30, 31) cause reduced pathology. Such studies have also unveiled a dominant role of nitric oxide in antimycobacterial immunity in the murine host, unlike in humans (32). In addition, they confirmed the relevance of subcellular pathogenicity events, such as cytosolic translocation of the bacilli, for Mtb pathogenicity during lung infection.

Research on TB immunology and pathology heavily relies on inbred and knock-out (KO) mouse strains and has recently been enriched by the addition of the collaborative cross (CC) lines (33, 34), diversity outbred (DO) (35–38) as well as humanized mice (39, 40). Whereas inbred animals and respective KO lines have permitted targeted characterization of host factors essential for the susceptibility to disease, genetic diversity has contributed to unbiased identification of host susceptibility or resistance traits. Of note, mice can be infected by various MTBC bacteria, and usage of transgenic knock-in models has facilitated analysis of particular cell types or molecules during infection (e.g. fluorescent-tagged reporters), or enabled targeted cell deletions (e.g. Cre-lox system). The transgenic mice used in TB research offer opportunities to decipher disease pathogenesis. They do not confer essential cell entry host factors to mycobacteria, a situation common for COVID-19 murine models where infection is usually abortive in wild type animals (see section 2.1.2).

TB outcome differs in various inbred mice which are classified as TB-resistant and TB-susceptible based on the time to death or bacterial outgrowth. TB-resistant mice, including C57BL/6 and Balb/c strains, control aerosol infection with relatively high doses (e.g. 500 colony forming units, CFU) of bacteria, do not develop

typical granulomas and succumb rather due to aging. As such, they have been proposed as potential latency TB models (41). Studies in C57BL/6 mice have been critical for defining kinetics of the immune events post exposure, requirements for priming of adaptive immunity as well as kinetics and plasticity of T-cell responses in TB. Very early in infection alveolar macrophages support Mtb replication, as demonstrated in depletion studies (42), likely due to their metabolic imprinting towards oxidative phosphorylation (43) and anti-oxidant features (44). Elegant fate-mapping studies have highlighted that alveolar macrophages translocate into the lung interstitium (45) and Mtb gains access to less permissive glycolytic lung macrophages (43). Subcellular virulence factors, notably mycobacterial ESX1 secretion system (31), and host determinants of susceptibility, for instance phagosomal proteolysis (46) have been unveiled also *in vivo* in the context of macrophage plasticity in TB in C57BL/6 mice. Cell types conferring an Mtb-permissive environment, including lung monocyte-derived macrophages and dendritic cells (47), and neutrophils (48, 49), have been defined also in this murine model. Kinetics of T-cell responses (50) and the impact of their localization on disease outcome (51, 52) have been established in C57BL/6 mice receiving transgenic cells expressing an Mtb-specific T-cell receptor (TCR). T-cell depletion alone or combined with adoptive transfer of antigen-specific T-cells has indicated an essential role of $CD4^+$ and $CD8^+$ lymphocytes for TB control (53, 54) and highlighted Mtb escape strategies related to dominant epitopes and misplaced T-cells (55). Thus, C57BL/6 mice have substantially contributed to the delineation of immune events in primary TB. The major caveat of the C57BL/6 model lies in the lack of human-like pathology. Of note, application of ultra-low dose infection (ULD) (56) may render these mice amenable for pathology studies (57). Upon ULD, mice develop single, structured lesions upon inhalation of 1-3 Mtb CFU of the laboratory strain H37Rv. Organized granulomas have also been reported in C57BL/6 mice challenged with low-dose hypervirulent HN878 Mtb (24, 58). These murine models mirror, to some extent, human TB lesions, have organized granuloma-like lesions which contain foamy macrophages, develop central necrosis, yet still miss certain cellular components such as multinucleated giant cells and do not show fibrosis and calcification. A further utility of C57BL/6 mice has recently been reported. Intra-dermal Mtb infection resulted in localized spread of Mtb (59, 60), unlike systemic dissemination seen upon aerosol or intravenous challenge (22), and thus may represent a refined experimental model for latent TB infection (LTBI). Besides mechanistic understanding of immunity and pathology, C57BL/6 and Balb/c strains are also gold standards for chemotherapy studies and TB vaccine development.

TB-susceptible inbred mice encompass the C3HeB/FeJ, 129Sv (129S2/SvPas), I/St and DBA/2 mouse strains. C3HeB/FeJ mice are best suited for investigating pathology. Exposure of these mice to Mtb leads to the formation of well-formed, necrotic granulomas showing hypoxic regions (61), with liquefaction observable particularly upon i.v. challenge (62). Necrosis of Mtb-infected macrophages is controlled by the sst1 locus (63) and has been linked to the *intracellular pathogen resistance 1* (*Ipr1*) gene (64). A role for neutrophils in susceptibly to TB in C3HeB/FeJ by controlling lesion progression (36, 62), likely via type I interferon (IFN-I)-driven NETosis (65), has been reported. The similarities to lesion progression in humans (66–68) make this model useful for pathology and immunopathogenesis studies. DBA/2 mice show a fast TB course with bronchogenic dissemination (69). Their susceptibility is driven by neutrophils (70) and limited accumulation of regulatory T-cells (Treg) within infected tissue (71). This phenotype is shared by the TB-susceptible inbred strain I/St (72, 73), which unlike their A/Sn counterparts cannot control TB. 129Sv (129S2) mice succumb early during TB (74) with extensive lung damage. Their susceptibility to TB is uncoupled from *natural resistance-associated macrophage protein 1* (*Nramp1*) allele gene polymorphism (75). Mtb-triggered lethality is due to early neutrophil recruitment (74), heightened necrotic cell death (76), and likely Mtb-driven and acetyl-coenzyme A dependent foamy cell differentiation (77). TB-susceptible mice are suitable for deciphering host traits which favor a poor outcome in TB. They are also helpful for testing drugs and host-directed interventions given the development of lesions, notably well-structured granulomas containing transformed cell types, and environments, such as hypoxia and necrosis, characteristic of active TB.

Understanding of the immune control of TB has been nurtured by failed immunity in KO and immunodeficient mice which mirror catastrophic human genetic defects. Examples are mice with full or cell-type specific deletion of IFN-γ (78) or TNF-α (79, 80). Just as reported in humans with mendelian susceptibility to mycobacterial disease (81) or on suppressive anti-TNF-α immunotherapy (82) these cannot control Mtb infection or reactivate LTBI, respectively. Since immunity can be investigated within organs, KO mice also enriched knowledge about *in situ* roles of host factors. For instance, IFN-γ was shown to regulate neutrophil apoptosis (83) and TNF-α to regulate lesion stability by signaling in myeloid and lymphoid cells (80). Mice lacking lymphocytes (Rag2 KO and Rag2/γc KO) have demonstrated essential yet differential roles of T- and NK-cell derived IFN-γ in TB control (84). KO mice have supported reverse translation investigations in TB, as exemplified for miRNAs. For instance, miR-223 is enriched in human TB lesions and susceptibility of miR-223 KO has been linked to the regulation of IL-6, as well as of CCL3 and CXCL2, during acute disease (85). Thus, various KO mice have supported the understanding of TB pathogenesis at a molecular level. In contrast, susceptibility of certain KO lines has not translated to observations in human TB. Some examples are heightened mortality associated with mice lacking the adaptors MyD88 (86) and CARD9 (87). Failed models with observations distinct from human data are also exemplified by mice lacking NADPH oxidase subunits (88, 89) or indolamin-2,3-dioxygenase (90). These mice do not show a strong phenotype in TB despite susceptibility being linked to deficiency in these pathways in humans (68, 91). The inbred features of the most common murine models may contribute to such discrepancies. In this context, CC lines and DO mice may better mirror genetic diversity of the human host. CC lines have uncovered genetic loci associated with uncontrolled infection, including IFN-γ-independent phenotypes (33). DO models have confirmed that neutrophils are detrimental in progressive TB and highlighted roles of neutrophil chemoattractants in this process (36). Since mice and humans show variabilities in immune components, for instance cell abundance (humans belong to neutrophil-high species) or molecular

constituents (mice lack granulysin and CD1-type-1 molecules), humanized murine models have been generated (39, 40). Their use is restricted due to financial and technological constraints as well as variability in immune reconstitution and persistence of a mixed human-mouse tissue environment. However, their usage could be critical for addressing co-infection of Mtb with viruses requiring human host factors for entry such as human immunodeficiency virus (HIV). Without doubt, KO models have substantially enriched the knowledge about immune cells and immune pathways in TB and provided causality proofs for disease pathogenesis. Embracing genetic diversity by usage of CC line and DO mice offers unique opportunities for mechanistic studies and may provide new ways to guide TB prophylaxis (92).

Collectively, murine models for TB are diverse and offer a spectrum of options to choose from (see Table 1). Experimental tools and feasibility of gene editing in mice, which permit cell fate mapping and tracing, will continue to support immunological research. There are yet several limitations related to the usage of mice in TB. Besides the drawback regarding TB pathology, mice are not suitable for transmission studies. They have been extensively used as models for primary TB. However, unlike humans, mice promptly allow Mtb dissemination to distal sites following aerogenic infection. Efforts to develop murine models for post-primary TB have been undertaken (41) and require additional evaluation.

2.1.2 Murine COVID-19 models

Inbred laboratory mouse strains such as Balb/C and C57BL/6 are not susceptible to ancestral (B.1) SARS-CoV-2 infection. With the emergence of SARS-CoV-2 variants (e.g. Alpha (B.1.1.7), Beta (B.1.351), and Gamma (P.1)) with extensive mutations in the spike protein, particularly the N501Y mutation, laboratory mouse strains became susceptible to infection and virus replication, although without showing significant pathology (93). However, the inbred mouse strain 129S2 develops clinical disease and has been employed to assess the efficacy of monoclonal antibodies and vaccines (94, 95). Two major approaches have been pursued to amend the murine model for COVID-19 study: genetic engineering of mice for expression of the human ACE2 (hACE2) receptor protein, and adaptation of SARS-CoV-2 to enter murine cells via endogenously expressed receptors (96, 97). A comprehensive summary of the frequently used genetically manipulated murine models as well as adapted virus strategies that substantially contributed to reproduce key characteristics of SARS-CoV-2 infection has been provided recently (98). For comparative evaluation we integrate the murine COVID-19 models with TB models and highlight benefits and disadvantages for each model and infection (Table 1).

The most commonly used K18-hACE2 model, where hACE2 is expressed under control of the human keratin 18 promotor, in addition to the murine ACE2, appears to be the most susceptible COVID-19 model reported to date using human SARS-CoV-2 isolates (99). This model has contributed to the clarification of disease pathophysiology. For instance, it provided evidence for SARS-CoV-2 invasion of sustentacular cells as the cause of subsequent anosmia (100). As inflammation drives severity of COVID-19 in humans, details on dynamics of inflammatory responses obtained in this mouse model could be valuable for the design of therapies. The K18-hACE2 mouse resembles severe COVID-19 disease (101), developing cytokine storm (102), prompt accumulation of immune cells within infected lung (103), loss of plasmacytoid dendritic cells (104) and alveolar macrophages paralleled by accumulation of monocyte-derived macrophages (105). The contribution of host genetics to inflammation control has been further evaluated using collaborative cross (CC) x K18-hACE2 F1 progeny mice (106). In this model survival was associated with early IFN-I expression and production of proinflammatory factors. Similarly, disease severity was driven by CXCR6 and CCR9 in a comparable mouse model approach (107). The K18-hACE2 model has been also useful to demonstrate the relevance of lymphoid cell depletion, which together with the impaired antigen presenting cells/T-cell axis, is a specific feature of severe SARS-CoV-2 infection (108). Furthermore, evidence for the protective roles of T-cells was demonstrated by the fact that vaccination with immunodominant T-cell epitopes provided partial or even full protection in K18-hACE2 mice in the absence of neutralizing antibodies (109, 110). Comparative RNAseq analysis (human vs. mouse) has revealed that at the broad level of immune responses and inflammation pathways, highly overlapping patterns between the two species exist suggesting that the K18-hACE2 mouse model emerges as a representative and relevant animal model of COVID-19 (111). It remains yet unclear whether innate immunity alone could under particular circumstances, for instance low inoculum, eliminate the virus in these transgenic mice. A disadvantage of this model is that it does not mirror mild disease, and interference of signaling from both murine and human ACE2 adds an additional layer of complexity when investigating SARS-COV-2 variants which bind the murine receptor (104). Further, the K18-hACE2 mouse model also has the disadvantage of hACE2 expression in the brain of the transgenic animals. The severity of the disease and the reason for humane endpoints are therefore usually the artificial occurrence of a severe infection of the brain with encephalitis (101, 112). Brain invasion has been demonstrated in humans (113), it is though not a common manifestation of COVID-19 (114). Of note, aerosol delivery in contrast to intranasal challenge bypasses brain involvement (115), suggesting that the route of infection may be relevant for the phenotype of the K18-hACE2 murine model. The K18-hACE mouse model has been essential for vaccine research and its preclinical value is impressive. The critical role of the murine models is highlighted by the fact that mRNA vaccine preparations were extensively tested in the mouse model before licensing in the U.S. under Emergency Use Authorization. Additionally, next generation SARS-CoV-2 vaccines covering multivalency or mucosal application have been similarly evaluated in mouse models [i.a (116–119)].

Adenovirus-, lentivirus- or adeno-associated virus-driven transient hACE2 expression in the murine lung has also been established multiple times in different laboratories [e.g (97, 120, 121)]. However, the virus-induced expression comes with the disadvantage of potential induction of unspecific inflammatory responses, non-uniform expression of hACE2 in the lung epithelium and interference with vector-based vaccines (121). Nevertheless, it has been utilized to study COVID-19 pathology and for preclinical vaccine investigations, including mechanism of

action studies. The pathology in this model is restricted to the respiratory tract, with milder disease and in most cases self-resolving inflammation (121, 122). Using this model, it has been shown that IFN-I responses are associated with inflammation and myeloid cell infiltration, but not with SARS-CoV-2 control (122, 123). The mild and localized pathology, along with the possibility to induce hACE2 expression in KO strains, have enabled to study mechanisms of SARS-CoV-2 clearance in naïve and vaccinated animals with different genetic backgrounds. These studies have confirmed the essential role of the adaptive immunity for resolution of inflammation and viral clearance (124). Furthermore, protection of neutralizing antibodies has been confirmed in this animal model (120).

Another approach that allows the use of standard laboratory mice and, more importantly, genetically modified mice, is to adapt SARS-CoV-2 to the mouse (96). These viral strains are therefore particularly suitable for studies in specific KO mouse lines. Thus, the age and sex dependency of human disease severity could be shown with an adapted ancestral SARS-CoV-2 strain (125, 126). However, such adaptations must be carried out separately for different virus variants which do not naturally infect mice and thus are disadvantageous due to the extensive time required for adaptation.

All mouse models come with a substantial drawback related to viral transmission. Even humanized and genetically modified mice are unable to transmit the virus to contact animals (127). Of note, recent investigations in a neonatal K18-hACE2 mouse model have reported virus transmission in a SARS-CoV-2 variant specific manner (128) and such promising observations require validation.

The murine models used for TB and COVID-19 differ substantially, primarily due to the distinct natural susceptibility of mice to Mtb and SARS-CoV-2 (Table 1). For both infections, mouse models are not amenable for investigating transmission and generally have limitations due to dissemination of infection at distal sites as well as at recapitulating human pulmonary pathology. Nonetheless, they are suitable for the mechanistical understanding of immune responses and thus have been extensively employed for vaccine studies. The diversification of the mouse models in TB during the last decade is remarkable (Figure 1), and attempts to employ systems approaches for vaccine discovery (129) further emphasize their value in pre-clinical research. Whereas transgenic knock-in mice have been essential for the progress of COVID-19 vaccines, such strains have rather targeted utility in TB. Irrespective of the peculiarities of the murine models, in both infections experimentation in mice has permitted evaluation of biological processes at subtissular and molecular scale and have advanced interventions.

2.2 Rat models for COVID-19 and TB

The rat is the animal species of choice in the pharmaceutical industry for pharmacokinetic and toxicological studies. Wistar rats are also generally employed in immunization studies given their

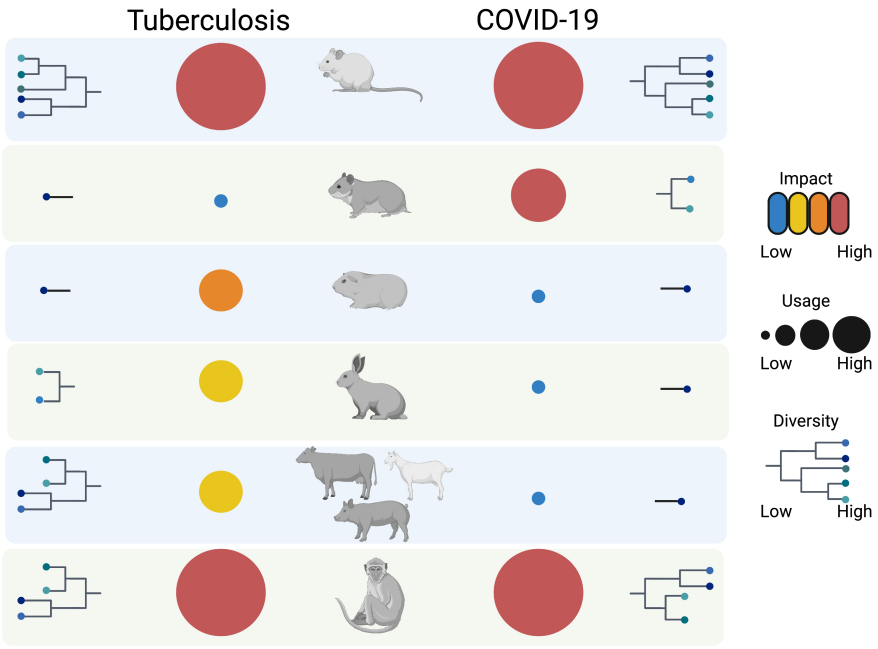

FIGURE 1
Overview of animal models for TB and COVID-19. The animal models used for the two respiratory infections are diversified and range from mouse to non-human primates. Whereas murine models show the highest diversity, guinea pigs, rabbits, hamsters and livestock show applicability for one of the two diseases. Non-human primates, just like mice, have the biggest impact in terms of knowledge gain with the former ones having the greatest translational value. All animal models have unique benefits and cumulatively contribute to the study of TB and COVID-19, and have potential to cross-fertilize understanding of other respiratory diseases. The figure was generated using the illustration software BioRender (BioRender.com).

broad availability, easy handling, defined physiology and potential to obtain larger samples compared to mice. They are infectible by selected SARS-CoV-2 variants, such as B.1.1.7 (130), but have not been used as a model for COVID-19. Instead, Wistar rats have been essential for investigating the pharmacokinetics of the lipid-nanoparticles used to formulate COVID-19 mRNA vaccines [EPAR – Comirnaty] (129–132). A limitation for vaccine studies in this model is the insufficient knowledge about SARS-CoV-2-induced pathology and the lack of appropriate immunological tools to monitor immune responses (e.g. T-cell responses) after vaccination and challenge.

Rats are generally susceptible to Mtb (132), and they have been used to distinguish bacteriostatic or bactericidal properties of investigational compounds (133). In the rat model, the decrease of T-cell reactivity to ESAT-6 has been proposed as a correlate of therapeutic efficacy (134) which principally sheds light on the maintenance of high-level T effector cell populations. Various rat models, including American cotton rats, Lewis rats, Wistar rats, and Sprague-Dawley rats, develop granulomatous lesions which do not liquefy (132, 135, 136), and thus human TB pathology is not fully mirrored.

Rats have proven valuable for TB diagnostic purposes, particularly in poor resource settings: African giant pouch rats have been trained to detect Mtb in sputum samples (137). Mycobacterial volatile organic compounds are detected by rats which recognize Mtb across different genotypes and discriminate it from related bacteria, including *M. avium* subsp. hominissuis or *M. intracellulare* (137). Although in this case the animal model does not immediately contribute to the understanding of TB pathophysiology, the approach has drawn attention to an entirely unexplored universe of small volatile bacterial compounds, and has brought forth a diagnostic method to detect TB in high burden regions with limited access to molecular diagnostics. Thus, the rat model could be useful to develop electronic nose devices for TB detection.

2.3 The guinea pig model for TB

Guinea pigs are resistant to SARS-CoV-2 (138), but are highly susceptible to TB. In his pioneering experiments Robert Koch used guinea pigs and rabbits to prove that a pure Mtb culture causes the disease (13). In the 1950s their susceptibility to TB prompted scientists to use guinea pigs as living air samplers to demonstrate aerial dissemination of mycobacteria (139). They not only take up mycobacteria by inhalation, but also expectorate them like humans and thus are amenable to transmission studies. Recently, guinea pigs have been used to reveal sulfolipid-1 as the activating factor for nociceptive neurons to trigger cough (140). The course of infection in guinea pigs varies with the Mtb strain and the initial dose, but invariably animals succumb to Mtb infection. After logarithmic growth in the lungs, Mtb loads remain stable over many weeks. Ultimately, the bacteria re-enter a logarithmic growth phase and this regularly coincides with the humane end point (141). Besides aerosol exposure also parenteral routes of infection are used. For example, the intramuscular route and the degree of generalized systemic dissemination has been used to assess the virulence of different Mtb isolates (142, 143). For batch potency testing of bovine tuberculin it is laid down in the corresponding monography of the European Pharmacopoeia that guinea pigs shall be sensitized by deep intramuscular injection of live *M. bovis* before defined amounts of the control and the test batch of the tuberculin are intradermally injected (Eur Ph 01/2008:0536). In guinea pigs, initial Mtb replication is confined to the site of entry, yet bacteria disseminate via lymphatic flow presumably by dendritic cells reaching the draining lymph node (144). Secondary to lymphadenitis, which is often a manifestation of TB in children and consistently developed in guinea pigs (144), there is systemic generalization and hematogenous spread to other organs. Ultimately, hematogenous reseeding of the lungs may occur which leads to progressive infection and tissue destruction. At these different tissue levels granulomatous infiltrates occur that develop to large, caseous, necrotizing granulomas in unsensitized animals (144). However, it is important to note that granulomas in guinea pigs barely show liquefaction and cavitation (145, 146). Such lesions are the hallmark of post-primary TB and, upon infection, can prominently be observed in pre-sensitized rabbits (see 2.4). In guinea pigs, granulomas rather reproduce primary lesions in humans. Accordingly, guinea pigs are not a suitable model to study mycobacterial latency (145). The vast, necrotizing lesions develop in the absence of preformed T-cell immunity and are probably due to early recruitment and decay of granulocytes (147). In the presence of antigen-specific T-cells, guinea pigs show fewer granulomas that are better structured and contain significantly smaller necrotic areas. This correlates with reduced bacterial burden (141). Hence, guinea pigs have been widely used to stringently test new vaccine candidates against TB (148–153). The observed protective effect can be achieved by immunizing guinea pigs with protein antigens, but also with mycobacterial lipids (154). In this context, it is of note that guinea pigs express a functional CD1-type1-system. This is another hallmark that distinguishes them from murine rodents and resembles humans (155–157). CD1 molecules are characterized by a deep, hydrophobic antigen binding groove which enables accommodation and presentation of long-chained lipids to T-cells. In contrast to CD1d-restricted NKT cells, the lymphocytes that recognize their antigen in the context of CD1-type-1-molecules bear a variable αβ-T TCR and truly belong to the adaptive immune system. They can be primed and develop an immunological memory (158). Because mycobacteria express a rich repertoire of glycolipids, lipoglycans and lipopeptides, which all represent or harbor potential CD1-ligands (159), the CD1-T-cell axis has always been of interest to TB vaccinologists. Due to their susceptibility to Mtb and the natural expression of CD1, guinea pigs are particularly well-suited to study the contribution of lipid-reactive T-cells to defense against Mtb (160). Accordingly, efforts have been undertaken to study the protective role of lipid-reactive T-cells (154, 160), but additional animal studies are required to better understand the complex interaction between mycobacterial lipids and the host's adaptive immunity (161). Guinea pigs are also suitable for evaluation of diagnostic skin tests and thus are an essential animal model for assessing delayed-type hypersensitivity to mycobacterial cognates (141, 162). They are in addition an

indispensable model for testing antimycobacterial compounds (163–165).

A drawback of the guinea pig model remains the scarcity of immunological tools and the lack of genetically modified strains. However, a number of guinea pig-specific, monoclonal antibodies have become available in recent years (166, 167). In addition, molecular screening techniques including gene arrays, qRT-PCR and classical immunological stimulation assays, have been developed to study guinea pig immune responses in more detail (168–170).

2.4 The rabbit model for TB

Rabbits can be infected with high doses of the ancestrally derived SARS-CoV-2, but are not suitable as model animals for COVID-19 because of their low susceptibility (171). On the other hand, they allow studying clinical features of TB since rabbits are relatively resistant to Mtb compared to *M. bovis* (172). Infection with Mtb strains can lead to a latent course of disease that can be reactivated by immunosuppressive drugs (173). Using this model, it has been shown that rapid innate immunity involving in particular an early activation of NK cells is essential for an early control of exponential bacterial growth. It has also been shown that T-cell activation is dampened once bacterial growth is controlled, leading to spontaneous latency (174). By contrast, infection with *M. bovis* results in extended lung tissue destruction ultimately leading to cavity formation (175). The rabbit model is amenable to closely reproduce post-primary TB. Animals develop cavities similar to lesions in humans, in a process that involves congestion of bronchioles, massive multiplication of mycobacteria and extensive, allergic necrotizing tissue destruction and depends on Mtb strains, previous sensitization, and host genotype (176). Sensitization of rabbits by multiple injections of heat-killed *M. bovis* in incomplete Freund's adjuvant and subsequently instillation of viable Mtb by bronchoscopy directly into the lung triggers cavitation (177). This approach has led to a better understanding of the role of matrix-metallo-proteases in TB cavity formation. Ability to induce cavitation depends also on Mtb strains, with hypervirulent W-Beijing Mtb causing cavities, while less virulent strains including CDC1551 rather trigger LTBI (173, 178). The outcome of Mtb exposure can be studied in rabbits and has unveiled that early innate inflammatory responses, inoculum size and bacillary aggregation facilitate progressive TB and development of pathology rather than establishment of LTBI (178, 179). Zonation of pro- and anti-inflammatory regions within granulomas, primarily due to variable abundancies of distinct eicosanoid species, are similar in rabbits and humans (180). TB pathology in rabbits, and specifically occurrence of cavities, reproduce this stage of the disease that is most critical for successful antibiotic treatment (181). Currently, the rabbit model has become instrumental to study the biodistribution of new and of well-known antimycobacterial compounds, such as rifampicin (182) and pyrazinamide (183). As for guinea pigs, lack of immunological reagents limits vaccinology studies in rabbits. The model has also limitations with regard to the clinical manifestation of TB. Moreover, genetic editing of rabbits is in its infancy and the high costs compared to rodent models restrict usage of the rabbit model to specific scientific questions.

2.5 Hamster models for COVID-19

Hamsters, including the golden Syrian hamster (*Mesocricetus auratus*), are susceptible to TB, but have not been extensively used for the study of this disease (184). They are naturally highly susceptible to SARS-CoV-2 infection (185–187). Experimental intranasal inoculation with SARS-CoV-2 results in a transient, self-limiting, epitheliotropic infection of the lungs with almost complete elimination of the virus within two weeks. Certain dwarf hamsters (e.g. the Roborovski dwarf hamster) are even more susceptible and usually die or have to be euthanized after SARS-CoV-2 challenge (187). In the Syrian hamster, SARS-CoV-2 infection is restricted to sites containing both ACE2 receptor protein and TMPRSS2 protease (188). Interestingly, the infectious dose 50 for Syrian hamsters is defined to be only five infectious particles, making the hamster a sensitive model for SARS-CoV-2 infectivity assessment (189). In this model, host factors have been investigated and variable influence of age on disease severity has been reported (189, 190). Syrian hamsters are also suitable to explore sex differences in the pathogenesis of SARS-CoV-2 and vaccine-induced immunity and protection (191, 192). Transmission to direct contact hamsters as well as airborne-based transmission occurs in this animal model [i.a (193, 194)]. Furthermore, the concept of super-spreading has been modelled in the Syrian hamster model (194, 195). These findings strengthen the superior value of the hamster model over other SARS-CoV-2 models for virology and disease pathogenesis studies. Besides utility in deciphering acute host responses to SARS-CoV-2, the Syrian hamster offers an alternative for modeling of long COVID-19. Despite the lack of detectable infectious virus hamsters exhibit altered long term systemic responses (196).

Although immunological tools are limited, SARS-CoV-2–specific T- and B-cells have been evaluated in a longitudinal study in infected and recovered hamsters (197). Adoptive T-cell transfer reduces virus loads and facilitates rapid induction of SARS-CoV-2–specific B-cells, demonstrating that both lymphocyte populations mutually contribute to protection in hamsters. Studies applying single-cell RNA and protein profiling have substantiated the utility of the hamster model for deciphering immune events in moderate COVID-19. Similar to human COVID-19 patients, early proinflammatory responses from lung-residing monocyte-derived macrophages have been detected in SARS-CoV-2 infected hamsters (198). The animals develop inflammatory profiles akin to the cytokine storm observed in humans (196). *In situ* accumulation of cytotoxic T-cells and release of IgM antibodies occur prior to viral elimination (198). Golden Syrian hamsters reproduce also the vasculopathy observed in human patients, including involvement of neutrophil extracellular traps (NETs) (199) which is observed in severe human cases (200). The hamster has been, and continues to be, instrumental for both COVID-19 vaccinology and therapy. Its translation value seems to exceed that of mice (15). More recently the hamster has provided mechanistic insights into the Th-2 basis of

vaccine-associated enhanced respiratory disease (201) and emphasized the value of tissue-resident memory T-cells (202) in protection against SARS-CoV-2 conferred by distinct live-vaccines.

Overall, the hamster is one of the most significant animal models for the study of SARS-CoV-2 pathogenesis and for vaccine development. In addition to usage of modern single-cell technologies, immunological tools are increasingly being developed for this species with the prospect of advancing hamster studies in the future.

3 Large animal models

Livestock species and non-human primates (NHP) are natural hosts for MTBC, with the latter ones being also prone to SARS-CoV-2 infection. Similarities to humans with respect to the anatomy of the respiratory tract and the structure of the lung, for instance lung lobulation, as well as commonalities in organization and functionality of the immune system are notable. The evolutionary relationship with humans confers large animal models additional assets and unique model values.

3.1 Non-human primate models

NHP have been essential for elucidating SARS-CoV-2 and TB disease pathogenesis as well as for vaccine studies. Three different NHP, Rhesus Macaques (RM), Cynomolgus Macaques (CM) and African Green Monkeys (AGM) have been primarily used for both pathogens with the rational that they are genetically and immunologically closely related to humans.

3.1.1 Non-human primates in TB

In the 1960s and 1970s RM were used for the first time in TB research for vaccine and drug efficacy testing. For two decades RM and CM have offered substantial novel insights into pathology, immunology, vaccine and therapies for TB. Nowadays the NHP model is considered the most relevant for translational human TB research (203).

Depending on the dose (10^1-10^5 CFU), Mtb strain (e.g. Erdman, H37Rv, CDC1551), and route of infection (intravenous, intratracheal or aerosol) RM and CM reflect the full TB spectrum (acute, LTBI and re-activation of LTBI) including all stages of human-like granuloma (204). NHP and human mature, adaptive granulomas, are structured into necrotic cores surrounded by layers of macrophages and lymphocyte zones (205), including immunocompromised microenvironments (68). Akin human lesions (206), NHP granulomas contain tertiary lymphoid structures with key roles in anti-mycobacterial immunity (207). Progression to active TB can be monitored in NHP by longitudinal MRI or PET-CT scans which correlate with bacterial burden and inflammation (208). Such clinical measurements revealed that even under non-clinical disease (e.g. LTBI) NHP lungs contain active, necrotic lesions and sterilized healing lesions at the same time (209), an observation which has been confirmed with similar methods in humans (210). RM are more susceptible to Mtb infection than CM, with RM showing increased pathology and progression to disease compared to CM (211). Evidence for variable baseline of anti- and pro-inflammatory status of the myeloid compartment resulting in increased anti-inflammatory responses in RM after Mtb infection compared to CM pro-inflammatory responses has been provided (212) and likely additional factors underlying diverging susceptibility exist.

Since the whole spectrum of human TB can be observed in NHP models, correlates of protection or susceptibility have been singled out by comparing progressor versus non-progressor animals and by comparing individual progressing versus sterile granulomas from the same animal (213). Treatment of NHP with an antibody against TNF-α leads to increased disease progression in line with observations in humans (214, 215). Similarly, co-infection of NHP with Mtb and Simian immunodeficiency virus (SIV) leads to active disease with pathological features comparable to HIV-1 co-infection in humans (216, 217). Consistent with human TB and many mammalian models, CD4+ T-cells play an essential role in protecting NHP against development of active TB (218, 219). Single-cell transcriptomic signatures of different granulomas from the same individual lung sample revealed that healing or sterile granuloma were associated with IFN-γ/IL17 producing Th1 CD4+ T-cells (213). However, T-cells alone do not seem to be sufficient to control Mtb infection in NHP and humans. Tertiary lymphoid structures (e.g. inducible Bronchus-Associated Lymphoid Tissues (iBALT) or granuloma-associated lymphoid tissue (GrALT)) are significantly associated with non-progressors for active TB (220). A recent study of these GrALT structures has revealed that Mtb-specific B-cells induce T follicular helper cells (Tfh cells) to promote such protection, while depletion of B-cells or impairment of Tfh cells would lead to reduction of GrALT and bacterial growth (207). These findings now require further investigations in human TB and highlight the power of the NHP model to advance knowledge about human TB.

Protection of NHP models against disease progression provided by BCG depends on the route of vaccination, the NHP model, the Mtb challenge strain and dose. Overall, intradermal BCG vaccination of NHP provides variable protection against pulmonary TB which might reflect BCG efficacy in humans (221–224). BCG appears to be more efficient when delivered via aerosol in low dose (225, 226) or when administered intravenously (224, 227). NHP have also been used extensively to test safety and efficacy of preclinical and clinical TB vaccine candidates (228). The vaccine candidate M72/AS01E, which showed 54% efficacy in a human clinical phase 2 trial (229) also showed efficacy in the CM model (230).

Thus, the NHP model greatly contributes to the understanding of TB pathology, correlates of protection and vaccine efficacy. NHP recapitulate active, latent TB, and TB reactivation, and are amenable to longitudinal studies with serial sampling, including imaging, as well as study of TB comorbidities. Limitations of this model are the high housing costs, ethical concerns and shortage of RM and CM for experimental studies. In addition, the variability in route of

infection, inoculum, Mtb strain and NHP model lead to heterogenous outcomes, making it challenging to select the most appropriate experimental setup for translational studies.

3.1.2 Non-human primates in COVID-19

The ACE2 receptor for SARS-CoV-2 in NHP is identical to hACE2 (231), which is an advantage over other mammalian models. Pathogenesis, vaccine and therapeutic studies have been primarily performed in RM (232), CM (233) and AGM (234) almost simultaneously and immediately after the start of the pandemic. In general, experimental infection resembles mild and/or moderate COVID-19 in humans. It reflects a mild to moderate disease course (235) including lung pathology, viral replication in the upper respiratory tract, vascular involvement including thrombosis (232) and more severe clinical symptoms in aged NHP (236). A direct comparison of RM and CM after SARS-CoV-2 challenge has demonstrated that both models are comparable in the clinical course of infection, viral replication, as well as humoral and cellular immune response (237).

The moderate clinical course in the NHP model allows investigations regarding the correlates of protection. The acute phase and viral replication peak at around 2-4 days post infection and virus genomic RNA and clinical signs decline rapidly afterwards (238). The dynamics of the viral burden are mirrored by influx of neutrophils, dendritic cells and monocyte/macrophage populations into the lung which peak around day 3 and resolve one week later (239). The inflammatory response in the lung of NHP seems dominated by infiltrated monocyte-derived macrophages and is required for clearance of infected pneumocytes and inflammation afterwards (240). This indicates that in NHP the innate immune system likely contributes to the control of virus replication and resolution of inflammation. In line with this, the decline of virus replication and inflammation was associated with IFN-I activated myeloid cells before the induction of adaptive immunity (241). The established immunity protects RM against re-infection, which is similar to observations in humans (242). However, in this case it is most likely mediated by humoral and cellular responses in the upper respiratory tract (243, 244).

NHP have been extensively used as a preclinical model for all currently licensed vaccines against COVID-19 (245). In this context NHP proved to be relevant to investigate correlates of protection and mechanisms of action of COVID-19 vaccines. Systemic neutralizing antibody titers have been found to provide protection induced by the mRNA-1273 vaccine in non-human primates and humans (246, 247). In case of declining antibody titers over time SARS-CoV-2-specific CD8+ T-cell responses provide additional protective immunity and T-cell responses correlate with protection (level of SARS-CoV-2 sgRNA) in RM vaccinated with mRNA-1273 (246). In summary, NHP serve as an excellent model for moderate human COVID-19 cases as well as for investigations of correlates of protection and vaccine efficacy. However, cost restraints and ethical concerns along with a shortage of RM for experimental studies (248) require complementation by other models for studying COVID-19 pathology and for vaccine development.

3.2 Livestock models for TB

Large livestock species have been tested for their susceptibility for COVID-19. However, SARS-CoV-2 does not establish productive infection, nor does it disseminate in farm species such as cattle, goats and pigs (249). In contrast, livestock species are natural hosts and are therefore used as models for human TB. While Mtb is a human-adapted strain, other members of this family such as *M. orygis*, *M. caprae* and *M. bovis* are zoonotic pathogens. The main reservoir for these MTBC members are livestock species, including cattle, goats and pigs (250–252). However, these bacteria can infect humans and cause undistinguishable pathology compared to Mtb-driven disease, yet more often extra-pulmonary disease (253, 254). Of note, Mtb can infect livestock, for instance cattle, but usually does not induce a comparable pathology. Especially under experimental conditions cattle, goats and pigs can eradicate Mtb (255–257). Therefore, livestock species may serve as a model for human TB to investigate pathology (e.g. *M. bovis*) and correlates of protection (Mtb).

Natural MTBC infections in cattle, goats and pigs cause granulomas of all stages as described in humans, including necrotic lesions containing extracellular mycobacteria (256, 258, 259), and well-contained fibrotic encapsulated granulomas (260). *M. bovis*-induced granulomas in cattle are characterized by a strong expansion of IFN-γ-producing CD4+ T-cells and *M. bovis*-specific B lymphocytes (261, 262). Like in humans, *M. bovis*-induced activation of CD8+ T cells seems low compared to CD4+ T cells, but their presence might support Th1 response (263). The lesions developed in minipigs encompass caseous, fibrotic to calcified granulomas within the lungs and lymph nodes. Granulomas progress to encapsulation in pigs. This fibrous cuff develops in close proximity to the fibrotic capsule which anatomically limits the lung lobules and seems to contribute to the containment of infection (260). Thus, the lobular partitioning of the lung which is seen in livestock and in NHP, but not in rodents, may significantly restrict bacillary dissemination. Of note, in pigs, bacilli can be transmitted from infected to naïve animals, possibly due to development of cavities (264). Tissue features and pathogen transmissibility underscore the value of pigs for transmission studies (265).

In all species, macrophages and their precursors (e.g. monocytes) are the main intracellular niche for *M. bovis* or Mtb (266). Bovine monocytes show functional and developmental similarities to monocyte subsets in humans (267). In line with monocyte analogies in man and cattle, bovine monocyte-derived macrophages are a niche for intracellular growth of *M. bovis*, respond with a pro-inflammatory response and contribute to early granuloma formation (268, 269). Likewise, neutrophils have been found in humans, mice, and cattle to be recruited early during infection with MTBC bacteria (270). Some anti-mycobacterial defense mechanisms might be species-specific with bovine myeloid cells being equipped with a high number of antimicrobial peptides, variable granules and pattern recognition receptors (PRRs) (271).

Experimental infection of cattle with *M. bovis* leads to an early development of pulmonary lesions and development of necrotic granulomas rich of bacteria, neutrophils and giant cells already 30

days post challenge (272). However, progression to clinical disease might take several years (273). Whether *M. bovis* becomes latent during this time and can be reactivated like in humans is not well understood (274). Strikingly, experimental infection of pigs, goats and cattle with Mtb results in recovery of low bacterial numbers and Mtb-associated lesions from infected animals (255–257). These findings indicate that Mtb is attenuated in other species. In pigs, using a high dose i.v. challenge model, induction of systemic IFN-γ responses was similar in *M. bovis* versus Mtb infected pigs suggesting that the abundance of Th1 responses does not correlate with disease outcome (257, 275). Strong Th1 responses also have been observed in miniature pigs aerogenically challenged with Mtb (260, 265). Systemic delivery of *M. bovis* results in early onset of clinical disease in piglets and development of TB granulomas in the wall of the meningeal vessels (275). Occurrence of brain pathology makes piglets appealing for modeling childhood meningeal TB, a disease form which is difficult to model in other experimental animals. The bovine immune system may tolerate low abundant Mtb or develop distinct T-cell responses against Mtb to restrict its replication. For example, T-cell responses against the Mtb/*M. bovis* antigen Rv3879c have been only detected in *M. bovis*-infected, but not in Mtb-infected cattle. This supports the hypothesis that the T-cell repertoire could differ and therefore also recognition and/or activation of infected macrophages by CD4+ T-cells (255, 276). Host tropism and lack of adaptation to ruminants likely confer to Mtb a limited replication advantage, and presumably immune-competent cattle and other mammalian species are dead-end hosts eliminating the human-adapted Mtb. Resistance of cattle to Mtb may also rely on differences in very early responses of lung cells to Mtb versus *M. bovis*. Variability in activation of the cytosolic DNA-sensing pathways (277) and subsequent IFN-I responses (278), as well as regulation of cytokines or receptors for pathogens (279) have been reported. In addition, Mtb and *M. bovis* seem to reside in different compartments in bovine and human macrophages and only *M. bovis* and *M. bovis*-derived MPB70 trigger multinucleation of macrophages (269). However, roles of the multinucleated giant cells in the resistance phenotype and in other species, such as pigs and goats, remain to be demonstrated. The two MTBC members could trigger distinct responses in other myeloid cells, too, or may differently alter immune responses, cell networking or tissue remodeling. In-depth characterization of protective immune responses in cattle, goats, pigs as models for human TB could unmask novel correlates of protection in natural hosts and inform rational design of therapeutics in humans.

Vaccine efficacy testing in several studies with BCG in cattle was similarly inconclusive to efficacy studies in humans. Like in humans, BCG supports the induction of an IFN-γ and CD4+ T-cell response, however it does not seem to prevent granuloma formation and disease progression in cows compared to calves (280), bearing similarities to age-imprinted protection in humans. Recent studies in calves have unveiled that BCG delivery via aerosol trained circulating monocytes, yet left antimycobacterial responses of alveolar macrophages unchanged (281). BCG-driven *ex vivo* training of cattle monocyte is similar to human counterparts, however aerogenic immunization seems inefficient at remodeling mucosal immune cells. In pigs, a study from 1932 suggested that BCG vaccination induces small healing lesions, but only limited protection against infection with Mtb (282). Recent data from this model have highlighted its value specifically for understanding neonatal and juvenile responses to BCG. Piglets receiving BCG show development of effector CD4+ lymphocytes and maintain frequencies of CD8+ T-cells constant over time. However, higher abundancies of activated monocytes persist after Mtb challenge (264). Whether the monocyte changes are associated with trained innate immunity, as known in human neonates, and have a critical role in protection remains to be investigated. Likewise, there is limited experimental data using BCG vaccinated goats. However, one report suggested that BCG has only a limited protective efficacy after challenge with *M. caprae* (259). More recent advanced goat models using video endoscopy for infection via intrabronchial spray inoculation (283) have demonstrated the relevance and suitability of goats for vaccine studies using BCG and new clinical candidates (284, 285). Considering that BCG is the only licensed vaccine against TB it still remains the gold standard when testing new vaccine concepts. BCG vaccination in cattle, pigs and goats might reflect outcome in humans, and therefore these are useful models for novel preclinical vaccine concepts. However, further studies in large livestock species are required.

Ruminants and pigs bring benefits for TB studies by offering unique opportunities to investigate disease susceptibility and resistance in natural hosts. Whereas experimentation in cattle is difficult due to their size and the high expenses related to the maintenance of infected animals for longer periods of time in high containment laboratories, goats offer a viable alternative given their smaller size, lower costs and easier maintenance. The immunology toolbox for ruminants is still limited. Immunological reagents available for pigs exceed those for ruminants. Moreover, pigs are smaller, largely available and relatively easy to sample and handle. Availability of outbred and inbred lines, as well as recent advances in gene editing make them appealing for TB research. Apparent limitations due to inversion of lymph nodes or immunological peculiarities related to lymphocyte subsets are compensated by similarities with regard to the organization of the immune system in pigs and humans (286) and the extensive experience from other medical fields, such as transplantation. Furthermore, pigs could be exploited for neonatal immunology in the context of BCG immunization and offer an experimental model for meningeal TB.

4 Perspectives

Animal models offer opportunities to investigate host responses in great detail and under controlled conditions, considering the interlinked reactivity of various organs over time. Describing currently used animal models for TB and COVID-19 it becomes obvious that there is no ideal model (Figure 1). Each model comes with benefits and limitations and only their purpose-oriented utilization or usage of multiple models can adequately clarify a specific scientific question and advance interventions. Since both infections affect the respiratory tissue, cross-fertilization from established animal models for TB and COVID-19 appear natural. Certainly, advances in investigational methodologies, for instance for

analysis of immunity in Mtb-infected NHP, have been swiftly translated from TB to COVID-19 (241). For other models, such as mice, translation of models from TB to COVID-19 was limited due to abortive viral infection in standard laboratory strains. Nonetheless, we envisage that these models may contribute to the elucidation of counter-regulation in TB and COVID-19 as it happens in co-infection. Mtb may change the host landscape for SARS-CoV-2 and vice-versa, and such cross-regulations are critical for the co-infected human host. Regarding interventions, the extensive expertise of BCG in pre-clinical research has paved the way for understanding whether its heterologous effects contribute to protection against SARS-CoV-2. Importantly, knowledge gain from coinfection studies or the value of BCG-triggered trained immunity for an emerging viral disease, notably COVID-19, may be valid for other pneumonias and could serve for rapid action in case of a future pandemic episode.

Animal models have been employed to decipher effects of SARS-CoV-2/Mtb coinfection, which is critical because both pathogens persist in the human and wildlife populations. Natural infections with each pathogen currently have been reported in certain species, although coinfection has been evaluated solely for humans (Table 2). Concerns about the severity of COVID-19 in the LTBI population or the risk of TB reactivation subsequent to infection with SARS-CoV-2 were raised shortly after COVID-19 emergence, and co-infection has been associated with higher mortality rates (299–301). Studies analyzing human cohorts report that subclinical and active TB may increase the risk of severe COVID-19 due to circulating myeloid subpopulations found in severe COVID-19 or impaired antiviral activity (12, 302, 303). Regarding effects of the viral pathogen on the control of bacterial replication, SARS-CoV-2 leads to reduced frequencies of Mtb-specific CD4+ T-cells which may facilitate TB progression (304). Of note, dysregulation of IFN-I is observed in both infections (305–307). The relevance of such cellular subsets and phenotypes as well as of the immune pathways relevant for TB outcome has been demonstrated in animal models (74, 308, 309). In line with clinical presumptions, the murine hepatitis virus, which is a mouse-adapted coronavirus, reactivates Mtb in a dormant mouse model using a streptomycin-auxotrophic mutant bacterial strain (310). Co-infection studies in mice addressing effects of TB on SARS-CoV-2 infection outcome so far have led to inconclusive results. K18-hACE2 mice chronically infected with Mtb limit SARS-CoV-2 loads (311) or become resistant to SARS-CoV-2 infection, presumably due to the strong Th1 milieu (312). These disparities may be due to imperfect modeling of the co-infection in the mouse and also to the spectra of disease for each infection. Thus, experimental co-infection of natural hosts of both pathogens might be more suitable for such investigations (Table 2). Since Mtb and SARS-CoV-2 infect multiple species aside from their host of choice, attempts to model them in other animal models or multiple species could be helpful. Following this approach, epidemiological observations from the human population could be explored to define molecular determinants controlling inflammation and cell death pathways which may co-regulate host-responses to both pathogens (313). A priority should be the elaboration of solutions for bottlenecks in mirroring diseases at various stages and certainly this becomes complex in co-infection and co-morbidity scenarios which are often associated with TB and COVID-19.

Modeling of potential unspecific benefits of BCG in surrogate animals generally has produced consistent results. Whereas systemic BCG protects mice from influenza A virus lethality (314), it does not protect hamsters from SARS-CoV-2 and its effects were inconsistent in K18-hACE2 transgenic mice (314, 315). The disparities in mice may stem from the usage of various BCG strains and variable study protocols. Aerosol delivery of BCG leaves the course of SARS-CoV-2 infection unchanged in RM (316). These results are overall supportive of observations from a large clinical trial: BCG (Denmark strain) did not reduce the risk of COVID-19 (317). Thus, the power of employing multiple animal models for devising interventions has been further substantiated in the context of BCG immunization for heterologous protection and represents a lesson learned from the COVID-19 pandemic.

For both TB and COVID-19 there are still knowledge gaps which should be addressed using experimentation in animal models. Current models do not fully allow to define determinants of TB latency, triggers of disseminated disease, mechanisms underlying tolerance to disease and molecular regulators of TB reactivation. Similarly, understanding factors which drive the development of long COVID-19, as well as multisystemic inflammatory syndrome in children (MIS-C), is a

TABLE 2 Currently known hosts with the potential of coinfection.

Species	MTBC strain	SARS-CoV-2 strain	References
Humans and non-human primates	*M. bovis* and Mtb	Ancestral and all variants	Hlavsa et al., 2008 (287) Wu et al., 2020 (288) Lerche et al., 2008 (289) Qiu et al., 2023 (290)
White-tailed deer	*M. bovis*	Alpha, Delta, Omicron	Vandergrift et al., 2022 (291) Marques et al., 2022 (292)
Minks, ferrets	*M. bovis*	Ancestral	Virtanen et al., 2022 (293) Shi et al., 2020 (294) Gupta et al., 2022 (295) Oude Munnink et al., 2021 (296)
Felidae	*M. bovis*	Ancestral	Giraldo-Ramirez et al., 2021 (297) Miller et al., 2019 (298)

The animal species and families from which virulent mycobacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC) as well as SARS-CoV-2 have been isolated are included. Details on the MTBC and virus strain and references reporting detection of the pathogen in respective animal species are provided.

priority. In the context of disease resolution, both for TB and COVID-19 reparatory processes as well as regulators of tissue sequelae remain largely elusive. Furthermore, the cellular and molecular basis of TB vaccine efficacy in young individuals, particularly neonates and infants are still not understood. Addressing these topics requires fit-for-purpose models and likely cross-species analysis. The multi-host disease feature and lung localization in both infections, along with the recent progress in single-cell technologies offer opportunities. The scientific community has initiated parallel deep profiling in multiple experimental models, and guidance for respiratory infections has recently been provided (318). Such agnostic approaches can be harnessed for the development of therapies and vaccines. Fit-for-purpose examples of animal models are juvenile pigs for early life conditions such as MIS-C and meningeal and miliary TB. Studies in juvenile pigs could also model vaccination in human neonates. Pigs already have provided robust results for disease pathogenesis, unveiling subtissular localization of virus-specific $CD8^+$ resident memory T-cells (319) and interventions, for instance mode of action of monoclonal antibodies (320) or various vaccine platforms (321), for flu. Pigs could also be a model for acute coronavirus infection (322). For the study of chronic COVID-19, engraftment of mice with human hematopoietic and stem cells (323) offers an alternative as these animals show lung pathology and fibrosis observed in severely ill patients.

Development of new animal models could clarify questions which cannot be addressed using available models. Acknowledging translatability issues from mice to humans, novel "wildlings" mice which combine the natural microbiome with genetic tractability of C57BL/6 mice emphasize the validity of this approach for reproducibility and translatability of immunological findings in biomedical studies (324). This model has not been applied yet in infectious disease research, but given the universality of housing mice it could be readily implemented. Studies of pathogen transmission are key for TB and COVID-19, however reliable and accessible animal models are scarce. The ferret is particularly suitable due to the anatomy of larger intranasal structures (325). SARS-CoV-2 infection foci with oligofocal pattern have been detected using a 3D microscopy approach in ferret conchae (326). Moreover, in characterizing SARS-CoV-2 variants of concern, it provides an additional model to investigate *in situ* viral competition (327) showing that, for instance Omicron BA.1 was no longer able to replicate in the presence of evolving variants (328). Recent studies have reported that ferrets successfully transmit Mtb and develop TB pathology (295), thus extending the model value of ferrets also to a bacterial respiratory infection. Housing and handling ferrets in high-containment laboratories requires adequate training and space, making experimentation feasible only at selected institutions. Other examples of novel model animals, particularly amenable to decipher disease tolerance, are bats. Bats harbor multiple viruses without showing signs of disease, and experimental challenge with SARS-CoV-2 has resulted in productive infection in the absence of disease (325). Understanding the basis of the resilience in bats could advance therapies, and high-end technologies have been applied recently to unmask the immune landscape in bats at steady state and during infection (329, 330). Access to bat colonies is restricted to only few research facilities worldwide and the value of bats does not rely in phenotyping a disease stage, but rather in recapitulating resilience in disease-free individuals. Thus, novel animal models with peculiar features are available for respiratory infections and the examples presented herein are not exhaustive. They could all contribute to uncovering the pathophysiology of maladaptive immune responses, including hyperinflammation and immunosuppression, as well as of the extensive lung destruction and dysfunction detected in TB and COVID-19.

In conclusion, for the understanding of infectious diseases as well as for testing of vaccines or therapeutics, targeted and well-considered use of animal models is still indispensable. It must be pointed out that it is essential to follow the 3R concepts to reduce, replace and refine usage of animals in experimental research. These ethical-driven approaches represent the foundation of animal experimentation around the world, and it is conceivable that in some cases newer systems, such as three-dimensional cell culture or organoids, will continue to proof themselves to be able to replace some of the animal testing. When considering two unrelated pathogens, as in our example with Mtb and SARS-CoV-2, it is noticeable that similar questions arise, which are then analyzed with the appropriate model in each case. Therefore, an important step is the selection of animal models to be used according to the available infrastructure, tools and scientific needs. However, common issues such as paucity of immunological reagents in non-murine models require solutions. Here, joint interdisciplinary (bacteriology and virology) and intersectoral (human and veterinary medicine) efforts are necessary to increment the value of non-conventional animal models and address societal needs, and state-of-the-art single cell technologies offer opportunities.

Author contributions

Conceived and designed the paper: BC and AD. Drafted the paper: BC, MBa, DH, MBe, AD. Revised the paper for critically intellectual content and review final manuscript: BC, MBa, DH, MBe, AD.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Cate TR, Couch RB. Live influenza A/Victoria/75 (H3N2) virus vaccines: reactogenicity, immunogenicity, and protection against wild-type virus challenge. *Infect Immun* (1982) 38(1):141–6. doi: 10.1128/iai.38.1.141-146.1982

2. Chulay JD, Schneider I, Cosgriff TM, Hoffman SL, Ballou WR, Quakyi IA, et al. Malaria transmitted to humans by mosquitoes infected from cultured Plasmodium falciparum. *Am J Trop Med Hyg* (1986) 35(1):66–8. doi: 10.4269/ajtmh.1986.35.66

3. Killingley B, Mann AJ, Kalinova M, Boyers A, Goonawardane N, Zhou J, et al. Safety, tolerability and viral kinetics during SARS-CoV-2 human challenge in young adults. *Nat Med* (2022) 28(5):1031–41. doi: 10.1038/s41591-022-01780-9

4. Minassian AM, Satti I, Poulton ID, Meyer J, Hill AV, McShane H. A human challenge model for Mycobacterium tuberculosis using Mycobacterium bovis bacille Calmette-Guerin. *J Infect Dis* (2012) 205(7):1035–42. doi: 10.1093/infdis/jis012

5. Davids M, Pooran A, Hermann C, Mottay L, Thompson F, Cardenas J, et al. A human lung challenge model to evaluate the safety and immunogenicity of PPD and live bacillus calmette-guerin. *Am J Respir Crit Care Med* (2020) 201(10):1277–91. doi: 10.1164/rccm.201908-1580OC

6. Gimenes Lima G, Portilho AI, De Gaspari E. Animal models to test SARS-CoV-2 vaccines: which ones are in use and future expectations. *Pathogens* (2022) 12(1). doi: 10.3390/pathogens12010020

7. Clever S, Volz A. Mouse models in COVID-19 research: analyzing the adaptive immune response. *Med Microbiol Immunol* (2023) 212(2):165–83. doi: 10.1007/s00430-022-00735-8

8. Team W. *Global Tubercolosis Report 2022*. Geneva: World Health Organization (2022). p. 68.

9. Programme WHOGT. *TB : a global emergency, WHO report on the TB epidemic*. Geneva: World Health Organization (1994).

10. Eurosurveillance Editorial Team. Note from the editors: World Health Organization declares novel coronavirus (2019-nCoV) sixth public health emergency of international concern. *Euro Surveill* (2020) 25(5):200131e. doi: 10.2807/1560-7917.ES.2020.25.5.200131e

11. Snouwaert JN, Jania LA, Nguyen T, Martinez DR, Schafer A, Catanzaro NJ, et al. Human ACE2 expression, a major tropism determinant for SARS-CoV-2, is regulated by upstream and intragenic elements. *PLoS Pathog* (2023) 19(2):e1011168. doi: 10.1371/journal.ppat.1011168

12. Sheerin D, Abhimanyu, Peton N, Vo W, Allison CC, Wang X, et al. Immunopathogenic overlap between COVID-19 and tuberculosis identified from transcriptomic meta-analysis and human macrophage infection. *iScience* (2022) 25(6):104464. doi: 10.1016/j.isci.2022.104464

13. Koch R. The etiology of tuberculosis by Dr. Robert Koch. From the Berliner Klinische Wochenschrift, Volume 19 (1882). *Zentralbl Bakteriol Mikrobiol Hyg A Med Mikrobiol Infekt Parasitol* (*1982*) 251(3):287-96.

14. Cooper AM. Mouse model of tuberculosis. *Cold Spring Harb Perspect Med* (2014) 5(2):a018556. doi: 10.1101/cshperspect.a018556

15. Munoz-Fontela C, Dowling WE, Funnell SGP, Gsell PS, Riveros-Balta AX, Albrecht RA, et al. Animal models for COVID-19. *Nature* (2020) 586(7830):509–15. doi: 10.1038/s41586-020-2787-6

16. Rydell-Tormanen K, Johnson JR. The applicability of mouse models to the study of human disease. *Methods Mol Biol* (2019) 1940:3–22. doi: 10.1007/978-1-4939-9086-3_1

17. Bru A, Cardona PJ. Mathematical modeling of tuberculosis bacillary counts and cellular populations in the organs of infected mice. *PLoS One* (2010) 5(9):e12985. doi: 10.1371/journal.pone.0012985

18. Kramnik I, Demant P, Bloom BB. Susceptibility to tuberculosis as a complex genetic trait: analysis using recombinant congenic strains of mice. *Novartis Found Symp* (1998) 217:120–31. doi: 10.1002/0470846526.ch9

19. Orme IM. Aging and immunity to tuberculosis: increased susceptibility of old mice reflects a decreased capacity to generate mediator T lymphocytes. *J Immunol* (1987) 138(12):4414–8. doi: 10.4049/jimmunol.138.12.4414

20. Harrison DE, Astle CM, Niazi MKK, Major S, Beamer GL. Genetically diverse mice are novel and valuable models of age-associated susceptibility to Mycobacterium tuberculosis. *Immun Ageing* (2014) 11(1):24. doi: 10.1186/s12979-014-0024-6

21. North RJ, Jung YJ. Immunity to tuberculosis. *Annu Rev Immunol* (2004) 22:599–623. doi: 10.1146/annurev.immunol.22.012703.104635

22. North RJ. Mycobacterium tuberculosis is strikingly more virulent for mice when given via the respiratory than via the intravenous route. *J Infect Dis* (1995) 172(6):1550–3. doi: 10.1093/infdis/172.6.1550

23. Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, Murray PJ, et al. Hypervirulent M. tuberculosis W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. *J Interferon Cytokine Res* (2005) 25(11):694–701. doi: 10.1089/jir.2005.25.694

24. Donovan ML, Bielefeldt-Ohmann H, Rollo RF, McPherson SJ, Schultz TE, Mori G, et al. Distinct contributions of the innate immune receptors TLR2 and RP105 to formation and architecture of structured lung granulomas in mice infected with Mycobacterium tuberculosis. *Immunology* (2023) 169(1):13–26. doi: 10.1111/imm.13606

25. Zha BS, Desvignes L, Fergus TJ, Cornelius A, Cheng TY, Moody DB, et al. Bacterial strain-dependent dissociation of cell recruitment and cell-to-cell spread in early M. *tuberculosis Infect mBio* (2022) 13(3):e0133222. doi: 10.1128/mbio.01332-22

26. Lovey A, Verma S, Kaipilyawar V, Ribeiro-Rodrigues R, Husain S, Palaci M, et al. Early alveolar macrophage response and IL-1R-dependent T cell priming determine transmissibility of Mycobacterium tuberculosis strains. *Nat Commun* (2022) 13(1):884. doi: 10.1038/s41467-022-28506-2

27. Sousa J, Ca B, Maceiras AR, Simoes-Costa L, Fonseca KL, Fernandes AI, et al. Mycobacterium tuberculosis associated with severe tuberculosis evades cytosolic surveillance systems and modulates IL-1beta production. *Nat Commun* (2020) 11(1):1949. doi: 10.1038/s41467-020-15832-6

28. Beisiegel M, Kursar M, Koch M, Loddenkemper C, Kuhlmann S, Zedler U, et al. Combination of host susceptibility and virulence of Mycobacterium tuberculosis determines dual role of nitric oxide in the protection and control of inflammation. *J Infect Dis* (2009) 199(8):1222–32. doi: 10.1086/597421

29. Pym AS, Brodin P, Brosch R, Huerre M, Cole ST. Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines Mycobacterium bovis BCG and Mycobacterium microti. *Mol Microbiol* (2002) 46(3):709–17. doi: 10.1046/j.1365-2958.2002.03237.x

30. Brodin P, Majlessi L, Marsollier L, de Jonge MI, Bottai D, Demangel C, et al. Dissection of ESAT-6 system 1 of Mycobacterium tuberculosis and impact on immunogenicity and virulence. *Infect Immun* (2006) 74(1):88–98. doi: 10.1128/IAI.74.1.88-98.2006

31. Simeone R, Sayes F, Song O, Groschel MI, Brodin P, Brosch R, et al. Cytosolic access of Mycobacterium tuberculosis: critical impact of phagosomal acidification control and demonstration of occurrence *in vivo*. *PLoS Pathog* (2015) 11(2):e1004650. doi: 10.1371/journal.ppat.1004650

32. Gilbertson SE, Walter HC, Gardner K, Wren SN, Vahedi G, Weinmann AS. Topologically associating domains are disrupted by evolutionary genome rearrangements forming species-specific enhancer connections in mice and humans. *Cell Rep* (2022) 39(5):110769. doi: 10.1016/j.celrep.2022.110769

33. Smith CM, Proulx MK, Lai R, Kiritsy MC, Bell TA, Hock P, et al. Functionally overlapping variants control tuberculosis susceptibility in collaborative cross mice. *mBio* (2019) 10(6). doi: 10.1128/mBio.02791-19

34. Smith CM, Proulx MK, Olive AJ, Laddy D, Mishra BB, Moss C, et al. Tuberculosis susceptibility and vaccine protection are independently controlled by host genotype. *mBio* (2016) 7(5). doi: 10.1128/mBio.01516-16

35. Kurtz SL, Rossi AP, Beamer GL, Gatti DM, Kramnik I, Elkins KL. The diversity outbred mouse population is an improved animal model of vaccination against tuberculosis that reflects heterogeneity of protection. *mSphere* (2020) 5(2). doi: 10.1128/mSphere.00097-20

36. Niazi MK, Dhulekar N, Schmidt D, Major S, Cooper R, Abeijon C, et al. Lung necrosis and neutrophils reflect common pathways of susceptibility to Mycobacterium tuberculosis in genetically diverse, immune-competent mice. *Dis Model Mech* (2015) 8(9):1141–53. doi: 10.1242/dmm.020867

37. Gopal R, Monin L, Torres D, Slight S, Mehra S, McKenna KC, et al. S100A8/A9 proteins mediate neutrophilic inflammation and lung pathology during tuberculosis. *Am J Respir Crit Care Med* (2013) 188(9):1137–46. doi: 10.1164/rccm.201304-0803OC

38. Koyuncu D, Niazi MKK, Tavolara T, Abeijon C, Ginese ML, Liao Y, et al. CXCL1: A new diagnostic biomarker for human tuberculosis discovered using Diversity Outbred mice. *PLoS Pathog* (2021) 17(8):e1009773. doi: 10.1371/journal.ppat.1009773

39. Arrey F, Lowe D, Kuhlmann S, Kaiser P, Moura-Alves P, Krishnamoorthy G, et al. Humanized mouse model mimicking pathology of human tuberculosis for *in vivo* evaluation of drug regimens. *Front Immunol* (2019) 10:89. doi: 10.3389/fimmu.2019.00089

40. Grover A, Troy A, Rowe J, Troudt JM, Creissen E, McLean J, et al. Humanized NOG mice as a model for tuberculosis vaccine-induced immunity: a comparative analysis with the mouse and Guinea pig models of tuberculosis. *Immunology* (2017) 152(1):150–62. doi: 10.1111/imm.12756

41. Hunter R, Actor J. The pathogenesis of post-primary tuberculosis. A game changer for vaccine development. *Tuberculosis (Edinb)* (2019) 116S:S114–S7. doi: 10.1016/j.tube.2019.04.018

42. Leemans JC, Juffermans NP, Florquin S, van Rooijen N, Vervoordeldonk MJ, Verbon A, et al. Depletion of alveolar macrophages exerts protective effects in pulmonary tuberculosis in mice. *J Immunol* (2001) 166(7):4604–11. doi: 10.4049/jimmunol.166.7.4604

43. Huang L, Nazarova EV, Tan S, Liu Y, Russell DG. Growth of Mycobacterium tuberculosis *in vivo* segregates with host macrophage metabolism and ontogeny. *J Exp Med* (2018) 215(4):1135–52. doi: 10.1084/jem.20172020

44. Rothchild AC, Olson GS, Nemeth J, Amon LM, Mai D, Gold ES, et al. Alveolar macrophages generate a noncanonical NRF2-driven transcriptional response to Mycobacterium tuberculosis *in vivo*. *Sci Immunol* (2019) 4(37). doi: 10.1126/sciimmunol.aaw6693

45. Cohen SB, Gern BH, Delahaye JL, Adams KN, Plumlee CR, Winkler JK, et al. Alveolar macrophages provide an early Mycobacterium tuberculosis niche and initiate

dissemination. *Cell Host Microbe* (2018) 24(3):439–46 e4. doi: 10.1016/j.chom.2018.08.001

46. Zheng W, Chang IC, Limberis J, Budzik JM, Zha BS, Howard Z, et al. Mycobacterium tuberculosis resides in lysosome-poor monocyte-derived lung cells during persistent infection. *bioRxiv* (2023). doi: 10.21203/rs.3.rs-3049913/v1

47. Lee J, Boyce S, Powers J, Baer C, Sassetti CM, Behar SM. CD11cHi monocyte-derived macrophages are a major cellular compartment infected by Mycobacterium tuberculosis. *PLoS Pathog* (2020) 16(6):e1008621. doi: 10.1371/journal.ppat.1008621

48. Blomgran R, Ernst JD. Lung neutrophils facilitate activation of naive antigen-specific CD4+ T cells during Mycobacterium tuberculosis infection. *J Immunol* (2011) 186(12):7110–9. doi: 10.4049/jimmunol.1100001

49. Repasy T, Lee J, Marino S, Martinez N, Kirschner DE, Hendricks G, et al. Intracellular bacillary burden reflects a burst size for Mycobacterium tuberculosis *in vivo*. *PLoS Pathog* (2013) 9(2):e1003190. doi: 10.1371/journal.ppat.1003190

50. Wolf AJ, Desvignes L, Linas B, Banaiee N, Tamura T, Takatsu K, et al. Initiation of the adaptive immune response to Mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs. *J Exp Med* (2008) 205(1):105–15. doi: 10.1084/jem.20071367

51. Gallegos AM, Pamer EG, Glickman MS. Delayed protection by ESAT-6-specific effector CD4+ T cells after airborne M. *tuberculosis infect J Exp Med* (2008) 205 (10):2359–68. doi: 10.1084/jem.20080353

52. Sakai S, Kauffman KD, Schenkel JM, McBerry CC, Mayer-Barber KD, Masopust D, et al. Cutting edge: control of Mycobacterium tuberculosis infection by a subset of lung parenchyma-homing CD4 T cells. *J Immunol* (2014) 192(7):2965–9. doi: 10.4049/jimmunol.1400019

53. Scanga CA, Mohan VP, Yu K, Joseph H, Tanaka K, Chan J, et al. Depletion of CD4(+) T cells causes reactivation of murine persistent tuberculosis despite continued expression of interferon gamma and nitric oxide synthase 2. *J Exp Med* (2000) 192 (3):347–58. doi: 10.1084/jem.192.3.347

54. Lu YJ, Barreira-Silva P, Boyce S, Powers J, Cavallo K, Behar SM. CD4 T cell help prevents CD8 T cell exhaustion and promotes control of Mycobacterium tuberculosis infection. *Cell Rep* (2021) 36(11):109696. doi: 10.1016/j.celrep.2021.109696

55. Reiley WW, Calayag MD, Wittmer ST, Huntington JL, Pearl JE, Fountain JJ, et al. ESAT-6-specific CD4 T cell responses to aerosol Mycobacterium tuberculosis infection are initiated in the mediastinal lymph nodes. *Proc Natl Acad Sci USA* (2008) 105(31):10961–6. doi: 10.1073/pnas.0801496105

56. Plumlee CR, Duffy FJ, Gern BH, Delahaye JL, Cohen SB, Stoltzfus CR, et al. Ultra-low dose aerosol infection of mice with Mycobacterium tuberculosis more closely models human tuberculosis. *Cell Host Microbe* (2021) 29(1):68–82 e5. doi: 10.1016/j.chom.2020.10.003

57. Plumlee CR, Barrett HW, Shao DE, Lien KA, Cross LM, Cohen SB, et al. Assessing vaccine-mediated protection in an ultra-low dose Mycobacterium tuberculosis murine model. *bioRxiv* (2023). doi: 10.1101/2023.03.22.533820

58. Choreno-Parra JA, Bobba S, Rangel-Moreno J, Ahmed M, Mehra S, Rosa B, et al. Mycobacterium tuberculosis HN878 infection induces human-like B-cell follicles in mice. *J Infect Dis* (2020) 221(10):1636–46. doi: 10.1093/infdis/jiz663

59. Kupz A, Zedler U, Staber M, Kaufmann SH. A mouse model of latent tuberculosis infection to study intervention strategies to prevent reactivation. *PLoS One* (2016) 11(7):e0158849. doi: 10.1371/journal.pone.0158849

60. Nemeth J, Olson GS, Rothchild AC, Jahn AN, Mai D, Duffy FJ, et al. Contained Mycobacterium tuberculosis infection induces concomitant and heterologous protection. *PLoS Pathog* (2020) 16(7):e1008655. doi: 10.1371/journal.ppat.1008655

61. Harper J, Skerry C, Davis SL, Tasneen R, Weir M, Kramnik I, et al. Mouse model of necrotic tuberculosis granulomas develops hypoxic lesions. *J Infect Dis* (2012) 205 (4):595–602. doi: 10.1093/infdis/jir786

62. Marzo E, Vilaplana C, Tapia G, Diaz J, Garcia V, Cardona PJ. Damaging role of neutrophilic infiltration in a mouse model of progressive tuberculosis. *Tuberculosis (Edinb)* (2014) 94(1):55–64. doi: 10.1016/j.tube.2013.09.004

63. Yan BS, Pichugin AV, Jobe O, Helming L, Eruslanov EB, Gutierrez-Pabello JA, et al. Progression of pulmonary tuberculosis and efficiency of bacillus Calmette-Guerin vaccination are genetically controlled via a common sst1-mediated mechanism of innate immunity. *J Immunol* (2007) 179(10):6919–32. doi: 10.4049/jimmunol.179.10.6919

64. Pan H, Yan BS, Rojas M, Shebzukhov YV, Zhou H, Kobzik L, et al. Ipr1 gene mediates innate immunity to tuberculosis. *Nature* (2005) 434(7034):767–72. doi: 10.1038/nature03419

65. Moreira-Teixeira L, Stimpson PJ, Stavropoulos E, Hadebe S, Chakravarty P, Ioannou M, et al. Type I IFN exacerbates disease in tuberculosis-susceptible mice by inducing neutrophil-mediated lung inflammation and NETosis. *Nat Commun* (2020) 11(1):5566. doi: 10.1038/s41467-020-19412-6

66. Ulrichs T, Kosmiadi GA, Jorg S, Pradl L, Titukhina M, Mishenko V, et al. Differential organization of the local immune response in patients with active cavitary tuberculosis or with nonprogressive tuberculoma. *J Infect Dis* (2005) 192(1):89–97. doi: 10.1086/430621

67. Panteleev AV, Nikitina IY, Burmistrova IA, Kosmiadi GA, Radaeva TV, Amansahedov RB, et al. Severe tuberculosis in humans correlates best with neutrophil abundance and lymphocyte deficiency and does not correlate with antigen-specific CD4 T-cell response. *Front Immunol* (2017) 8:963. doi: 10.3389/fimmu.2017.00963

68. McCaffrey EF, Donato M, Keren L, Chen Z, Delmastro A, Fitzpatrick MB, et al. The immunoregulatory landscape of human tuberculosis granulomas. *Nat Immunol* (2022) 23(2):318–29. doi: 10.1038/s41590-021-01121-x

69. Cardona PJ, Gordillo S, Diaz J, Tapia G, Amat I, Pallares A, et al. Widespread bronchogenic dissemination makes DBA/2 mice more susceptible than C57BL/6 mice to experimental aerosol infection with Mycobacterium tuberculosis. *Infect Immun* (2003) 71(10):5845–54. doi: 10.1128/IAI.71.10.5845-5854.2003

70. Keller C, Hoffmann R, Lang R, Brandau S, Hermann C, Ehlers S. Genetically determined susceptibility to tuberculosis in mice causally involves accelerated and enhanced recruitment of granulocytes. *Infect Immun* (2006) 74(7):4295–309. doi: 10.1128/IAI.00057-06

71. Leepiyasakulchai C, Ignatowicz L, Pawlowski A, Kallenius G, Skold M. Failure to recruit anti-inflammatory CD103+ dendritic cells and a diminished CD4+ Foxp3+ regulatory T cell pool in mice that display excessive lung inflammation and increased susceptibility to Mycobacterium tuberculosis. *Infect Immun* (2012) 80(3):1128–39. doi: 10.1128/IAI.05552-11

72. Lavebratt C, Apt AS, Nikonenko BV, Schalling M, Schurr E. Severity of tuberculosis in mice is linked to distal chromosome 3 and proximal chromosome 9. *J Infect Dis* (1999) 180(1):150–5. doi: 10.1086/314843

73. Yeremeev V, Linge I, Kondratieva T, Apt A. Neutrophils exacerbate tuberculosis infection in genetically susceptible mice. *Tuberculosis (Edinb)* (2015) 95(4):447–51. doi: 10.1016/j.tube.2015.03.007

74. Dorhoi A, Yeremeev V, Nouailles G, Weiner J3rd, Jorg S, Heinemann E, et al. Type I IFN signaling triggers immunopathology in tuberculosis-susceptible mice by modulating lung phagocyte dynamics. *Eur J Immunol* (2014) 44(8):2380–93. doi: 10.1002/eji.201344219

75. Medina E, North RJ. The Bcg gene (Nramp1) does not determine resistance of mice to virulent Mycobacterium tuberculosis. *Ann N Y Acad Sci* (1996) 797:257–9. doi: 10.1111/j.1749-6632.1996.tb52970.x

76. Zhang L, Jiang X, Pfau D, Ling Y, Nathan CF. Type I interferon signaling mediates Mycobacterium tuberculosis-induced macrophage death. *J Exp Med* (2021) 218(2). doi: 10.1084/jem.20200887

77. Brandenburg J, Marwitz S, Tazoll SC, Waldow F, Kalsdorf B, Vierbuchen T, et al. WNT6/ACC2-induced storage of triacylglycerols in macrophages is exploited by Mycobacterium tuberculosis. *J Clin Invest* (2021) 131(16). doi: 10.1172/JCI141833

78. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. *J Exp Med* (1993) 178(6):2249–54. doi: 10.1084/jem.178.6.2249

79. Bean AG, Roach DR, Briscoe H, France MP, Korner H, Sedgwick JD, et al. Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol Mycobacterium tuberculosis infection, which is not compensated for by lymphotoxin. *J Immunol* (1999) 162(6):3504–11. doi: 10.4049/jimmunol.162.6.3504

80. Allie N, Grivennikov SI, Keeton R, Hsu NJ, Bourigault ML, Court N, et al. Prominent role for T cell-derived tumour necrosis factor for sustained control of Mycobacterium tuberculosis infection. *Sci Rep* (2013) 3:1809. doi: 10.1038/srep01809

81. Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinical features of inborn errors of IFN-gamma immunity. *Semin Immunol* (2014) 26(6):454–70. doi: 10.1016/j.smim.2014.09.008

82. Dorhoi A, Kaufmann SH. Tumor necrosis factor alpha in mycobacterial infection. *Semin Immunol* (2014) 26(3):203–9. doi: 10.1016/j.smim.2014.04.003

83. Nandi B, Behar SM. Regulation of neutrophils by interferon-gamma limits lung inflammation during tuberculosis infection. *J Exp Med* (2011) 208(11):2251–62. doi: 10.1084/jem.20110919

84. Feng CG, Kaviratne M, Rothfuchs AG, Cheever A, Hieny S, Young HA, et al. NK cell-derived IFN-gamma differentially regulates innate resistance and neutrophil response in T cell-deficient hosts infected with Mycobacterium tuberculosis. *J Immunol* (2006) 177(10):7086–93. doi: 10.4049/jimmunol.177.10.7086

85. Dorhoi A, Iannaccone M, Farinacci M, Fae KC, Schreiber J, Moura-Alves P, et al. MicroRNA-223 controls susceptibility to tuberculosis by regulating lung neutrophil recruitment. *J Clin Invest* (2013) 123(11):4836–48. doi: 10.1172/JCI67604

86. Fremond CM, Yeremeev V, Nicolle DM, Jacobs M, Quesniaux VF, Ryffel B. Fatal Mycobacterium tuberculosis infection despite adaptive immune response in the absence of MyD88. *J Clin Invest* (2004) 114(12):1790–9. doi: 10.1172/JCI200421027

87. Dorhoi A, Desel C, Yeremeev V, Pradl L, Brinkmann V, Mollenkopf HJ, et al. The adaptor molecule CARD9 is essential for tuberculosis control. *J Exp Med* (2010) 207(4):777–92. doi: 10.1084/jem.20090067

88. Cooper AM, Segal BH, Frank AA, Holland SM, Orme IM. Transient loss of resistance to pulmonary tuberculosis in p47(phox-/-) mice. *Infect Immun* (2000) 68 (3):1231–4. doi: 10.1128/IAI.68.3.1231-1234.2000

89. Olive AJ, Smith CM, Kiritsy MC, Sassetti CM. The phagocyte oxidase controls tolerance to Mycobacterium tuberculosis infection. *J Immunol* (2018) 201(6):1705–16. doi: 10.4049/jimmunol.1800202

90. Blumenthal A, Nagalingam G, Huch JH, Walker L, Guillemin GJ, Smythe GA, et al. M. tuberculosis induces potent activation of IDO-1, but this is not essential for the immunological control of infection. *PLoS One* (2012) 7(5):e37314. doi: 10.1371/journal.pone.0037314

91. Bustamante J, Arias AA, Vogt G, Picard C, Galicia LB, Prando C, et al. Germline CYBB mutations that selectively affect macrophages in kindreds with X-linked predisposition to tuberculous mycobacterial disease. *Nat Immunol* (2011) 12(3):213–21. doi: 10.1038/ni.1992

92. Lai R, Gong D, Williams T, Ogunsola AF, Cavallo K, Arlehamn CSL, et al. Host genetic background is a barrier to broadly effective vaccine protection: Relevance to BCG and Mycobacterium tuberculosis Infection. *bioRxiv* (2022). doi: 10.1101/2022.09.19.508548

93. Chen Q, Huang XY, Liu Y, Sun MX, Ji B, Zhou C, et al. Comparative characterization of SARS-CoV-2 variants of concern and mouse-adapted strains in mice. *J Med Virol* (2022) 94(7):3223–32. doi: 10.1002/jmv.27735

94. Chen RE, Winkler ES, Case JB, Aziati ID, Bricker TL, Joshi A, et al. *In vivo* monoclonal antibody efficacy against SARS-CoV-2 variant strains. *Nature* (2021) 596 (7870):103–8. doi: 10.1038/s41586-021-03720-y

95. Ying B, Whitener B, VanBlargan LA, Hassan AO, Shrihari S, Liang CY, et al. Protective activity of mRNA vaccines against ancestral and variant SARS-CoV-2 strains. *Sci Transl Med* (2022) 14(630):eabm3302. doi: 10.1126/scitranslmed.abm3302

96. Dinnon KH3rd, Leist SR, Schafer A, Edwards CE, Martinez DR, Montgomery SA, et al. A mouse-adapted model of SARS-CoV-2 to test CO7VID-19 countermeasures. *Nature* (2020) 586(7830):560–6. doi: 10.1038/s41586-020-2708-8

97. Winkler ES, Chen RE, Alam F, Yildiz S, Case JB, Uccellini MB, et al. SARS-CoV-2 causes lung infection without severe disease in human ACE2 knock-in mice. *J Virol* (2022) 96(1):e0151121. doi: 10.1128/JVI.01511-21

98. Zeiss CJ, Compton S, Veenhuis RT. Animal models of COVID-19. I. Comparative virology and disease pathogenesis. *ILAR J* (2021) 62(1-2):35–47. doi: 10.1093/ilar/ilab007

99. Dong W, Mead H, Tian L, Park JG, Garcia JI, Jaramillo S, et al. The K18-human ACE2 transgenic mouse model recapitulates non-severe and severe COVID-19 in response to an infectious dose of the SARS-CoV-2 virus. *J Virol* (2022) 96(1):e0096421. doi: 10.1128/JVI.00964-21

100. Zheng J, Wong LR, Li K, Verma AK, Ortiz ME, Wohlford-Lenane C, et al. COVID-19 treatments and pathogenesis including anosmia in K18-hACE2 mice. *Nature* (2021) 589(7843):603–7. doi: 10.1038/s41586-020-2943-z

101. Arce VM, Costoya JA. SARS-CoV-2 infection in K18-ACE2 transgenic mice replicates human pulmonary disease in COVID-19. *Cell Mol Immunol* (2021) 18 (3):513–4. doi: 10.1038/s41423-020-00616-1

102. Oladunni FS, Park JG, Pino PA, Gonzalez O, Akhter A, Allue-Guardia A, et al. Lethality of SARS-CoV-2 infection in K18 human angiotensin-converting enzyme 2 transgenic mice. *Nat Commun* (2020) 11(1):6122. doi: 10.1038/s41467-020-19891-7

103. Winkler ES, Bailey AL, Kafai NM, Nair S, McCune BT, Yu J, et al. SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. *Nat Immunol* (2020) 21(11):1327–35. doi: 10.1038/s41590-020-0778-2

104. Stolp B, Stern M, Ambiel I, Hofmann K, Morath K, Gallucci L, et al. SARS-CoV-2 variants of concern display enhanced intrinsic pathogenic properties and expanded organ tropism in mouse models. *Cell Rep* (2022) 38(7):110387. doi: 10.1016/j.celrep.2022.110387

105. Yinda CK, Port JR, Bushmaker T, Offei Owusu I, Purushotham JN, Avanzato VA, et al. K18-hACE2 mice develop respiratory disease resembling severe COVID-19. *PLoS Pathog* (2021) 17(1):e1009195. doi: 10.1371/journal.ppat.1009195

106. Robertson S, Bedard O, McNally K, Shaia C, Clancy C, Lewis M, et al. Genetically diverse mouse models of SARS-CoV-2 infection reproduce clinical variation in type I interferon and cytokine responses in COVID-19. *bioRxiv* (2023). doi: 10.1101/2021.09.17.460664

107. Schafer A, Leist SR, Gralinski LE, Martinez DR, Winkler ES, Okuda K, et al. A multitrait locus regulates sarbecovirus pathogenesis. *mBio* (2022) 13(4):e0145422. doi: 10.1128/mbio.01454-22

108. Lee YJ, Seok SH, Lee NY, Choi HJ, Lee YW, Chang HJ, et al. Murine coronavirus disease 2019 lethality is characterized by lymphoid depletion associated with suppressed antigen-presenting cell functionality. *Am J Pathol* (2023) 193(7):866–82. doi: 10.1016/j.ajpath.2023.03.008

109. Mattoo SU, Myoung J. T cell responses to SARS-CoV-2 in humans and animals. *J Microbiol* (2022) 60(3):276–89. doi: 10.1007/s12275-022-1624-z

110. Pardieck IN, van der Sluis TC, van der Gracht ETI, Veerkamp DMB, Behr FM, van Duikeren S, et al. A third vaccination with a single T cell epitope confers protection in a murine model of SARS-CoV-2 infection. *Nat Commun* (2022) 13(1):3966. doi: 10.1038/s41467-022-31721-6

111. Bishop CR, Dumenil T, Rawle DJ, Le TT, Yan K, Tang B, et al. Mouse models of COVID-19 recapitulate inflammatory pathways rather than gene expression. *PLoS Pathog* (2022) 18(9):e1010867. doi: 10.1371/journal.ppat.1010867

112. Kumari P, Rothan HA, Natekar JP, Stone S, Pathak H, Strate PG, et al. Neuroinvasion and encephalitis following intranasal inoculation of SARS-CoV-2 in K18-hACE2 mice. *Viruses* (2021) 13(1). doi: 10.3390/v13010132

113. Shen WB, Elahi M, Logue J, Yang P, Baracco L, Reece EA, et al. SARS-CoV-2 invades cognitive centers of the brain and induces Alzheimer's-like neuropathology. *bioRxiv* (2022). doi: 10.1101/2022.01.31.478476

114. Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo PA, Cuapio A, et al. More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. *Sci Rep* (2021) 11(1):16144. doi: 10.1038/s41598-021-95565-8

115. Fumagalli V, Rava M, Marotta D, Di Lucia P, Laura C, Sala E, et al. Administration of aerosolized SARS-CoV-2 to K18-hACE2 mice uncouples respiratory infection from fatal neuroinvasion. *Sci Immunol* (2022) 7(67):eabl9929. doi: 10.1126/sciimmunol.abl9929

116. Afkhami S, D'Agostino MR, Zhang A, Stacey HD, Marzok A, Kang A, et al. Respiratory mucosal delivery of next-generation COVID-19 vaccine provides robust protection against both ancestral and variant strains of SARS-CoV-2. *Cell* (2022) 185 (5):896–915 e19. doi: 10.1016/j.cell.2022.02.005

117. An Y, Li S, Jin X, Han JB, Xu K, Xu S, et al. A tandem-repeat dimeric RBD protein-based covid-19 vaccine zf2001 protects mice and nonhuman primates. *Emerg Microbes Infect* (2022) 11(1):1058–71. doi: 10.1080/22221751.2022.2056524

118. Xu K, Gao P, Liu S, Lu S, Lei W, Zheng T, et al. Protective prototype-Beta and Delta-Omicron chimeric RBD-dimer vaccines against SARS-CoV-2. *Cell* (2022) 185 (13):2265–78 e14. doi: 10.1016/j.cell.2022.04.029

119. Corleis B, Hoffmann D, Rauch S, Fricke C, Roth N, Gergen J, et al. Efficacy of an unmodified bivalent mRNA vaccine against SARS-CoV-2 variants in female small animal models. *Nat Commun* (2023) 14(1):816. doi: 10.1038/s41467-023-36110-1

120. Hassan AO, Case JB, Winkler ES, Thackray LB, Kafai NM, Bailey AL, et al. A SARS-CoV-2 infection model in mice demonstrates protection by neutralizing antibodies. *Cell* (2020) 182(3):744–53.e4. doi: 10.1016/j.cell.2020.06.011

121. Rathnasinghe R, Strohmeier S, Amanat F, Gillespie VL, Krammer F, Garcia-Sastre A, et al. Comparison of transgenic and adenovirus hACE2 mouse models for SARS-CoV-2 infection. *Emerg Microbes Infect* (2020) 9(1):2433–45. doi: 10.1080/22221751.2020.1838955

122. Israelow B, Song E, Mao T, Lu P, Meir A, Liu F, et al. Mouse model of SARS-CoV-2 reveals inflammatory role of type I interferon signaling. *J Exp Med* (2020) 217 (12). doi: 10.1084/jem.20201241

123. Rawle DJ, Le TT, Dumenil T, Yan K, Tang B, Nguyen W, et al. ACE2-lentiviral transduction enables mouse SARS-CoV-2 infection and mapping of receptor interactions. *PLoS Pathog* (2021) 17(7):e1009723. doi: 10.1371/journal.ppat.1009723

124. Israelow B, Mao T, Klein J, Song E, Menasche B, Omer SB, et al. Adaptive immune determinants of viral clearance and protection in mouse models of SARS-CoV-2. *Sci Immunol* (2021) 6(64):eabl4509. doi: 10.1126/sciimmunol.abl4509

125. Beer J, Crotta S, Breithaupt A, Ohnemus A, Becker J, Sachs B, et al. Impaired immune response drives age-dependent severity of COVID-19. *J Exp Med* (2022) 219 (12). doi: 10.1084/jem.20220621

126. Davis MA, Voss K, Turnbull JB, Gustin AT, Knoll M, Muruato A, et al. A C57BL/6 mouse model of SARS-CoV-2 infection recapitulates age- and sex-based differences in human COVID-19 disease and recovery. *Vaccines (Basel)* (2022) 11(1). doi: 10.3390/vaccines11010047

127. Da Costa CBP, Cruz ACM, Penha JCQ, Castro HC, Da Cunha LER, Ratcliffe NA, et al. Using *in vivo* animal models for studying SARS-CoV-2. *Expert Opin Drug Discovery* (2022) 17(2):121–37. doi: 10.1080/17460441.2022.1995352

128. Rodriguez-Rodriguez BA, Ciabattoni GO, Duerr R, Valero-Jimenez AM, Yeung ST, Crosse KM, et al. A neonatal mouse model characterizes transmissibility of SARS-CoV-2 variants and reveals a role for ORF8. *bioRxiv* (2023). doi: 10.1101/2022.10.04.510658

129. Muruganandah V, Sathkumara HD, Pai S, Rush CM, Brosch R, Waardenberg AJ, et al. A systematic approach to simultaneously evaluate safety, immunogenicity, and efficacy of novel tuberculosis vaccination strategies. *Sci Adv* (2020) 6(10):eaaz1767. doi: 10.1126/sciadv.aaz1767

130. Wang Y, Lenoch J, Kohler D, DeLiberto TJ, Tang CY, Li T, et al. SARS-CoV-2 exposure in Norway rats (Rattus norvegicus) from New York City. *mBio* (2023) 14(2): e0362122. doi: 10.1128/mbio.03621-22

131. Agency EM. *Assessment Report EMA/707383/2020 Corr.1*: Comirnaty COVID-19 MRNA Vaccine (Nucleoside-Modified) Assessment Report*, Vol. 31. Amsterdam, Netherlands: EMA (2021). pp. 1–140.

132. Singhal A, Aliouat el M, Herve M, Mathys V, Kiass M, Creusy C, et al. Experimental tuberculosis in the Wistar rat: a model for protective immunity and control of infection. *PLoS One* (2011) 6(4):e18632. doi: 10.1371/journal.pone.0018632

133. Kumar N, Vishwas KG, Kumar M, Reddy J, Parab M, Manikanth CL, et al. Pharmacokinetics and dose response of anti-TB drugs in rat infection model of tuberculosis. *Tuberculosis (Edinb)* (2014) 94(3):282–6. doi: 10.1016/j.tube.2014.02.004

134. Foo DG, Tay HC, Siew JY, Singhal A, Camacho L, Bifani P, et al. T cell monitoring of chemotherapy in experimental rat tuberculosis. *Antimicrob Agents Chemother* (2011) 55(8):3677–83. doi: 10.1128/AAC.00136-11

135. Elwood RL, Wilson S, Blanco JC, Yim K, Pletneva L, Nikonenko B, et al. The American cotton rat: a novel model for pulmonary tuberculosis. *Tuberculosis (Edinb)* (2007) 87(2):145–54. doi: 10.1016/j.tube.2006.07.001

136. Sugawara I, Yamada H, Mizuno S. Pathological and immunological profiles of rat tuberculosis. *Int J Exp Pathol* (2004) 85(3):125–34. doi: 10.1111/j.0959-9673.2004.00379.x

137. Mgode GF, Cohen-Bacrie S, Bedotto M, Weetjens BJ, Cox C, Jubitana M, et al. Mycobacterium genotypes in pulmonary tuberculosis infections and their detection by trained African giant pouched rats. *Curr Microbiol* (2015) 70(2):212–8. doi: 10.1007/s00284-014-0705-6

138. Hewitt JA, Lutz C, Florence WC, Pitt MLM, Rao S, Rappaport J, et al. ACTIVating resources for the COVID-19 pandemic: *in vivo* models for vaccines and therapeutics. *Cell Host Microbe* (2020) 28(5):646–59. doi: 10.1016/j.chom.2020.09.016

139. Riley RL. Aerial dissemination of pulmonary tuberculosis. *Am Rev Tuberc* (1957) 76(6):931–41. doi: 10.1164/artpd.1957.76.6.931

140. Ruhl CR, Pasko BL, Khan HS, Kindt LM, Stamm CE, Franco LH, et al. Mycobacterium tuberculosis sulfolipid-1 activates nociceptive neurons and induces cough. *Cell* (2020) 181(2):293–305 e11. doi: 10.1016/j.cell.2020.02.026

141. Clark S, Hall Y, Williams A. Animal models of tuberculosis: Guinea pigs. *Cold Spring Harb Perspect Med* (2014) 5(5):a018572. doi: 10.1101/cshperspect.a018572

142. Dharmadhikari AS, Nardell EA. What animal models teach humans about tuberculosis. *Am J Respir Cell Mol Biol* (2008) 39(5):503–8. doi: 10.1165/rcmb.2008-0154TR

143. Mitchison DA, Wallace JG, Bhatia AL, Selkon JB, Subbaiah TV, Lancaster MC. A comparison of the virulence in Guinea-pigs of South Indian and British tubercle bacilli. *Tubercle* (1960) 41:1–22. doi: 10.1016/S0041-3879(60)80019-0

144. Basaraba RJ, Dailey DD, McFarland CT, Shanley CA, Smith EE, McMurray DN, et al. Lymphadenitis as a major element of disease in the Guinea pig model of tuberculosis. *Tuberculosis (Edinb)* (2006) 86(5):386–94. doi: 10.1016/j.tube.2005.11.003

145. McMurray DN. Guinea pig model of tuberculosis. In: Bloom BR, editors. *Tuberculosis* (1994). doi: 10.1128/9781555818357.ch9

146. Smith DW, Harding GE. Animal model of human disease. Pulmonary tuberculosis. Animal model: Experimental airborne tuberculosis in the Guinea pig. *Am J Pathol* (1977) 89(1):273–6.

147. Turner OC, Basaraba RJ, Orme IM. Immunopathogenesis of pulmonary granulomas in the Guinea pig after infection with Mycobacterium tuberculosis. *Infect Immun* (2003) 71(2):864–71. doi: 10.1128/IAI.71.2.864-871.2003

148. Cardona PJ, Williams A. Experimental animal modelling for TB vaccine development. *Int J Infect Dis* (2017) 56:268–73. doi: 10.1016/j.ijid.2017.01.030

149. Commandeur S, van den Eeden SJ, Dijkman K, Clark SO, van Meijgaarden KE, Wilson L, et al. The *in vivo* expressed Mycobacterium tuberculosis (IVE-TB) antigen Rv2034 induces CD4(+) T-cells that protect against pulmonary infection in HLA-DR transgenic mice and Guinea pigs. *Vaccine* (2014) 32(29):3580–8. doi: 10.1016/j.vaccine.2014.05.005

150. Skeiky YA, Dietrich J, Lasco TM, Stagliano K, Dheenadhayalan V, Goetz MA, et al. Non-clinical efficacy and safety of HyVac4:IC31 vaccine administered in a BCG prime-boost regimen. *Vaccine* (2010) 28(4):1084–93. doi: 10.1016/j.vaccine.2009.10.114

151. Aagaard C, Hoang TT, Izzo A, Billeskov R, Troudt J, Arnett K, et al. Protection and polyfunctional T cells induced by Ag85B-TB10.4/IC31 against Mycobacterium tuberculosis is highly dependent on the antigen dose. *PLoS One* (2009) 4(6):e5930. doi: 10.1371/journal.pone.0005930

152. Clark S, Cross ML, Nadian A, Vipond J, Court P, Williams A, et al. Oral vaccination of Guinea pigs with a Mycobacterium bovis bacillus Calmette-Guerin vaccine in a lipid matrix protects against aerosol infection with virulent M. bovis. *Infect Immun* (2008) 76(8):3771–6. doi: 10.1128/IAI.00052-08

153. Watanabe Y, Watari E, Matsunaga I, Hiromatsu K, Dascher CC, Kawashima T, et al. BCG vaccine elicits both T-cell mediated and humoral immune responses directed against mycobacterial lipid components. *Vaccine* (2006) 24(29-30):5700–7. doi: 10.1016/j.vaccine.2006.04.049

154. Larrouy-Maumus G, Layre E, Clark S, Prandi J, Rayner E, Lepore M, et al. Protective efficacy of a lipid antigen vaccine in a Guinea pig model of tuberculosis. *Vaccine* (2017) 35(10):1395–402. doi: 10.1016/j.vaccine.2017.01.079

155. Dascher CC, Hiromatsu K, Naylor JW, Brauer PP, Brown KA, Storey JR, et al. Conservation of a CD1 multigene family in the Guinea pig. *J Immunol* (1999) 163 (10):5478–88. doi: 10.4049/jimmunol.163.10.5478

156. Dascher CC, Hiromatsu K, Xiong X, Sugita M, Buhlmann JE, Dodge IL, et al. Conservation of CD1 intracellular trafficking patterns between mamMalian species. *J Immunol* (2002) 169(12):6951–8. doi: 10.4049/jimmunol.169.12.6951

157. Hiromatsu K, Dascher CC, Sugita M, Gingrich-Baker C, Behar SM, LeClair KP, et al. Characterization of Guinea-pig group 1 CD1 proteins. *Immunology* (2002) 106 (2):159–72. doi: 10.1046/j.1365-2567.2002.01422.x

158. Van Rhijn I, Godfrey DI, Rossjohn J, Moody DB. Lipid and small-molecule display by CD1 and MR1. *Nat Rev Immunol* (2015) 15(10):643–54. doi: 10.1038/nri3889

159. Minnikin DE, Kremer L, Dover LG, Besra GS. The methyl-branched fortifications of Mycobacterium tuberculosis. *Chem Biol* (2002) 9(5):545–53. doi: 10.1016/S1074-5521(02)00142-4

160. Hiromatsu K, Dascher CC, LeClair KP, Sugita M, Furlong ST, Brenner MB, et al. Induction of CD1-restricted immune responses in Guinea pigs by immunization with mycobacterial lipid antigens. *J Immunol* (2002) 169(1):330–9. doi: 10.4049/jimmunol.169.1.330

161. Eckhardt E, Bastian M. Animal models for human group 1 CD1 protein function. *Mol Immunol* (2021) 130:159–63. doi: 10.1016/j.molimm.2020.12.018

162. Haslov K, Andersen A, Nagai S, Gottschau A, Sorensen T, Andersen P. Guinea pig cellular immune responses to proteins secreted by Mycobacterium tuberculosis. *Infect Immun* (1995) 63(3):804–10. doi: 10.1128/iai.63.3.804-810.1995

163. Eckhardt E, Li Y, Mamerow S, Schinkothe J, Sehl-Ewert J, Dreisbach J, et al. Pharmacokinetics and Efficacy of the Benzothiazinone BTZ-043 against Tuberculous Mycobacteria inside Granulomas in the Guinea Pig Model. *Antimicrob Agents Chemother* (2023) 67(4):e0143822. doi: 10.1128/aac.01438-22

164. Ordway DJ, Shanley CA, Caraway ML, Orme EA, Bucy DS, Hascall-Dove L, et al. Evaluation of standard chemotherapy in the Guinea pig model of tuberculosis. *Antimicrob Agents Chemother* (2010) 54(5):1820–33. doi: 10.1128/AAC.01521-09

165. Ahmad Z, Klinkenberg LG, Pinn ML, Fraig MM, Peloquin CA, Bishai WR, et al. Biphasic kill curve of isoniazid reveals the presence of drug-tolerant, not drug-resistant, Mycobacterium tuberculosis in the Guinea pig. *J Infect Dis* (2009) 200 (7):1136–43. doi: 10.1086/605605

166. Schafer H, Burger R. Tools for cellular immunology and vaccine research the in the Guinea pig: monoclonal antibodies to cell surface antigens and cell lines. *Vaccine* (2012) 30(40):5804–11. doi: 10.1016/j.vaccine.2012.07.012

167. Schafer H, Kliem G, Kropp B, Burger R. Monoclonal antibodies to Guinea pig interferon-gamma: tools for cytokine detection and neutralization. *J Immunol Methods* (2007) 328(1-2):106–17. doi: 10.1016/j.jim.2007.08.012

168. McMurray DN, Allen SS, Jeevan A, Lasco T, Cho H, Skwor T, et al. Vaccine-induced cytokine responses in a Guinea pig model of pulmonary tuberculosis. *Tuberculosis (Edinb)* (2005) 85(5-6):295–301. doi: 10.1016/j.tube.2005.08.012

169. Allen SS, McMurray DN. Coordinate cytokine gene expression *in vivo* following induction of tuberculous pleurisy in Guinea pigs. *Infect Immun* (2003) 71 (8):4271–7. doi: 10.1128/IAI.71.8.4271-4277.2003

170. Spohr C, Kaufmann E, Battenfeld S, Duchow K, Cussler K, Balks E, et al. A new lymphocyte proliferation assay for potency determination of bovine tuberculin PPDs. *Altex* (2015) 32(3):201–10. doi: 10.14573/altex.1502101

171. Mykytyn AZ, Lamers MM, Okba NMA, Breugem TI, Schipper D, van den Doel PB, et al. Susceptibility of rabbits to SARS-CoV-2. *Emerg Microbes Infect* (2021) 10 (1):1–7. doi: 10.1080/22221751.2020.1868951

172. Lurie MB. The fate of human and bovine tubercle bacilli in various organs of the rabbit. *J Exp Med* (1928) 48(2):155–82. doi: 10.1084/jem.48.2.155

173. Subbian S, Tsenova L, O'Brien P, Yang G, Kushner NL, Parsons S, et al. Spontaneous latency in a rabbit model of pulmonary tuberculosis. *Am J Pathol* (2012) 181(5):1711–24. doi: 10.1016/j.ajpath.2012.07.019

174. Subbian S, O'Brien P, Kushner NL, Yang G, Tsenova L, Peixoto B, et al. Molecular immunologic correlates of spontaneous latency in a rabbit model of pulmonary tuberculosis. *Cell Commun Signal* (2013) 11(1):16. doi: 10.1186/1478-811X-11-16

175. Nedeltchev GG, Raghunand TR, Jassal MS, Lun S, Cheng QJ, Bishai WR. Extrapulmonary dissemination of Mycobacterium bovis but not Mycobacterium tuberculosis in a bronchoscopic rabbit model of cavitary tuberculosis. *Infect Immun* (2009) 77(2):598–603. doi: 10.1128/IAI.01132-08

176. Hunter RL, Actor JK, Hwang SA, Khan A, Urbanowski ME, Kaushal D, et al. Pathogenesis and animal models of post-primary (Bronchogenic) tuberculosis, A review. *Pathogens* (2018) 7(1). doi: 10.3390/pathogens7010019

177. Kubler A, Luna B, Larsson C, Ammerman NC, Andrade BB, Orandle M, et al. Mycobacterium tuberculosis dysregulates MMP/TIMP balance to drive rapid cavitation and unrestrained bacterial proliferation. *J Pathol* (2015) 235(3):431–44. doi: 10.1002/path.4432

178. Subbian S, Bandyopadhyay N, Tsenova L, O'Brien P, Khetani V, Kushner NL, et al. Early innate immunity determines outcome of Mycobacterium tuberculosis pulmonary infection in rabbits. *Cell Commun Signal* (2013) 11:60. doi: 10.1186/1478-811X-11-60

179. Tsenova L, Fallows D, Kolloli A, Singh P, O'Brien P, Kushner N, et al. Inoculum size and traits of the infecting clinical strain define the protection level against Mycobacterium tuberculosis infection in a rabbit model. *Eur J Immunol* (2020) 50 (6):858–72. doi: 10.1002/eji.201948448

180. Marakalala MJ, Raju RM, Sharma K, Zhang YJ, Eugenin EA, Prideaux B, et al. Inflammatory signaling in human tuberculosis granulomas is spatially organized. *Nat Med* (2016) 22(5):531–8. doi: 10.1038/nm.4073

181. Sarathy JP, Dartois V. Caseum: a niche for Mycobacterium tuberculosis drug-tolerant persisters. *Clin Microbiol Rev* (2020) 33(3). doi: 10.1128/CMR.00159-19

182. Rifat D, Prideaux B, Savic RM, Urbanowski ME, Parsons TL, Luna B, et al. Pharmacokinetics of rifapentine and rifampin in a rabbit model of tuberculosis and correlation with clinical trial data. *Sci Transl Med* (2018) 10(435). doi: 10.1126/scitranslmed.aai7786

183. Blanc L, Sarathy JP, Alvarez Cabrera N, O'Brien P, Dias-Freedman I, Mina M, et al. Impact of immunopathology on the antituberculous activity of pyrazinamide. *J Exp Med* (2018) 215(8):1975–86. doi: 10.1084/jem.20180518

184. Dennis EW, Gaboe FC. Experimental tuberculosis of the Syrian hamster, Cricetus auratus. *Ann N Y Acad Sci* (1949) 52(5):646–61. doi: 10.1111/j.1749-6632.1949.tb53954.x

185. Imai M, Iwatsuki-Horimoto K, Hatta M, Loeber S, Halfmann PJ, Nakajima N, et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc Natl Acad Sci USA* (2020) 117(28):16587–95. doi: 10.1073/pnas.2009799117

186. Sia SF, Yan LM, Chin AWH, Fung K, Choy KT, Wong AYL, et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature* (2020) 583(7818):834–8. doi: 10.1038/s41586-020-2342-5

187. Trimpert J, Vladimirova D, Dietert K, Abdelgawad A, Kunec D, Dokel S, et al. The roborovski dwarf hamster is A highly susceptible model for a rapid and fatal course of SARS-CoV-2 infection. *Cell Rep* (2020) 33(10):108488. 10.1016/j.celrep.2020.108488

188. Tomris I, Bouwman KM, Adolfs Y, Noack D, van der Woude R, Kerster G, et al. Distinct spatial arrangements of ACE2 and TMPRSS2 expression in Syrian hamster lung lobes dictates SARS-CoV-2 infection patterns. *PLoS Pathog* (2022) 18(3): e1010340. doi: 10.1371/journal.ppat.1010340

189. Rosenke K, Meade-White K, Letko M, Clancy C, Hansen F, Liu Y, et al. Defining the Syrian hamster as a highly susceptible preclinical model for SARS-CoV-2 infection. *Emerg Microbes Infect* (2020) 9(1):2673–84. doi: 10.1080/22221751.2020.1858177

190. Osterrieder N, Bertzbach LD, Dietert K, Abdelgawad A, Vladimirova D, Kunec D, et al. Age-dependent progression of SARS-CoV-2 infection in Syrian hamsters. *Viruses* (2020) 12(7). doi: 10.3390/v12070779

191. Dhakal S, Ruiz-Bedoya CA, Zhou R, Creisher PS, Villano JS, Littlefield K, et al. Sex differences in lung imaging and SARS-CoV-2 antibody responses in a COVID-19 golden Syrian hamster model. *mBio* (2021) 12(4):e0097421. doi: 10.1128/mBio.00974-21

192. Castellan M, Zamperin G, Franzoni G, Foiani G, Zorzan M, Drzewniokova P, et al. Host Response of Syrian Hamster to SARS-CoV-2 Infection including Differences with Humans and between Sexes. *Viruses* (2023) 15(2). doi: 10.3390/v15020428

193. Chan JF, Zhang AJ, Yuan S, Poon VK, Chan CC, Lee AC, et al. Simulation of the clinical and pathological manifestations of coronavirus disease 2019 (COVID-19) in a golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clin Infect Dis* (2020) 71(9):2428–46. doi: 10.1093/cid/ciaa325

194. Port JR, Morris DH, Riopelle JC, Yinda CK, Avanzato VA, Holbrook MG, et al. Host and viral determinants of airborne transmission of SARS-CoV-2 in the Syrian hamster. *bioRxiv* (2023). doi: 10.7554/eLife.87094.1

195. Ulrich L, Halwe NJ, Taddeo A, Ebert N, Schon J, Devisme C, et al. Enhanced fitness of SARS-CoV-2 variant of concern Alpha but not Beta. *Nature* (2022) 602 (7896):307–13. doi: 10.1038/s41586-021-04342-0

196. Frere JJ, Serafini RA, Pryce KD, Zazhytska M, Oishi K, Golynker I, et al. SARS-CoV-2 infection in hamsters and humans results in lasting and unique systemic perturbations after recovery. *Sci Transl Med* (2022) 14(664):eabq3059. doi: 10.1126/scitranslmed.abq3059

197. Horiuchi S, Oishi K, Carrau L, Frere J, Moller R, Panis M, et al. Immune memory from SARS-CoV-2 infection in hamsters provides variant-independent protection but still allows virus transmission. *Sci Immunol* (2021) 6(66):eabm3131. doi: 10.1126/sciimmunol.abm3131

198. Nouailles G, Wyler E, Pennitz P, Postmus D, Vladimirova D, Kazmierski J, et al. Temporal omics analysis in Syrian hamsters unravel cellular effector responses to moderate COVID-19. *Nat Commun* (2021) 12(1):4869. doi: 10.1038/s41467-021-25030-7

199. Becker K, Beythien G, de Buhr N, Stanelle-Bertram S, Tuku B, Kouassi NM, et al. Vasculitis and neutrophil extracellular traps in lungs of golden Syrian hamsters with SARS-CoV-2. *Front Immunol* (2021) 12:640842. doi: 10.3389/fimmu.2021.640842

200. Veras FP, Pontelli MC, Silva CM, Toller-Kawahisa JE, de Lima M, Nascimento DC, et al. SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19 pathology. *J Exp Med* (2020) 217(12). doi: 10.1084/jem.20201129

201. Ebenig A, Muraleedharan S, Kazmierski J, Todt D, Auste A, Anzaghe M, et al. Vaccine-associated enhanced respiratory pathology in COVID-19 hamsters after T(H)2-biased immunization. *Cell Rep* (2022) 40(7):111214. doi: 10.1016/j.celrep.2022.111214

202. Nouailles G, Adler JM, Pennitz P, Peidli S, Teixeira Alves LG, Baumgardt M, et al. Live-attenuated vaccine sCPD9 elicits superior mucosal and systemic immunity to SARS-CoV-2 variants in hamsters. *Nat Microbiol* (2023) 8(5):860–74. doi: 10.1038/s41564-023-01352-8

203. Pena JC, Ho WZ. Monkey models of tuberculosis: lessons learned. *Infect Immun* (2015) 83(3):852–62. doi: 10.1128/IAI.02850-14

204. Lin PL, Rodgers M, Smith L, Bigbee M, Myers A, Bigbee C, et al. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. *Infect Immun* (2009) 77(10):4631–42. doi: 10.1128/IAI.00592-09

205. Mattila JT, Ojo OO, Kepka-Lenhart D, Marino S, Kim JH, Eum SY, et al. Microenvironments in tuberculous granulomas are delineated by distinct populations of macrophage subsets and expression of nitric oxide synthase and arginase isoforms. *J Immunol* (2013) 191(2):773–84. doi: 10.4049/jimmunol.1300113

206. Ulrichs T, Kosmiadi GA, Trusov V, Jorg S, Pradl L, Titukhina M, et al. Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. *J Pathol* (2004) 204(2):217–28. doi: 10.1002/path.1628

207. Swanson RV, Gupta A, Foreman TW, Lu L, Choreno-Parra JA, Mbandi SK, et al. Antigen-specific B cells direct T follicular-like helper cells into lymphoid follicles to mediate Mycobacterium tuberculosis control. *Nat Immunol* (2023) 24(5):855–68. doi: 10.1038/s41590-023-01476-3

208. Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, Ioerger T, et al. Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. *Nat Med* (2014) 20(1):75–9. doi: 10.1038/nm.3412

209. Coleman MT, Maiello P, Tomko J, Frye LJ, Fillmore D, Janssen C, et al. Early Changes by (18)Fluorodeoxyglucose positron emission tomography coregistered with computed tomography predict outcome after Mycobacterium tuberculosis infection in cynomolgus macaques. *Infect Immun* (2014) 82(6):2400–4. doi: 10.1128/IAI.01599-13

210. Barry CE3rd, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol* (2009) 7(12):845–55. doi: 10.1038/nrmicro2236

211. Maiello P, DiFazio RM, Cadena AM, Rodgers MA, Lin PL, Scanga CA, et al. Rhesus macaques are more susceptible to progressive tuberculosis than cynomolgus macaques: a quantitative comparison. *Infect Immun* (2018) 86(2). doi: 10.1128/IAI.00505-17

212. Dijkman K, Vervenne RAW, Sombroek CC, Boot C, Hofman SO, van Meijgaarden KE, et al. Disparate tuberculosis disease development in macaque species is associated with innate immunity. *Front Immunol* (2019) 10:2479. doi: 10.3389/fimmu.2019.02479

213. Gideon HP, Hughes TK, Tzouanas CN, Wadsworth MH2nd, Tu AA, Gierahn TM, et al. Multimodal profiling of lung granulomas in macaques reveals cellular correlates of tuberculosis control. *Immunity* (2022) 55(5):827–46 e10. doi: 10.1016/j.immuni.2022.04.004

214. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* (2001) 345(15):1098–104. doi: 10.1056/NEJMoa011110

215. Lin PL, Myers A, Smith L, Bigbee C, Bigbee M, Fuhrman C, et al. Tumor necrosis factor neutralization results in disseminated disease in acute and latent Mycobacterium tuberculosis infection with normal granuloma structure in a cynomolgus macaque model. *Arthritis Rheum* (2010) 62(2):340–50. doi: 10.1002/art.27271

216. Diedrich CR, Flynn JL. HIV-1/mycobacterium tuberculosis coinfection immunology: how does HIV-1 exacerbate tuberculosis? *Infect Immun* (2011) 79 (4):1407–17. doi: 10.1128/IAI.01126-10

217. Diedrich CR, Rutledge T, Maiello P, Baranowski TM, White AG, Borish HJ, et al. SIV and Mycobacterium tuberculosis synergy within the granuloma accelerates the reactivation pattern of latent tuberculosis. *PLoS Pathog* (2020) 16(7):e1008413. doi: 10.1371/journal.ppat.1008413

218. Lin PL, Rutledge T, Green AM, Bigbee M, Fuhrman C, Klein E, et al. CD4 T cell depletion exacerbates acute Mycobacterium tuberculosis while reactivation of latent infection is dependent on severity of tissue depletion in cynomolgus macaques. *AIDS Res Hum Retroviruses* (2012) 28(12):1693–702. doi: 10.1089/aid.2012.0028

219. Yao S, Huang D, Chen CY, Halliday L, Wang RC, Chen ZW. CD4+ T cells contain early extrapulmonary tuberculosis (TB) dissemination and rapid TB progression and sustain multieffector functions of CD8+ T and CD3- lymphocytes: mechanisms of CD4+ T cell immunity. *J Immunol* (2014) 192(5):2120–32. doi: 10.4049/jimmunol.1301373

220. Slight SR, Rangel-Moreno J, Gopal R, Lin Y, Fallert Junecko BA, Mehra S, et al. CXCR5(+) T helper cells mediate protective immunity against tuberculosis. *J Clin Invest* (2013) 123(2):712–26. doi: 10.1172/JCI65728

221. Verreck FAW, Tchilian EZ, Vervenne RAW, Sombroek CC, Kondova I, Eissen OA, et al. Variable BCG efficacy in rhesus populations: Pulmonary BCG provides protection where standard intra-dermal vaccination fails. *Tuberculosis (Edinb)* (2017) 104:46–57. doi: 10.1016/j.tube.2017.02.003

222. Langermans JA, Andersen P, van Soolingen D, Vervenne RA, Frost PA, van der Laan T, et al. Divergent effect of bacillus Calmette-Guerin (BCG) vaccination on Mycobacterium tuberculosis infection in highly related macaque species: implications for primate models in tuberculosis vaccine research. *Proc Natl Acad Sci USA* (2001) 98 (20):11497–502. doi: 10.1073/pnas.201404898

223. Scanga CA, Flynn JL. Modeling tuberculosis in nonhuman primates. *Cold Spring Harb Perspect Med* (2014) 4(12):a018564. doi: 10.1101/cshperspect.a018564

224. Darrah PA, Zeppa JJ, Maiello P, Hackney JA, Wadsworth MH2nd, Hughes TK, et al. Prevention of tuberculosis in macaques after intravenous BCG immunization. *Nature* (2020) 577(7788):95–102. doi: 10.1038/s41586-019-1817-8

225. Dijkman K, Sombroek CC, Vervenne RAW, Hofman SO, Boot C, Remarque EJ, et al. Prevention of tuberculosis infection and disease by local BCG in repeatedly exposed rhesus macaques. *Nat Med* (2019) 25(2):255–62. doi: 10.1038/s41591-018-0319-9

226. White AD, Sarfas C, Sibley LS, Gullick J, Clark S, Rayner E, et al. Protective efficacy of inhaled BCG vaccination against ultra-low dose aerosol M. tuberculosis challenge in rhesus macaques. *Pharmaceutics* (2020) 12(5). doi: 10.3390/pharmaceutics12050394

227. Barclay WR, Anacker RL, Brehmer W, Leif W, Ribi E. Aerosol-induced tuberculosis in subhuman primates and the course of the disease after intravenous BCG vaccination. *Infect Immun* (1970) 2(5):574–82. doi: 10.1128/iai.2.5.574-582.1970

228. Foreman TW, Mehra S, Lackner AA, Kaushal D. Translational research in the nonhuman primate model of tuberculosis. *ILAR J* (2017) 58(2):151–9. doi: 10.1093/ilar/ilx015

229. Tait DR, Hatherill M, van der Meeren O, Ginsberg AM, Van Brakel E, Salaun B, et al. Final analysis of a trial of M72/AS01(E) vaccine to prevent tuberculosis. *N Engl J Med* (2019) 381(25):2429–39. doi: 10.1056/NEJMoa1909953

230. Reed SG, Coler RN, Dalemans W, Tan EV, DeLa Cruz EC, Basaraba RJ, et al. Defined tuberculosis vaccine, Mtb72F/AS02A, evidence of protection in cynomolgus monkeys. *Proc Natl Acad Sci USA* (2009) 106(7):2301–6. doi: 10.1073/pnas.0712077106

231. Damas J, Hughes GM, Keough KC, Painter CA, Persky NS, Corbo M, et al. Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. *Proc Natl Acad Sci U.S.A.* (2020) 117(36):22311–22. doi: 10.1073/pnas.2010146117

232. Aid M, Busman-Sahay K, Vidal SJ, Maliga Z, Bondoc S, Starke C, et al. Vascular disease and thrombosis in SARS-CoV-2-infected rhesus macaques. *Cell* (2020) 183 (5):1354–66 e13. doi: 10.1016/j.cell.2020.10.005

233. Koo BS, Oh H, Kim G, Hwang EH, Jung H, Lee Y, et al. Transient lymphopenia and interstitial pneumonia with endotheliitis in SARS-CoV-2-infected macaques. *J Infect Dis* (2020) 222(10):1596–600. doi: 10.1093/infdis/jiaa486

234. Cross RW, Agans KN, Prasad AN, Borisevich V, Woolsey C, Deer DJ, et al. Intranasal exposure of African green monkeys to SARS-CoV-2 results in acute phase pneumonia with shedding and lung injury still present in the early convalescence phase. *Virol J* (2020) 17(1):125. doi: 10.1186/s12985-020-01396-w

235. Munster VJ, Feldmann F, Williamson BN, van Doremalen N, Perez-Perez L, Schulz J, et al. Respiratory disease in rhesus macaques inoculated with SARS-CoV-2. *Nature* (2020) 585(7824):268–72. doi: 10.1038/s41586-020-2324-7

236. Yu P, Qi F, Xu Y, Li F, Liu P, Liu J, et al. Age-related rhesus macaque models of COVID-19. *Anim Model Exp Med* (2020) 3(1):93–7. doi: 10.1002/ame2.12108

237. Salguero FJ, White AD, Slack GS, Fotheringham SA, Bewley KR, Gooch KE, et al. Comparison of rhesus and cynomolgus macaques as an infection model for COVID-19. *Nat Commun* (2021) 12(1):1260. doi: 10.1038/s41467-021-21389-9

238. Goncalves A, Maisonnasse P, Donati F, Albert M, Behillil S, Contreras V, et al. SARS-CoV-2 viral dynamics in non-human primates. *PLoS Comput Biol* (2021) 17(3): e1008785. doi: 10.1371/journal.pcbi.1008785

239. Singh DK, Singh B, Ganatra SR, Gazi M, Cole J, Thippeshappa R, et al. Responses to acute infection with SARS-CoV-2 in the lungs of rhesus macaques, baboons and marmosets. *Nat Microbiol* (2021) 6(1):73–86. doi: 10.1038/s41564-021-00867-2

240. Speranza E, Williamson BN, Feldmann F, Sturdevant GL, Perez-Perez L, Meade-White K, et al. Single-cell RNA sequencing reveals SARS-CoV-2 infection dynamics in lungs of African green monkeys. *Sci Transl Med* (2021) 13(578). doi: 10.1126/scitranslmed.abe8146

241. Nelson CE, Namasivayam S, Foreman TW, Kauffman KD, Sakai S, Dorosky DE, et al. Mild SARS-CoV-2 infection in rhesus macaques is associated with viral control prior to antigen-specific T cell responses in tissues. *Sci Immunol* (2022) eabo0535. doi: 10.1126/sciimmunol.abo0535

242. Nordstrom P, Ballin M, Nordstrom A. Risk of SARS-CoV-2 reinfection and COVID-19 hospitalisation in individuals with natural and hybrid immunity: a retrospective, total population cohort study in Sweden. *Lancet Infect Dis* (2022) 22 (6):781–90. doi: 10.1016/S1473-3099(22)00143-8

243. Deng W, Bao L, Liu J, Xiao C, Liu J, Xue J, et al. Primary exposure to SARS-CoV-2 protects against reinfection in rhesus macaques. *Science* (2020) 369(6505):818–23. doi: 10.1126/science.abc5343

244. McMahan K, Yu J, Mercado NB, Loos C, Tostanoski LH, Chandrashekar A, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature* (2021) 590(7847):630–4. doi: 10.1038/s41586-020-03041-6

245. Klasse PJ, Nixon DF, Moore JP. Immunogenicity of clinically relevant SARS-CoV-2 vaccines in nonhuman primates and humans. *Sci Adv* (2021) 7(12). doi: 10.1126/sciadv.abe8065

246. Corbett KS, Nason MC, Flach B, Gagne M, O'Connell S, Johnston TS, et al. Immune correlates of protection by mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. *Science* (2021) 373(6561):eabj0299. doi: 10.1126/science.abj0299

247. Goldblatt D, Alter G, Crotty S, Plotkin SA. Correlates of protection against SARS-CoV-2 infection and COVID-19 disease. *Immunol Rev* (2022) 310(1):6–26. doi: 10.1111/imr.13091

248. Trichel AM. Overview of nonhuman primate models of SARS-CoV-2 infection. *Comp Med* (2021) 71(5):411–32. doi: 10.30802/AALAS-CM-20-000119

249. Michelitsch A, Wernike K, Ulrich L, Mettenleiter TC, Beer M. SARS-CoV-2 in animals: From potential hosts to animal models. *Adv Virus Res* (2021) 110:59–102. doi: 10.1016/bs.aivir.2021.03.004

250. Rahim Z, Thapa J, Fukushima Y, van der Zanden AGM, Gordon SV, Suzuki Y, et al. Tuberculosis caused by Mycobacterium orygis in dairy cattle and captured monkeys in Bangladesh: a new scenario of tuberculosis in South Asia. *Transbound Emerg Dis* (2017) 64(6):1965–9. doi: 10.1111/tbed.12596

251. Bailey SS, Crawshaw TR, Smith NH, Palgrave CJ. Mycobacterium bovis infection in domestic pigs in Great Britain. *Vet J* (2013) 198(2):391–7. doi: 10.1016/j.tvjl.2013.08.035

252. Rodriguez S, Bezos J, Romero B, de Juan L, Alvarez J, Castellanos E, et al. Mycobacterium caprae infection in livestock and wildlife, Spain. *Emerg Infect Dis* (2011) 17(3):532–5. doi: 10.3201/eid1703.100618

253. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, et al. A new evolutionary scenario for the Mycobacterium tuberculosis complex. *Proc Natl Acad Sci USA* (2002) 99(6):3684–9. doi: 10.1073/pnas.052548299

254. Bayraktar B, Bulut E, Baris AB, Toksoy B, Dalgic N, Celikkan C, et al. Species distribution of the Mycobacterium tuberculosis complex in clinical isolates from 2007 to 2010 in Turkey: a prospective study. *J Clin Microbiol* (2011) 49(11):3837–41. doi: 10.1128/JCM.01172-11

255. Whelan AO, Coad M, Cockle PJ, Hewinson G, Vordermeier M, Gordon SV. Revisiting host preference in the Mycobacterium tuberculosis complex: experimental infection shows M. tuberculosis H37Rv to be avirulent in cattle. *PLoS One* (2010) 5(1): e8527. doi: 10.1371/journal.pone.0008527

256. Bezos J, Casal C, Diez-Delgado I, Romero B, Liandris E, Alvarez J, et al. Goats challenged with different members of the Mycobacterium tuberculosis complex display different clinical pictures. *Vet Immunol Immunopathol* (2015) 167(3-4):185–9. doi: 10.1016/j.vetimm.2015.07.009

257. Niroula N, Lim ZL, Walker S, Huang Y, Gerdts V, Zriba S, et al. Domestic pigs experimentally infected with Mycobacterium bovis and Mycobacterium tuberculosis exhibit different disease outcomes. *Tuberculosis (Edinb)* (2022) 133:102167. doi: 10.1016/j.tube.2022.102167

258. Villarreal-Ramos B, Berg S, Whelan A, Holbert S, Carreras F, Salguero FJ, et al. Experimental infection of cattle with Mycobacterium tuberculosis isolates shows the attenuation of the human tubercle bacillus for cattle. *Sci Rep* (2018) 8(1):894. doi: 10.1038/s41598-017-18575-5

259. Perez de Val B, Villarreal-Ramos B, Nofrarias M, Lopez-Soria S, Romera N, Singh M, et al. Goats primed with Mycobacterium bovis BCG and boosted with a recombinant adenovirus expressing Ag85A show enhanced protection against tuberculosis. *Clin Vaccine Immunol* (2012) 19(9):1339–47. doi: 10.1128/CVI.00275-12

260. Gil O, Diaz I, Vilaplana C, Tapia G, Diaz J, Fort M, et al. Granuloma encapsulation is a key factor for containing tuberculosis infection in minipigs. *PLoS One* (2010) 5(4):e10030. doi: 10.1371/journal.pone.0010030

261. Waters WR, Whelan AO, Lyashchenko KP, Greenwald R, Palmer MV, Harris BN, et al. Immune responses in cattle inoculated with Mycobacterium bovis, Mycobacterium tuberculosis, or Mycobacterium kansasii. *Clin Vaccine Immunol* (2010) 17(2):247–52. doi: 10.1128/CVI.00442-09

262. Waters WR, Palmer MV, Thacker TC, Bannantine JP, Vordermeier HM, Hewinson RG, et al. Early antibody responses to experimental Mycobacterium bovis infection of cattle. *Clin Vaccine Immunol* (2006) 13(6):648–54. doi: 10.1128/CVI.00061-06

263. Villarreal-Ramos B, McAulay M, Chance V, Martin M, Morgan J, Howard CJ. Investigation of the role of CD8+ T cells in bovine tuberculosis *in vivo*. *Infect Immun* (2003) 71(8):4297–303. doi: 10.1128/IAI.71.8.4297-4303.2003

264. Ramos L, Obregon-Henao A, Henao-Tamayo M, Bowen R, Izzo A, Lunney JK, et al. Minipigs as a neonatal animal model for tuberculosis vaccine efficacy testing. *Vet Immunol Immunopathol* (2019) 215:109884. doi: 10.1016/j.vetimm.2019.109884

265. Ramos L, Obregon-Henao A, Henao-Tamayo M, Bowen R, Lunney JK, Gonzalez-Juarrero M. The minipig as an animal model to study Mycobacterium tuberculosis infection and natural transmission. *Tuberculosis (Edinb)* (2017) 106:91–8. doi: 10.1016/j.tube.2017.07.003

266. Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. *Immunol Rev* (2015) 264(1):182–203. doi: 10.1111/imr.12266

267. Talker SC, Barut GT, Lischer HEL, Rufener R, von Munchow L, Bruggmann R, et al. Monocyte biology conserved across species: Functional insights from cattle. *Front Immunol* (2022) 13:889175. doi: 10.3389/fimmu.2022.889175

268. Jensen K, Gallagher IJ, Johnston N, Welsh M, Skuce R, Williams JL, et al. Variation in the early host-pathogen interaction of bovine macrophages with divergent Mycobacterium bovis strains in the United Kingdom. *Infect Immun* (2018) 86(3). doi: 10.1128/IAI.00385-17

269. Queval CJ, Fearns A, Botella L, Smyth A, Schnettger L, Mitermite M, et al. Macrophage-specific responses to human- and animal-adapted tubercle bacilli reveal pathogen and host factors driving multinucleated cell formation. *PLoS Pathog* (2021) 17(3):e1009410. doi: 10.1371/journal.ppat.1009410

270. Borkute RR, Woelke S, Pei G, Dorhoi A. Neutrophils in tuberculosis: cell biology, cellular networking and multitasking in host defense. *Int J Mol Sci* (2021) 22 (9). doi: 10.3390/ijms22094801

271. Bassel LL, Caswell JL. Bovine neutrophils in health and disease. *Cell Tissue Res* (2018) 371(3):617–37. doi: 10.1007/s00441-018-2789-y

272. Cassidy JP, Bryson DG, Pollock JM, Evans RT, Forster F, Neill SD. Early lesion formation in cattle experimentally infected with Mycobacterium bovis. *J Comp Pathol* (1998) 119(1):27–44. doi: 10.1016/S0021-9975(98)80069-8

273. Hancox M. Latency and the control of bovine TB in man and other animals. *Respir Med* (2003) 97(9):1075–7. doi: 10.1016/S0954-6111(03)00135-5

274. McIlroy SG, Neill SD, McCracken RM. Pulmonary lesions and Mycobacterium bovis excretion from the respiratory tract of tuberculin reacting cattle. *Vet Rec* (1986) 118(26):718–21. doi: 10.1136/vr.118.26.718

275. Bolin CA, Whipple DL, Khanna KV, Risdahl JM, Peterson PK, Molitor TW. Infection of swine with Mycobacterium bovis as a model of human tuberculosis. *J Infect Dis* (1997) 176(6):1559–66. doi: 10.1086/514155

276. Meikle V, Alito A, Llera AS, Gioffre A, Peralta A, Buddle BM, et al. Identification of novel Mycobacterium bovis antigens by dissection of crude protein fractions. *Clin Vaccine Immunol* (2009) 16(9):1352–9. doi: 10.1128/CVI.00211-09

277. Malone KM, Rue-Albrecht K, Magee DA, Conlon K, Schubert OT, Nalpas NC, et al. Comparative 'omics analyses differentiate Mycobacterium tuberculosis and Mycobacterium bovis and reveal distinct macrophage responses to infection with the human and bovine tubercle bacilli. *Microb Genom* (2018) 4(3). doi: 10.1099/mgen.0.000163

278. Remot A, Carreras F, Coupe A, Doz-Deblauwe E, Boschiroli ML, Browne JA, et al. Mycobacterial Infection of Precision-Cut Lung Slices Reveals Type 1 Interferon Pathway Is Locally Induced by Mycobacterium bovis but Not M. tuberculosis in a Cattle Breed. *Front Vet Sci* (2021) 8:696525. doi: 10.3389/fvets.2021.696525

279. Magee DA, Conlon KM, Nalpas NC, Browne JA, Pirson C, Healy C, et al. Innate cytokine profiling of bovine alveolar macrophages reveals commonalities and

divergence in the response to Mycobacterium bovis and Mycobacterium tuberculosis infection. *Tuberculosis (Edinb)* (2014) 94(4):441–50. doi: 10.1016/j.tube.2014.04.004

280. Buddle BM, Vordermeier HM, Chambers MA, de Klerk-Lorist LM. Efficacy and safety of BCG vaccine for control of tuberculosis in domestic livestock and wildlife. *Front Vet Sci* (2018) 5:259. doi: 10.3389/fvets.2018.00259

281. Guerra-Maupome M, Vang DX, McGill JL. Aerosol vaccination with Bacille Calmette-Guerin induces a trained innate immune phenotype in calves. *PLoS One* (2019) 14(2):e0212751. doi: 10.1371/journal.pone.0212751

282. Hayes FM, Haring CM, Traum J. Vaccination of swine against tuberculosis with calmette-guerin culture, BCG. *Hilgardia* (1932) 7:235–61. doi: 10.3733/hilg.v07n06p235

283. Wedlich N, Figl J, Liebler-Tenorio EM, Kohler H, von Puckler K, Rissmann M, et al. Video endoscopy-guided intrabronchial spray inoculation of Mycobacterium bovis in goats and comparative assessment of lung lesions with various imaging methods. *Front Vet Sci* (2022) 9:877322. doi: 10.3389/fvets.2022.877322

284. Figl J, Kohler H, Wedlich N, Liebler-Tenorio EM, Grode L, Parzmair G, et al. Safety and immunogenicity of recombinant bacille calmette-guerin strain VPM1002 and its derivatives in a goat model. *Int J Mol Sci* (2023) 24(6). doi: 10.3390/ijms24065509

285. Liebler-Tenorio EM, Heyl J, Wedlich N, Figl J, Kohler H, Krishnamoorthy G, et al. Vaccine-induced subcutaneous granulomas in goats reflect differences in host-Mycobacterium interactions between BCG- and recombinant BCG-derivative vaccines. *Int J Mol Sci* (2022) 23(19). doi: 10.3390/ijms231910992

286. Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V. The pig: a model for human infectious diseases. *Trends Microbiol* (2012) 20(1):50–7. doi: 10.1016/j.tim.2011.11.002

287. Hlavsa MC, Moonan PK, Cowan LS, Navin TR, Kammerer JS, Morlock GP, et al. Human tuberculosis due to Mycobacterium bovis in the United States, 1995-2005. *Clin Infect Dis* (2008) 47(2):168–75. doi: 10.1086/589240

288. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. *Nature* (2020) 579(7798):265–9. doi: 10.1038/s41586-020-2008-3

289. Lerche NW, Yee JL, Capuano SV, Flynn JL. New approaches to tuberculosis surveillance in nonhuman primates. *ILAR J* (2008) 49(2):170–8. doi: 10.1093/ilar.49.2.170

290. Qiu X, Liu Y, Sha A. SARS-CoV-2 and natural infection in animals. *J Med Virol* (2023) 95(1):e28147. doi: 10.1002/jmv.28147

291. Vandegrift KJ, Yon M, Surendran Nair M, Gontu A, Ramasamy S, Amirthalingam S, et al. SARS-CoV-2 omicron (B.1.1.529) infection of wild white-tailed deer in New York City. *Viruses* (2022) 14(12). doi: 10.3390/v14122770

292. Marques AD, Sherrill-Mix S, Everett JK, Adhikari H, Reddy S, Ellis JC, et al. Multiple introductions of SARS-CoV-2 alpha and delta variants into white-tailed deer in Pennsylvania. *mBio* (2022) 13(5):e0210122. doi: 10.1128/mbio.02101-22

293. Virtanen J, Aaltonen K, Kegler K, Venkat V, Niamsap T, Kareinen L, et al. Experimental infection of mink with SARS-COV-2 omicron variant and subsequent clinical disease. *Emerg Infect Dis* (2022) 28(6):1286–8. doi: 10.3201/eid2806.220328

294. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science* (2020) 368(6494):1016–20. doi: 10.1126/science.abb7015

295. Gupta T, SOmanna N, Rowe T, LaGatta M, Helms S, Owino SO, et al. Ferrets as a model for tuberculosis transmission. *Front Cell Infect Microbiol* (2022) 12:873416. doi: 10.3389/fcimb.2022.873416

296. Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, Molenaar RJ, Munger E, Molenkamp R, et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science* (2021) 371(6525):172–7. doi: 10.1126/science.abe5901

297. Giraldo-Ramirez S, Rendon-Marin S, Jaimes JA, Martinez-Gutierrez M, Ruiz-Saenz J. SARS-CoV-2 clinical outcome in domestic and wild cats: A systematic review. *Anim (Basel)* (2021) 11(7). doi: 10.3390/ani11072056

298. Miller MA, Buss P, Sylvester TT, Lyashchenko KP, deKlerk-Lorist LM, Bengis R, et al. Mycobacterium bovis in free-ranging lions (Panthera leo) - evaluation of serological and tuberculin skin tests for detection of infection and disease. *J Zoo Wildl Med* (2019) 50(1):7–15. doi: 10.1638/2017-0187

299. GroupTC-GS. Tuberculosis and COVID-19 co-infection: description of the global cohort. *Eur Respir J* (2022) 59(3). doi: 10.1183/13993003.02538-2021

300. Jassat W, Mudara C, Ozougwu L, Tempia S, Blumberg L, Davies MA, et al. Difference in mortality among individuals admitted to hospital with COVID-19 during the first and second waves in South Africa: a cohort study. *Lancet Glob Health* (2021) 9(9):e1216–25. doi: 10.1016/S2214-109X(21)00289-8

301. Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases SA. Risk factors for coronavirus disease 2019 (COVID-19) death in a population cohort study from the Western Cape Province, South Africa. *Clin Infect Dis* (2021) 73(7):e2005–e15. doi: 10.1093/cid/ciaa1198

302. Schulte-Schrepping J, Reusch N, Paclik D, Bassler K, Schlickeiser S, Zhang B, et al. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. *Cell* (2020) 182(6):1419–40 e23. doi: 10.1016/j.cell.2020.08.001

303. Petrone L, Petruccioli E, Vanini V, Cuzzi G, Gualano G, Vittozzi P, et al. Coinfection of tuberculosis and COVID-19 limits the ability to *in vitro* respond to SARS-CoV-2. *Int J Infect Dis* (2021) 113(Suppl 1):S82–S7. doi: 10.1016/j.ijid.2021.02.090

304. Riou C, du Bruyn E, Stek C, Daroowala R, Goliath RT, Abrahams F, et al. Relationship of SARS-CoV-2-specific CD4 response to COVID-19 severity and impact of HIV-1 and tuberculosis coinfection. *J Clin Invest* (2021) 131(12). doi: 10.1172/JCI149125

305. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Moller R, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* (2020) 181(5):1036–45.e9. doi: 10.1016/j.cell.2020.04.026

306. Banerjee A, El-Sayes N, Budylowski P, Jacob RA, Richard D, Maan H, et al. Experimental and natural evidence of SARS-CoV-2-infection-induced activation of type I interferon responses. *iScience* (2021) 24(5):102477. doi: 10.1016/j.isci.2021.102477

307. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* (2010) 466(7309):973–7. doi: 10.1038/nature09247

308. Knaul JK, Jorg S, Oberbeck-Mueller D, Heinemann E, Scheuermann L, Brinkmann V, et al. Lung-residing myeloid-derived suppressors display dual functionality in murine pulmonary tuberculosis. *Am J Respir Crit Care Med* (2014) 190(9):1053–66. doi: 10.1164/rccm.201405-0828OC

309. Moreira-Teixeira L, Tabone O, Graham CM, Singhania A, Stavropoulos E, Redford PS, et al. Mouse transcriptome reveals potential signatures of protection and pathogenesis in human tuberculosis. *Nat Immunol* (2020) 21(4):464–76. doi: 10.1038/s41590-020-0610-z

310. Pathak L, Gayan S, Pal B, Talukdar J, Bhuyan S, Sandhya S, et al. Coronavirus activates an altruistic stem cell-mediated defense mechanism that reactivates dormant tuberculosis: implications in coronavirus disease 2019 pandemic. *Am J Pathol* (2021) 191(7):1255–68. doi: 10.1016/j.ajpath.2021.03.011

311. Hildebrand RE, Chandrasekar SS, Riel M, Touray BJB, Aschenbroich SA, Talaat AM. Superinfection with SARS-CoV-2 Has Deleterious Effects on Mycobacterium bovis BCG Immunity and Promotes Dissemination of Mycobacterium tuberculosis. *Microbiol Spectr* (2022) 10(5):e0307522. doi: 10.1128/spectrum.03075-22

312. Rosas Mejia O, Gloag ES, Li J, Ruane-Foster M, Claeys TA, Farkas D, et al. Mice infected with Mycobacterium tuberculosis are resistant to acute disease caused by secondary infection with SARS-CoV-2. *PLoS Pathog* (2022) 18(3):e1010093. doi: 10.1371/journal.ppat.1010093

313. Chiok KR, Dhar N, Banerjee A. Mycobacterium tuberculosis and SARS-CoV-2 co-infections: The knowns and unknowns. *iScience* (2023) 26(5):106629. doi: 10.1016/j.isci.2023.106629

314. Kaufmann E, Khan N, Tran KA, Ulndreaj A, Pernet E, Fontes G, et al. BCG vaccination provides protection against IAV but not SARS-CoV-2. *Cell Rep* (2022) 38(10):110502. doi: 10.1016/j.celrep.2022.110502

315. Hilligan KL, Namasivayam S, Clancy CS, O'Mard D, Oland SD, Robertson SJ, et al. Intravenous administration of BCG protects mice against lethal SARS-CoV-2 challenge. *J Exp Med* (2022) 219(2). doi: 10.1084/jem.20211862

316. White AD, Sibley L, Sarfas C, Morrison AL, Bewley K, Churchward C, et al. Influence of aerosol delivered BCG vaccination on immunological and disease parameters following SARS-CoV-2 challenge in rhesus macaques. *Front Immunol* (2021) 12:801799. doi: 10.3389/fimmu.2021.801799

317. Pittet LF, Messina NL, Orsini F, Moore CL, Abruzzo V, Barry S, et al. Randomized trial of BCG vaccine to protect against covid-19 in health care workers. *N Engl J Med* (2023) 388(17):1582–96. doi: 10.1056/NEJMoa2212616

318. Pennitz P, Kirsten H, Friedrich VD, Wyler E, Goekeri C, Obermayer B, et al. A pulmonologist's guide to perform and analyse cross-species single lung cell transcriptomics. *Eur Respir Rev* (2022) 31(165). doi: 10.1183/16000617.0056-2022

319. Paudyal B, McNee A, Rijal P, Carr BV, Nunez A, McCauley J, et al. Low dose pig anti-influenza virus monoclonal antibodies reduce lung pathology but do not prevent virus shedding. *Front Immunol* (2021) 12:790918. doi: 10.3389/fimmu.2021.790918

320. Martini V, Edmans M, Gubbins S, Jayaraman S, Paudyal B, Morgan S, et al. Spatial, temporal and molecular dynamics of swine influenza virus-specific CD8 tissue resident memory T cells. *Mucosal Immunol* (2022) 15(3):428–42. doi: 10.1038/s41385-021-00478-4

321. Vatzia E, Allen ER, Manjegowda T, Morris S, McNee A, Martini V, et al. Respiratory and intramuscular immunization with chAdOx2-NPM1-NA induces distinct immune responses in H1N1pdm09 pre-exposed pigs. *Front Immunol* (2021) 12:763912. doi: 10.3389/fimmu.2021.763912

322. Keep S, Carr BV, Lean FZX, Fones A, Newman J, Dowgier G, et al. Porcine respiratory coronavirus as a model for acute respiratory coronavirus disease. *Front Immunol* (2022) 13:867707. doi: 10.3389/fimmu.2022.867707

323. Sefik E, Israelow B, Mirza H, Zhao J, Qu R, Kaffe E, et al. A humanized mouse model of chronic COVID-19. *Nat Biotechnol* (2022) 40(6):906–20. doi: 10.1038/s41587-021-01155-4

324. Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH, et al. Laboratory mice born to wild mice have natural microbiota and model human immune responses. *Science* (2019) 365(6452). doi: 10.1126/science.aaw4361

325. Schlottau K, Rissmann M, Graaf A, Schon J, Sehl J, Wylezich C, et al. SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *Lancet Microbe* (2020) 1(5):e218–25. doi: 10.1016/S2666-5247(20)30089-6

326. Zaeck LM, Scheibner D, Sehl J, Muller M, Hoffmann D, Beer M, et al. Light sheet microscopy-assisted 3D analysis of SARS-CoV-2 infection in the respiratory tract of the ferret model. *Viruses* (2021) 13(3). doi: 10.3390/v13030529

327. Zhou B, Thao TTN, Hoffmann D, Taddeo A, Ebert N, Labroussaa F, et al. SARS-CoV-2 spike D614G change enhances replication and transmission. *Nature* (2021) 592(7852):122–7. doi: 10.1038/s41586-021-03361-1

328. Pulit-Penaloza JA, Belser JA, Sun X, Pappas C, Brock N, Kieran TJ, et al. Comparative assessment of severe acute respiratory syndrome coronavirus 2 variants in the ferret model. *mBio* (2022) 13(5):e0242122. doi: 10.1128/mbio.02421-22

329. Friedrichs V, Toussaint C, Schafer A, Rissmann M, Dietrich O, Mettenleiter TC, et al. Landscape and age dynamics of immune cells in the Egyptian rousette bat. *Cell Rep* (2022) 40(10):111305. doi: 10.1016/j.celrep.2022.111305

330. Gamage AM, Chan WOY, Zhu F, Lim YT, Long S, Ahn M, et al. Single-cell transcriptome analysis of the *in vivo* response to viral infection in the cave nectar bat Eonycteris spelaea. *Immunity* (2022) 55(11):2187–205 e5. doi: 10.1016/j.immuni.2022.10.008

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Initial immune response after exposure to *Mycobacterium tuberculosis* or to SARS-COV-2: similarities and differences

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb) and Coronavirus disease-2019 (COVID-19), whose etiologic agent is severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), are currently the two deadliest infectious diseases in humans, which together have caused about more than 11 million deaths worldwide in the past 3 years. TB and COVID-19 share several aspects including the droplet- and aerosol-borne transmissibility, the lungs as primary target, some symptoms, and diagnostic tools. However, these two infectious diseases differ in other aspects as their incubation period, immune cells involved, persistence and the immunopathological response. In this review, we highlight the similarities and differences between TB and COVID-19 focusing on the innate and adaptive immune response induced after the exposure to Mtb and SARS-CoV-2 and the pathological pathways linking the two infections. Moreover, we provide a brief overview of the immune response in case of TB-COVID-19 co-infection highlighting the similarities and differences of each individual infection. A comprehensive understanding of the immune response involved in TB and COVID-19 is of utmost importance for the design of effective therapeutic strategies and vaccines for both diseases.

KEYWORDS

SARS-CoV-2, *M. tuberculosis*, COVID-19, tuberculosis, innate response, T cell response, antibody response, co-infection

Introduction

Coronavirus disease-2019 (COVID-19), whose etiologic agent is severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and tuberculosis (TB), that is caused by the bacterial pathogen *Mycobacterium tuberculosis* (Mtb), are the two-leading causes of death from a single infectious agent in humans. In the past 3 years, SARS-CoV-2 has been responsible for more than 7 million deaths, and Mtb for 4.5 million worldwide (1, 2).

SARS-CoV-2 is an enveloped RNA-based single-stranded virus recently emerged belonging to the Betacoronavirus genus. The first case of COVID-19 dates back to 2019

in Wuhan, China, and it is thought to be the result of a zoonotic spill-over event that likely occurred from bats and humans and finally caused the global pandemic (3). More than 700 million SARS-CoV-2 infections have been reported worldwide (to date, as of June 2023) (1). According to WHO, the largest number of confirmed cases are in Europe, Western Pacific and Americas (Table 1) (1). The spread of the virus was probably aided also by the onset of highly mutated forms of SARS-CoV-2, defined as "variants of concern" (VOCs), with enhanced transmission rate and with relatively lower morbidity and mortality compared to the ancestral strain (94, 95).

On the contrary, Mtb is an ancient slow growing bacterium that has plagued the human population for thousand years. To date, it is estimated that one third of the world population is infected with Mtb (2), and about 5-10% of the Mtb-exposed and -infected individuals will progress to TB disease. In most of them bacilli

TABLE 1 Comparison of the features of SARS-CoV-2 and M. *tuberculosis* in terms of cell tropism, disease development and diagnosis.

Characteristics	COVID-19	Pulmonary TB disease
Etiologic agent	SARS-CoV-2	*Mycobacterium tuberculosis*
Epidemiology	Incidence rate in 2021: 206 million (Africa: 5 million; Americas: 66 million; Eastern Mediterranean: 12 million; Europe: 75.5 million; South-Est Asia: 32.7 million and Western Pacific: 10.7 million) Mortality in 2021: 3.5 million (1)	Incidence rate in 2021: 10.6 million (Africa: 2.46 million; Americas: 309.000; Eastern Mediterranean: 860.000; Europe: 230.000; South-Est Asia: 4.82 million and Western Pacific: 1.89 million) Mortality in 2021: 1.6 million (2)
Incubation period	2-14 days (average 5 days) (1)	From 8 weeks to a lifetime (2)
Time to develop a T cell specific response	From day 5 after infection (4, 5)	From 4-6 weeks on (6, 7)
Correlate of protective immune response	Neutralizing antibodies (8, 9)	Likely T cell-mediated response (10)
Route of transmission	Aerosols, droplets and contaminated surfaces (11–14)	Aerosols and droplets (15, 16)
Cell tropism	Primary targets: respiratory epithelial cells, such as ciliated cells, secretory goblet cells and alveolar epithelial type II cells within the nasal cavity and the upper and lower respiratory tract. Secondary targets: kidneys, small intestines, pancreas, blood vessels, testes and other tissues expressing ACE2 (3, 17, 18).	Primary target: alveolar macrophages, pneumocytes, epithelial cells (19–21) Secondary targets: lymph nodes, central nervous system, bones/joints, genitourinary tract, abdomen (intra-abdominal organs, peritoneum), and pericardium (22–25).
Entry mechanisms	Plasma membrane fusion, endocytic pathway, cell-to-cell transmission (26–28)	Phagocytosis (29, 30)
Main receptors	ACE2 as primary receptor and TMPRSS2 for the activation of the spike protein. Other receptors include integrins, neuropilin 1 (NRP1), phosphatidylserine receptors, the C-type lectins, asialoglycoprotein receptor 1 (ASGR1), Kringle Containing Transmembrane Protein 1 (KREMEN1), and CD147 (3, 26–28, 31).	Dectin-1, the complement receptor 3, TLRs, mannose receptor, the dendritic cell-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN), Fc receptors, scavenger receptors and CD14 (29, 30).
Innate immune response	Early production of type I IFN, IL-1β, IL-6, TNF-α and chemokines. Cytokine storm and late IFN-I production in severe COVID-19 patients (4, 5, 32–34). Neutrophilia, NET generation (35–38)	Early production of IL-1β, IL-1α, IL-6, TNF-α, IFN-γ and chemokines (21, 39). High monocyte/lymphocyte ratio (40)
Adaptive immune response	Lymphocytopenia, increased T cell activation, T cell dysfunctions, neutralizing antibodies (IgM, IgA and IgG) (41–51).	Lymphocytopenia, granuloma formation high T cell activation and finally exhaustion, antibody production (IgG) (52–58).
Detection tools for T cell response	IGRA, Flow cytometry Evaluated antigens: spike, N and M proteins/peptides (45, 59–63)	TST, IGRA, Flow cytometry Evaluated antigens: PPD, ESAT-6, CFP-10, Ag85 B, HBHA, Rv2628, MTB300 proteins/peptides (2, 6, 56, 64–68).
Main evasion mechanisms	Autoantibodies against IFN-I, mutations in spike protein (32, 69–75).. The envelope (E) protein down-regulates the CD1d, an antigen-presenting molecule of invariant NKT (iNKT) cells, and suppresses these cells (76).	Inhibition of phagosome maturation, induction of TLR2 antagonist glycolipids, NET formation for Mtb replication, and suppression of the production of pro-inflammatory cytokines or release of anti-inflammatory cytokines (77–84).

(Continued)

TABLE 1 Continued

Characteristics	COVID-19	Pulmonary TB disease
Clinical manifestation	Cough, fatigue, fever, sneezing, runny nose, sore throat, and anosmia in the first few days followed by shortness of breath, diarrhea, vomiting etc. (1, 85)	Cough, fatigue, fever, weight loss, night sweats, chest pain and hemoptysis (2, 86).
Comorbidities may influence clinical outcome	Old age, hypertension, diabetes, biological therapy based on CD20 inhibitors (1, 85, 87–89).	HIV, diabetes, malnutrition, biological therapy based on TNF-α inhibitors, extreme age (children below 5 age or elderly) (2, 86, 87, 90).
Diagnostics	RT-PCR or rapid antigenic tests (1, 91).	Microscopy, culture, molecular tests such as Gene-Xpert, and chest X-ray (2, 92, 93).
Samples	Naso- and -oropharyngeal swabs and saliva (1, 91)	Sputum or bronchoalveolar lavage (2, 92, 93)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease-19; Mtb; Mycobacterium tuberculosis; ACE2, angiotensin-converting enzyme 2; TMPRSS2, type 2 transmembrane serine protease; TLR, toll-like receptor; N, nucleocapsid; M, membrane; IFNs, interferons; IL, interleukin; TNF, tumor necrosis factor; NET, Neutrophil extracellular traps; Ig, immunoglobulin; IGRA, IFN-γ release assay; TST, tuberculin skin test; PPD, purified protein derivative; ESAT-6, early secretory antigenic target; CFP-10, 10-kDa culture filtrate protein; HBHA, heparin-binding hemagglutinin antigen.

are detectable in the sputum (15). According to WHO, the largest number of confirmed cases are in Africa and South-Est Asia (Table 1) (2).

Although Mtb and SARS-CoV-2 are distinct pathogens, they share several features summarized in Table 1. The main transmission route for both pathogens is *via* droplets (> 100 μm particles) or aerosols (< 100 μm particles) that are expelled by an ill individual by coughing, sneezing, talking, and breathing (11, 16). These particles can travel short distances in the air before being inhaled (12). However, for SARS-CoV-2, the infection can also occur as a result of contact with contaminated surfaces or objects on which virions can persist even for 72 hours (13, 14). Regarding Mtb, infection can also occur during autopsies (96) or during the spill of caseus material, i.e. from a scrofula when the cervical tuberculous lymphadenitis drains the material outside (97–99).

While SARS-CoV-2 shows a short incubation period (2-14 days) before symptoms onset, in Mtb infection it can range from eight weeks to a lifetime (1, 2) (Table 1 and Figure 1).

Considering the route of transmission, it is not surprising that both SARS-CoV-2 and Mtb firstly infect the respiratory system causing symptoms such as cough, fatigue and fever. In addition, SARS-CoV-2-infected subjects also experience sneezing, runny nose, sore throat, and anosmia in the first few days followed by shortness of breath, diarrhea, vomiting, etc. (85), whereas in TB patients weight loss, night sweats, chest pain and coughing up of blood were reported (86). This similarity in symptoms might make the diagnosis difficult; however, in most cases the COVID-19 symptoms are short-lived compared to those of TB, which has a long incubation with long-lasting symptoms duration.

Both agents can be detected in respiratory samples such as nasopharyngeal swab or saliva for SARS-CoV-2, and sputum or bronchoalveolar lavage (BAL) for Mtb.

The diagnosis can require different tools. For SARS-CoV-2 infection, molecular swab is the first choice in case of suspected symptomatic individuals, contacts of confirmed cases with symptoms and for the screening of health workers. In other contexts, it is recommended to use rapid antigenic tests that are less labor-intensive and costly and can provide results in less than half an hour (91) (Table 1).

Regarding Mtb, two main types of tests are used to determine the traditionally called latent infection, now defined "tuberculosis infection" (2): the tuberculin skin test (TST) and interferon (IFN)-γ release assays (IGRA). For patients with suspected pulmonary TB, the Center for Disease Control (CDC) recommends performing an acid-fast-bacilli smear on three different sputum specimens (92). Moreover, Gene-Xpert (Cepheid, Sunnyvale, CA, USA) is a widely accepted diagnostic test for TB detection in direct smear negative cases (93).

Notably, SARS-CoV-2 and Mtb-infected individuals show a diverse spectrum of clinical manifestations. Patients infected with SARS-CoV-2 can experience a clinical outcome ranging from asymptomatic to mild/moderate infection up to severe disease (particularly with Wuhan strain and in those not vaccinated), which can also progress to acute respiratory distress syndrome (ARDS) (1). Indeed, SARS-CoV-2 can interfere with the host immune system leading to hyperinflammatory state, immune dysregulation, and extensive lung damage (100, 101).

Differently, Mtb-exposed individuals remain clinically asymptomatic due to the development of an immune response that controls Mtb replication (102, 103). It has been shown that some individuals heavily exposed to Mtb can clear the infection early before the emergence of the adaptive immune response, can keep a negative score to the TST and IGRA, and therefore do not show any evidence of infection (104). The lack of a detectable adaptive immune response in these resistant individuals suggests the key role mediated by the local innate immunity. The difficulty of treating and eradicating Mtb is related to the ability of the mycobacteria to survive and replicate within human cells.

In both infections, the clinical manifestations may be more severe in presence of comorbidities. In this regard, they share similar risk factors in terms of comorbidities as advanced age (87), and diabetes (90), although they have specific peculiarities as hypertension and biological therapy with CD20 inhibitors for COVID-19 and HIV infection, malnourishment and biological therapy based on TNF-α inhibitors for TB (90) (Table 1).

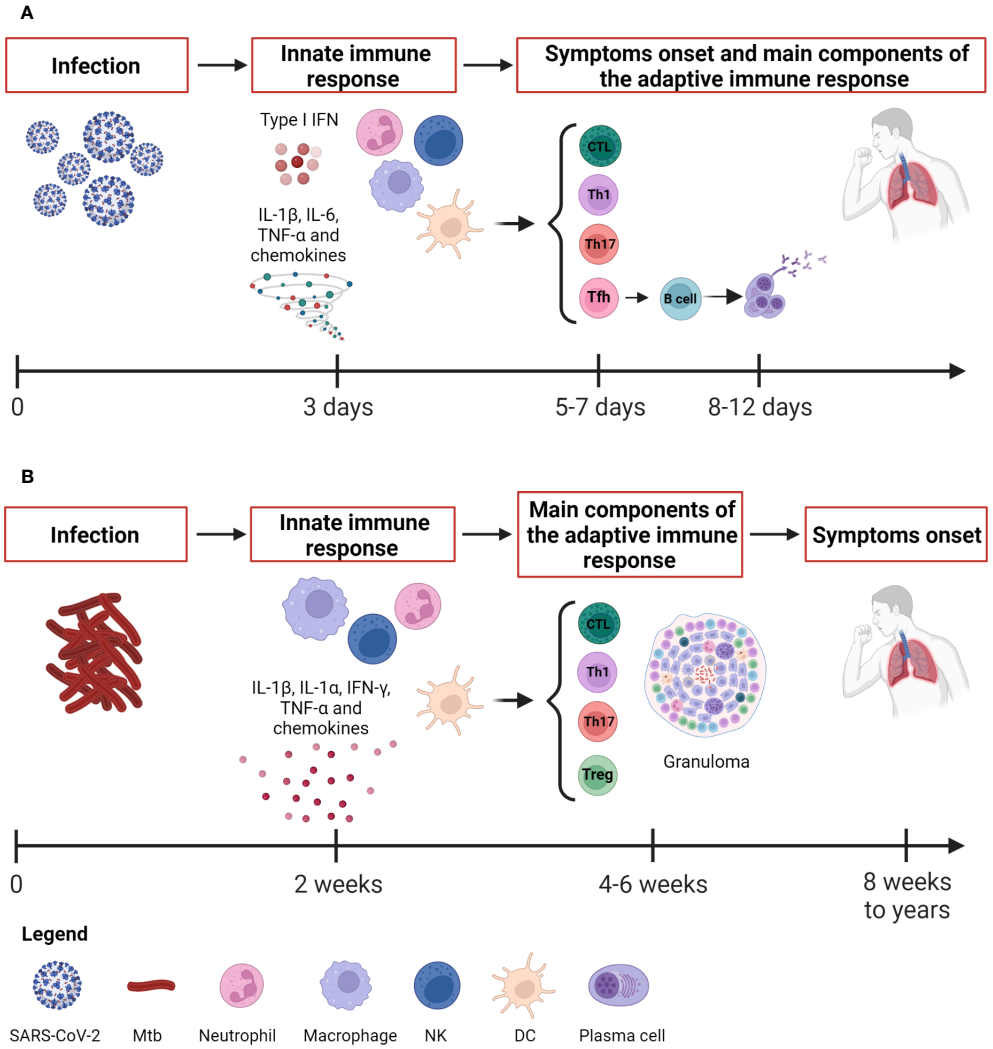

FIGURE 1
Kinetic of the immune response to SARS-CoV-2 and Mtb. **(A)** SARS-CoV-2 infection evolves rapidly. The innate immune response occurs after about 3 days and is detectable through immunoenzymatic assays and flow cytometry. The antigen-specific T cell response appears around 5-7 days concurrently also with the onset of symptoms, whereas the antibody response appears later around 8-12 days. The adaptive immune response is detectable by immunoenzymatic assays, flow cytometry and IGRA. **(B)** Mtb causes a slow-progressing infection that might result in the development of TB disease even after many years. The innate immune response occurs after about 2 weeks and is detectable through immunoenzymatic assays and flow cytometry as for SARS-CoV-2. The antigen-specific T cell response is detectable around 4-6 weeks by means IGRA, TST, immunoenzymatic assays and flow cytometry. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Mtb, *Mycobacterium tuberculosis*; IFNs, interferons; DCs, dendritic cells; NK, natural killer; Tfh, T follicular helper lymphocytes; Th, T helper; IGRA, IFN-γ release assay; TST, tuberculin skin test. Created with BioRender.com.

An effective and timely immune response plays a pivotal role in affecting the clinical course of both COVID-19 and TB. This review aims to provide an overview of innate and adaptive immune responses induced after the exposure to Mtb and SARS-CoV-2 highlighting the similarities and differences of each individual infection and their crosstalk in TB-COVID-19 co-infection.

Cell tropism and entry mechanisms

Viral entry is the first and pivotal step for the viral life cycle. Not surprisingly, blocking virus entry is a primary target of several therapeutic strategies to prevent the subsequent steps and inhibit viral replication and host cell pathology (3). Although both SARS-CoV-2 and Mtb are airborne pathogen entering *via* droplets, and primarily infect the human respiratory system, they differ by cellular tropism and entry mechanisms.

SARS-CoV-2 and cell tropism and entry mechanisms

SARS-CoV-2 has a broad spectrum of tropism. The human angiotensin-converting enzyme 2 (ACE2) represents the major cellular entry point for the virus, thus the expression of ACE2 defines which tissues can be potentially infected by SARS-CoV-2 (3,

26). The epithelial cells such as subset of ciliated cells, secretory goblet cells and alveolar epithelial type II cells within the nasal cavity and the upper and lower respiratory tract, represent the primary targets for the initial infection and spread of SARS-CoV-2. In this regard, the higher amount of viral RNA was found in ciliated and epithelial progenitors (105). Interestingly, although the human respiratory tract is the main target for the virus due to its airborne transmissibility, ACE2 expression in kidneys and gastrointestinal tract is even higher than the lungs (17). Notably, extrapulmonary organs such as the kidneys, small intestines, pancreas, blood vessels, testes and other tissues can be additional targets for SARS-CoV-2, thus explaining the variety of symptoms associated to the infection (17, 18).

SARS-CoV-2 gains access to cells mainly through two possible routes, the plasma membrane fusion and the endocytic pathway. The entry route used by the virus is dependent on the expression of cell surface proteases, which are needed for the activation of the viral protein (27, 28), and it is primarily mediated by the structural protein spike, a trimeric glycoprotein that binds to the ACE2 (26). After binding, spike undergoes a conformational change that allows the proteolytic cleavage before membrane fusion (Figure 2).

Spike activation can occur either at the cell surface or in endosomes and consists of two different proteolytic events. The first proteolytic event occurs during spike biosynthesis and it is mediated by the host pro-protein convertase furin that cleaves the polybasic S1/S2 junction (106) generating the two subunits S1 and S2 non-covalently linked and with different roles in the viral entry (107). The amino-terminal S1 subunit includes a receptor-binding domain (RBD) that is involved in the initial recognition of ACE2 receptor (108), whereas the carboxy-terminal S2 presents highly conserved regions that catalyse the fusion between viral and host cell membranes, crucial to release the viral RNA genome and start the replication in the target cell. A further cleavage at the S2' site is needed to expose the S2' fragment, a highly hydrophobic fusion peptide that starts the fusion of membranes (109, 110).

Interestingly, TMPRSS2, which is a type 2 transmembrane serine protease (TTSPs) expressed in the human upper and lower respiratory tract, heart, prostate and gastrointestinal tracts (111–113), has been shown to prime spikes on cell surface thus allowing the entry *via* membrane fusion (26). In the absence or insufficient availability of cell surface proteases, in particular TMPRSS2, SARS-CoV-2 prefers to enter *via* clathrin-mediated endocytosis (114). In this case, the conformational modifications of the spike occur in the acidic environment of endosomes and its cleavage is mediated by the members of the cathepsin family (e.g. B and L). While the virus takes 10 minutes to enter the cells *via* cell surface membrane fusion, the pH-dependent endocytosis process needs about 40–60 minutes after infection (28).

The cleavage of S1/S2 can have an impact on viral fitness and transmission, thus affecting viral infectivity (115). Notably, during the COVID-19 pandemic, several mutations have accumulated in S1 and S2 subunits of the spike causing the emergence of several SARS-CoV-2 VOCs capable of escaping the immune system, while preserving the steps of activation of the spike protein. The different infectivity rate in the epithelial cells of the nose, bronchi, and lung by SARS-CoV-2 VOC is correlated with the different protease expression, subsequent transmissibility, and severity of disease (18, 87, 116–118).

Emerged Omicron subvariants are less dependent on TMPRSS2-mediated spike activation at the plasma membrane, showing a reduced replication of the virus in the lung and intestinal cultures, while a similar replication rate was observed in the nasal epithelia compared to the Delta variant (117, 119, 120). Likely, this modified tropism allowed a major air transmission of the virus, in accordance with the highest rate of spread observed in the latest variants compared with the ancestral one (69). Moreover, the different spike protease tropism resulted in the diminished pathogenesis in the lung.

Besides TMPRSS2, other TTSPs or metalloproteases can mediate SARS-CoV-2 entry. For instance, TMPRSS2 and TMPRSS4 promote viral entry into human enterocytes of the proximal digestive tract (121), and matrix metalloproteases (MMPs), such as ADAM10 and ADAM17, seem to be involved in the cleavage at the S2 site in cells lacking TMPRSS2 (122–124). Moreover, coagulation factors, such as factor Xa and thrombin, can directly cleave spike protein at both cleavage sites and thus further contributing to infection at the stage of viral entry (125, 126).

Furthermore, other molecules have been suggested as alternative receptors for the SARS-CoV-2 entry process including integrins, neuropilin 1 (NRP1), phosphatidylserine receptors, the C-type lectins, asialoglycoprotein receptor 1 (ASGR1), Kringle Containing Transmembrane Protein 1 (KREMEN1), and CD147, as reviewed by Jackson and colleagues (27, 31).

Notably, SARS-CoV-2 could also infect cells through other mechanisms that allow the virus to escape the immune recognition favoring its spread in the host. In this regard, SARS-CoV-2-infected cells can directly fuse with adjacent cells expressing ACE2 through S1/S2 cleaved SARS-CoV-2 spikes resulting in the formation of multinucleated cells or syncytia (127, 128). The syncytia formation favors a cell-to-cell transmission of the virus without even the need to assemble viral particles or to release the virus in the extracellular environment (129). SARS-CoV-2-induced multinucleated pneumocytes and syncytia formation is a feature of severe COVID-19 patients, suggesting their involvement in the COVID-19 pathogenesis (130–132). Moreover, these structures might cause direct cytopathic effects to lymphocytes. In this regard, Zhang and colleagues reported that lymphocytes could be internalized by syncytia by forming cell-in-cell structures and leading to cell death (133).

Another possible mechanism for viral entry is mediated by extracellular vesicles (EVs) containing particles or viral components well documented in SARS-CoV-2-infected cells (134).

Regardless of the mechanism and molecules involved in SARS-CoV-2 entry, the virus replicates triggering the host immune response.

M. tuberculosis and cell tropism and entry mechanisms

As for SARS-CoV-2, the first interactions between bacteria and host occur in the lungs after the inhalation of the aerosolized Mtb.

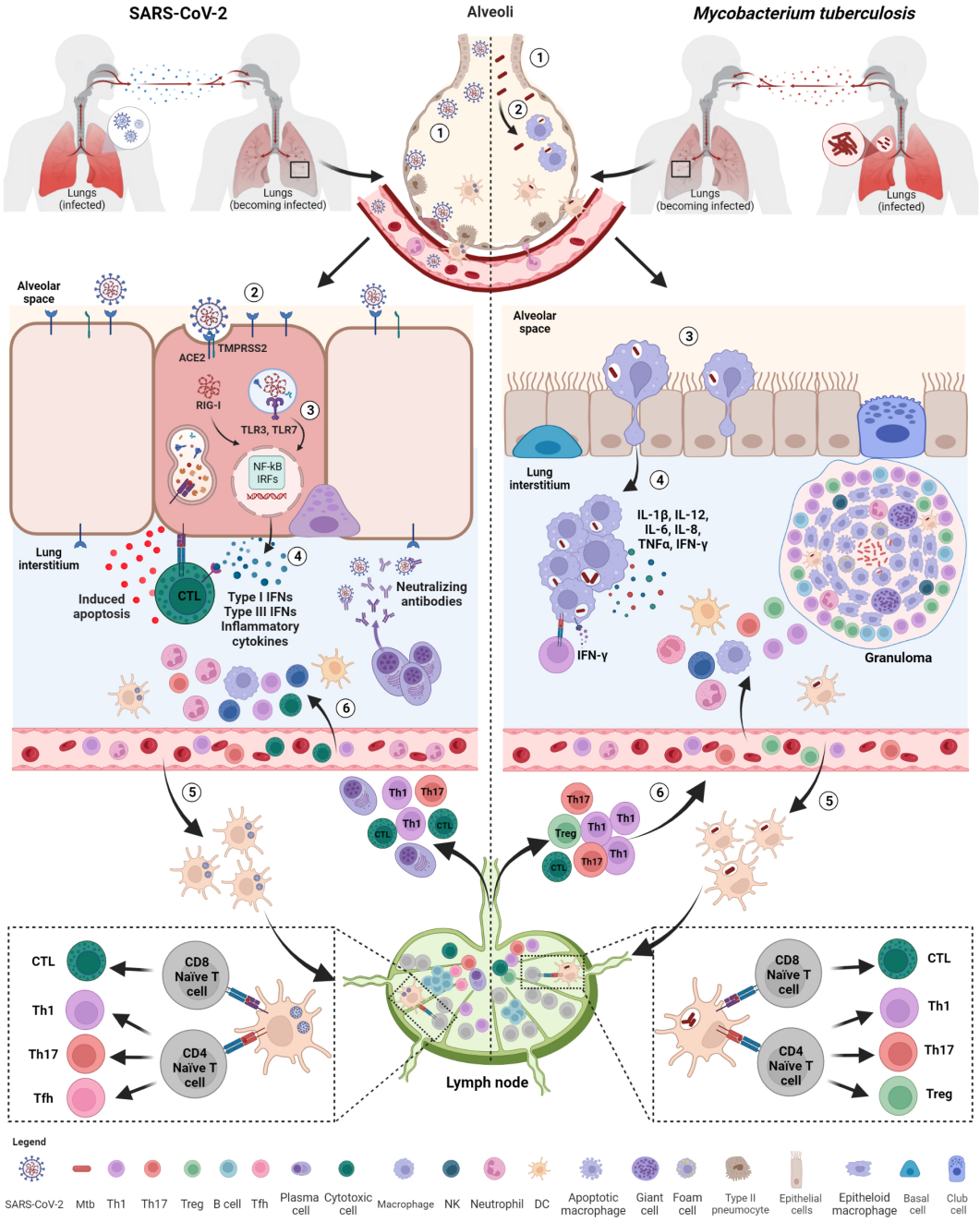

FIGURE 2
Initial immune response after exposure to SARS-CoV-2 and Mtb. Both SARS-CoV-2 and *M. tuberculosis* (Mtb) are transmitted by aerosols or droplets. SARS-CoV-2 infection (1): virions enter into the airways and (2), once arrived in the lung, infect epithelial lung cells *via* recognition and binding of the spike protein to the ACE2 cell receptor. (3) Viral RNA, once released inside the cells, is recognized by endosomal (TLR3, TLR7) or cytosolic (RIG-I) receptors and activate downstream signaling pathways (NF-kB and IRFs) (4) leading to the release of IFNs, pro-inflammatory cytokines and chemokines favoring immune cell recruitment, including neutrophils and DCs. (5) Infected DCs migrate to the lymph nodes for T and B cell priming. (6) Primed T cells and plasma cells go back to the infection site *via* blood where they exert their functions, including apoptosis induced by cytotoxic T cells and viral neutralization. Mtb infection: (1) Mtb bacilli enter into the airways and (2) are phagocytosed by alveolar macrophages. (3) Alveolar macrophages migrate to lung interstitium, where they form aggregates and (4) release cytokines promoting the recruitment of immune cells, such neutrophils, macrophages and DCs. (5) Infected DCs migrate to lymph nodes to prime T cells that are recruited at the infection sites where they contribute to the formation of the organized granuloma. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Mtb, *Mycobacterium tuberculosis*; ACE2, angiotensin-converting enzyme 2; TMPRSS2, type 2 transmembrane serine protease; TLR, toll-like receptor; IFNs, interferons; RIG, retinoic acid-inducible gene-I; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; IRFs, interferon regulatory factors; DCs, dendritic cells; NK, natural killer; Tfh, T follicular helper lymphocytes; Th, T helper. Created with BioRender.com.

The size of Mtb droplets (2–5 μm particles) is important to ensure the passage through the upper respiratory tract into the alveolar space, where bacilli primarily encounter pneumocytes, epithelial cells (AEC), and alveolar macrophages (AMs) with anti-bacterial capacities (19–21). On the other hand, larger droplets can be stuck in the upper airways or oropharynx probably explaining the onset of the extrapulmonary forms of TB localized in the oropharynx but lacking evidence of concurrent pulmonary disease (135).

Once entered into the airways, Mtb is phagocytosed by AMs, which are permissive for infection establishment. In the upper airway, Mtb invades the specialized epithelial cells called microfold cell (M cell) through the binding to the scavenger receptor B1 in both mouse and human tissue (136, 137). Similar to SARS-CoV-2, Mtb can disseminate to other organs including the lymphatics and lymph nodes that are the main sites of extrapulmonary TB (22). Lymphatic endothelial cells, the adipose tissue and the bone marrow have been identified as extrapulmonary niches where Mtb may persist for long time (23–25).

The receptors involved in the Mtb entry into cells have not been fully demonstrated. Phagocytosis of Mtb by macrophages seems not occur *via* a single receptor-mediated pathway, but rather it seems to be mediated by multiple receptors including dectin-1, the complement receptor 3, mannose receptor, the dendritic cell-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN), Fc receptors, scavenger receptors and CD14 (29). Other receptors, such as Toll-like receptors (TLRs) are involved in the recognition of mycobacteria. To enable their entrance into AMs, mycobacteria exploit a group of pathogen-associated molecular patterns (PAMPs) expressed on its surface, including mycobacterial lipoproteins such as the 19 kDa surface antigen LpqH, which acts as an adhesin playing a crucial role in both host-pathogen interactions and pleiotropic immune regulation through the engagement of the TLR1/TLR2 (30). The downstream signaling and the phagosomal fate depend on the type of receptor engaged during the phagocytosis.

Macrophages containing Mtb then migrate from the air space to the lung interstitium in an IL1-R signaling- and ESX-1 secretion system-dependent manner (138, 139). This is the first step preceding the formation of the granuloma, the pathologic hallmark of TB (Figure 2).

Innate immune response

Whereas Mtb causes a slow-progressing infection that might result in the development of TB disease even after many years, the SARS-CoV-2 infection evolves rapidly causing COVID-19 (Figure 1). Within the immunological response to Mtb and SARS-CoV-2, both the innate and adaptive responses play an important role. The innate immune response is a nonspecific response that serves as initial defense against pathogens. It consists of humoral components (cytokines, chemokines, interferons, complement and coagulation-fibrinolysis systems, and naturally occurring antibodies) and cellular components (natural killer cells, macrophages, dendritic cells and other innate lymphocytes). Innate immunity aids in controlling the infection, in the identification and eradication of infected cells as well as in the development of the adaptive immunity (59, 140).

Innate immune response to SARS-CoV-2

The heterogeneous course of SARS-CoV-2 infection depends on the immune response at the early stages of infection (141). Considering the rapid course of COVID-19, the capability of patients with asymptomatic or mild disease to control the infection is likely due to the innate immune response since the adaptive response occurs days later, with the T cell immunity preceding the B cell response occurring after 2 weeks (Figure 1).

Early on, an effective control of SARS-CoV-2 spread depends on the induction of a robust antiviral response and on the ability of alveolar macrophages to eliminate the virus and the infected cells through phagocytosis.

Immune cells resident within the lung recognize SARS-CoV-2 through several pathogen-recognition receptors (PRRs), such as TLRs (TL3 and TLR7), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and inflammasomes. As a result, downstream signaling pathways involving nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and interferon regulatory factors (IRFs) are activated inducing the production of multiple pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, several chemokines (CCL20, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8 and CXCL16) (32, 33) and antiviral IFNs resulting in the initial inflammation. The local innate immune response attracts and activates into the site of infection further innate immune cells such as neutrophils, monocytes, dendritic cells (DCs), natural killer (NK), and innate lymphoid cells aimed to promote viral clearance (142) (Figure 2). Consequently, the combined action of innate immune cells, cytokines, and chemokines may have an impact on the outcome of SARS-CoV-2 infection (143).

Although SARS-CoV-2 induces a pro-inflammatory state, there are reports of reduced IFN release (70, 144); in fact, SARS-CoV-2 is more effective at suppressing IFN responses compared to other respiratory viruses (71). Type I IFN, which includes IFN-α and IFN-β, represents the primary defensive response against viral infections by the induction of antiviral effector molecules encoded by IFN-stimulated genes (ISGs) and immunomodulatory responses (145). In SARS-CoV-2-infected individuals, the presence of a quick type I IFN production soon after infection contributes to protection against critical illness as observed in studies conducted in individuals exposed to COVID-19 cases (4, 5, 34).

On the contrary, if a strong and rapid antiviral response is lacking, the ongoing infection can lead to an exuberant release of cytokines and chemokines that is amplified by the further infiltration of circulating immune cells, finally provoking the so-called "cytokine storm", which can be caused by infectious and non-infectious agents, and which in COVID-19 is responsible for the immunopathology associated with its severe presentation (141). Based on the evidence, individuals with highly compromised IFN-I response, which means no IFN-β and low IFN-α production and

activity due to neutralizing auto-antibodies or inherited errors of type I IFN immunity, do not control the primary SARS-CoV-2 infection and they are more at risk of fatal COVID-19 (70, 146–148). Moreover, a low number and an impaired functionality of plasmacytoid dendritic cells (pDCs), which are the main IFN producers, have been found in bronchoalveolar lavage fluid (BALF) from severe or critical patients compared to the moderate ones (149). Also, a lower frequency of circulating pDCs was found in samples from SARS-CoV-2-infected individuals than in controls (150).

In vitro studies have shown the presence of a huge amount of NF-kB-dependent proinflammatory mediators in BALF (CCL2, CCL3, CCL4, and CXCL10) (151) and in circulation (IP-10, IL-6, and IL-8, IL-1, IFN-γ, IL-17, TNF-α, MCP-1, G-CSF, GM-CSF, IL-1RA, CCL2, CCL3, CCL5, CCL8, CXCL2, CXCL8, CXCL9, and CXCL16) (32, 60, 70, 152–155).

Patients with COVID-19 generally show migration of neutrophils and monocytes into the nasopharyngeal mucosa in response to chemokines released by infected epithelial cells (e.g. CXCL1, CXCL3, CXCL6, CXCL15, CXCL16, and CXCL17) (156).

Once reached the lung, neutrophils as phagocytes may exert a protective role in the clearance of the infection by secreting leukotrienes, reactive oxygen species (ROS), and forming neutrophil extracellular traps (NET), which are aggregates of extracellular DNA, histones, microbicidal proteins and proteases aimed to entrap and kill pathogens. However, neutrophils are known to be implicated in COVID-19 pathology as hyperinflammation drivers through increased cytokine production and cell degranulation (35). Indeed, their extensive and prolonged activation causes an hyperinflammatory environment and cellular infiltrations that may result in the tissue damage observed in the ARDS and increased mortality (36, 37). Indeed, a high neutrophil-to-lymphocyte ratio (NLR), that is a marker of inflammation and infection, and NET DNA complexes have been found in severe COVID-19 compared with mild/moderate cases or healthy controls (38).

In addition to NET generation, another source of hyperinflammation associated with COVID-19 is the activation of the NLRP3-inflammasome due to the interaction of the nucleoprotein (N) with NLRP3 (157). In this regard, a study conducted in an ACE2 humanized mouse model of COVID-19 showed that, in response to infection, macrophages activate inflammasomes causing the release of IL-1β and IL-18 and undergo pyroptosis, thus favoring the pathogenesis of acute lung injury (158).

During SARS-CoV-2 infection, monocytes/macrophages are involved either as virus target or as producer of inflammatory cytokines and undergo phenotypical changes (159). Alterations in the phenotype of monocytes consisting of reduced antigenic presentation and dysregulated immune response have been observed (35). In the peripheral blood of COVID-19 patients there are cell subsets of mixed M1/M2 macrophages secreting IL-6, TNF-α and IL-10 and characterized by higher expression of CD80 and CD86 (35, 160–162).

NK cells are innate lymphocytes that are recruited along with macrophages and neutrophils in the lungs as confirmed by the analysis on BALF samples of COVID-19 patients (163). NK cells usually exert an antiviral activity through the production of the effector cytokines IFN-γ and TNF-α and limit tissue fibrosis (164). Regarding the protective role of NK cells against infection, Witkowski and colleagues reported that SARS-CoV-2-infected individuals with a higher NK cell number at hospitalization showed a more rapid clearance of viral load (165). Although during early stages of infection NK cells may contribute to control viral replication and dissemination, their migration in affected tissue may favor the enhancement of inflammation. In this context, a reduced peripheral cell count and functional impairment of NK cells with an enhanced expression of the cytolytic proteins perforin and granzyme B have been found in patients with severe COVID-19 (166–168).

CD1d-restricted NKT cells are other types of innate lymphocytes that are involved in antiviral immunity (169). To counteract their function, the envelope (E) protein of SARS-CoV-2 reduces the expression of the antigen-presenting molecule CD1d thus inhibiting the activation of innate NKT cells and enhancing SARS-CoV-2 virulence (76).

The activation of the innate immune system is essential to mount an effective adaptive immune response. In this regard, DCs, as professional antigen presenting cells (APCs), represent a point of junction between innate and adaptive immune response as they migrate to lymph nodes to activate naïve T lymphocytes (170).

Innate immune response to M. tuberculosis

The innate immune response to Mtb infection is multifaceted with several different cell types and functions involved. Upon pattern recognition, a variety of cellular functions, including phagocytosis, autophagy, and apoptosis will be launched by the host to clear or control Mtb (171–173). In particular, macrophages with antimicrobial mechanisms such as nitric oxide synthesis and antimicrobial peptides such as cathelicidin represent the first defense line against Mtb infection (174).

The investigation of the early events and host responses against Mtb in humans is very challenging and difficult as the progression of infection is generally slow and individuals often do not know the exact time of exposure or infection (175). Therefore, a validated model that recapitulates TB in human lungs is critical to support TB research. In this regard, a number of *in vitro* systems (176), spheroids (177), human airway organoids (178), and experimental animal models of TB such as zebrafish (179), mouse (180), guinea pig (181), rabbit (182) and rat (183) have provided new insights into the local events that occur during few days and weeks post Mtb infection. In particular, Mtb infection in nonhuman primates closely recapitulates human TB and these models can be used to study the full spectrum of infection outcome and pathology of TB (184).

Early in infection, the infected cells are activated and start to release some early mediators of inflammation such as TNF-α, IL-1α, IL-1β, IFN-γ and chemo-attractant molecules (e.g. CXCL5, CXCL8), some of which also characterize the early stages of SARS-CoV-2 infection (Figure 2). These soluble factors mediate the

recruitment to the site of infection of different blood cell types including neutrophils, monocytes, macrophages and DCs (21, 39), which are necessary for starting early granuloma formation (139). These innate granulomas include cells that are not yet fully activated, thus favoring the dissemination of mycobacteria from infected macrophages to uninfected cells.

Notably, the EVs released from infected cells containing mycobacterial components, including lipoarabinomannan, the Ag85 complex and lipoproteins, have been shown to contribute to the migration of immune cells to the lungs (185). Moreover, EVs can modulate immune response by promoting the release of proinflammatory cytokines and by increasing autophagy and superoxide production (185, 186).

During the first 10 days post-infection, Mtb almost exclusively resides and replicates inside AMs, suggesting that these cells provide an early niche for Mtb growth (139, 187, 188). In a murine model of TB, Mtb was reported to be equally distributed between AMs, DCs and neutrophils 14 days post-aerosol challenge (189).

As already mentioned for SARS-CoV-2 and also known for other infections including Mtb, DCs play a crucial role by transporting bacteria from the site of infection to the draining lymph nodes (64) in order to prime naïve T cells and start an adaptive immune response (190, 191). An involvement of $CCR2^+$ inflammatory monocytes in the Mtb delivery to pulmonary lymph nodes has also been reported (192). Notably, a higher monocyte/lymphocyte ratio is observed in Mtb-infected patients (40).

Neutrophils are other professional phagocytes that have been shown to be involved in the early innate immune response against Mtb through a direct antimicrobial activity and chemokines/cytokines production (193). They readily phagocytose Mtb and can destroy it *via* ROS, proteases and antimicrobial peptides (AMPs). They can also undergo apoptosis and microbe-containing apoptotic neutrophils can be phagocytosed by macrophages and DCs and then transported to the lymph nodes (194, 195).

In addition, mucosal-associated invariant T cells (MAITs) are a group of T cells restricted to a nonclassical molecule MR-1 and not to the classical major histocompatibility complex (MHC) molecules. MAITs are also involved in the early responses to Mtb by producing IFN-γ and TNF-α, and showing cytotoxic activity upon recognition of microbe-derived riboflavin metabolites (196).

Moreover, there is evidence for a role of NK cells in controlling Mtb infection, by killing the pathogen through antibody-dependent cellular cytotoxicity, directly targeting the Mtb by binding to cell wall components such as mycolic acid, arabinogalactan, peptidoglycan through receptors including TLR-2, NKp44, NKp46, and NK group 2D (NKG2D), promoting the maturation of phagolysosome and phagocytosis by producing cytokines such as IFN-γ and TNF-α and by killing Mtb-infected macrophages through the release of granules (perforin, granulysin, and granzyme) (175, 197–199). However, it is not well known whether the role of NK cells is as important as that of macrophages or cytokines such as IFN-γ or TNF-α.

Nonetheless, Mtb has evolved several strategies to evade the host's immune system through its unique cell wall structure, intracellular survival, dormancy and the ability to modulate immune response. Mtb has adapted to survive and replicate in macrophages by inhibiting phagosome maturation (77–80) and promoting necrosis over apoptosis (200). Several types of programmed necrosis in response to Mtb infection, such as inflammasome-mediated pyroptosis and NET-associated NETosis have been identified (201–203). However, NETosis may facilitate the interactions between neutrophils and other immune cells rather than killing Mtb directly (81). Moreover, the formation of NETs can be induced *via* type I IFN signaling to favor MTB replication (82). Mtb inhibits also innate immune response by induction of TLR2 antagonist glycolipids (83). It also modulates the immune response through the release of molecules that suppress the production of proinflammatory cytokines or even by inducing the production of anti-inflammatory cytokines (84).

As for SARS-CoV-2, the control of Mtb infection requires a timely innate response as well as an effective adaptive response.

Adaptive immune response

The adaptive immune response comprises antibody and cell-mediated responses and takes approximately 2 to 3 weeks before we can measure it (59). It is involved in the specific recognition of pathogens and in the establishment of the immunological memory. Notwithstanding the importance of innate responses, a coordinated cellular immunity is crucial for disease control in both SARS-CoV-2 and Mtb infection.

T cell response to SARS-CoV-2

In the majority of cases, SARS-CoV-2 infection induces adaptive antigen-specific responses, viral clearance and immunological memory finally resulting in an asymptomatic or mild disease. However, a failure of the first line defense mechanisms, particularly of innate IFN, may act as triggering factor for viral proliferation and immune dysregulation. Indeed, the delayed/ineffective adaptive responses and exaggerated inflammatory response can promote immunopathogenesis of COVID-19, particularly ARDS (204–207).

Several lines of evidence from both human studies and animal model systems have shown that an effective T cell response is required to control and eradicate SARS-CoV-2 infection by releasing cytokines and other anti-inflammatory factors (208).

During the infection, subepithelial DCs present SARS-CoV-2-specific peptides through MHC class I and II molecules on the cell surface, thus promoting the activation of $CD8^+$ and $CD4^+$ T cells, respectively, which migrate to the lung after antigen exposure. Indeed, the lung is characterized by the presence of tissue-resident T cells with a memory phenotype ($CD69^+$, $CD103^{+/-}$, $CD45RA,CCR7^-$) originated from the priming of naïve T cells (209). Interestingly, an involvement of EVs in the regulation of antigen presentation and T cell activation has also been reported (210).

While $CD8^+$ T cells recognize and kill the infected cells, $CD4^+$ T cells contribute to activate B cells for antibody secretion and $CD8^+$

T cells to exert the cytotoxic activity, and to produce cytokines that favor immune cell migration at the site of infection (143).

Initial studies conducted by Grifoni and colleagues, and subsequently confirmed by others showed $CD4^+$ and $CD8^+$ viral specific T cell responses in most infected individuals mainly against spike antigen, although present also against other structural (nucleocapsid and membrane proteins) and non-structural SARS-CoV-2 antigens (61–63). Since spike protein has been identified as the most immunogenic antigen, it has been employed for many of the currently used SARS-CoV-2 vaccines (61, 62).

Unlike Mtb infection, the early development of antigen-specific T cell responses is generally observed within 7 days after the onset of COVID-19 symptoms, peaks at 14 days and may be detectable even if SARS-CoV-2 specific antibodies are lacking (5) (Figure 1). Several studies have shown that asymptomatic or pauci-symptomatic individuals are characterized by a strong SARS-CoV-2-specific $CD4^+$ T cell response (41, 211–213). Surprisingly, $CD4^+$ T cell responses were also observed in 40% to 60% of unexposed individuals likely because of the cross-recognition between SARS-CoV-2 and other "common cold" coronaviruses (63).

T cell activity has been associated with a less disease severity (8, 59). The critical role played by T cells in the protection against the severe disease has been highlighted also with the occurrence of different VOCs with an increased ability to escape neutralizing antibodies (214–216). Indeed, the spike-specific T cell response induced by both vaccination and natural infection seems to be not affected by the amino acid mutations that characterize the VOCs, including Omicron, in healthy subjects and in the vulnerable populations (217–221). Indeed, the availability of thousands of SARS-CoV-2 epitopes that may be recognized by T cells makes unlikely that the virus may successfully escape the T cell response by mutating the epitopes.

SARS-CoV-2 infection mainly support the differentiation of $CD4^+$ T lymphocytes toward T helper 1 (Th1), T helper 17 (Th17) and T follicular helper (Tfh) cells (Figure 2).

An appropriate Th1 immune response is necessary for protection against COVID-19, as an early and rapid expansion of IFN-γ-secreting SARS-CoV-2-specific T cells was detected over the course of acute infection and was associated with viral clearance (42, 222) and mild disease (43, 223, 224).

Chauss and colleagues showed that asymptomatic SARS-CoV-2-infected individuals present in the BALF $CD4^+$ T cells switched from a predominantly pro-inflammatory Th1 phenotype toward an IFN-γ and IL-10-producing phenotype that enable them the viral control without causing pathology (225). The mechanism behind the switching phenotype is triggered by cell-intrinsic complement that orchestrates an autocrine/paracrine autoregulatory vitamin D (VitD) loop to initiate Th1 shutdown. During this process, Vitamin D induces epigenetic changes in the $CD4^+$ T cells and recruits transcriptional factors, including c-JUN, STAT3 and BACH2 finally resulting in the switch off of Th1 programs and in the IL-10 induction (225). In patients with severe COVID-19 these regulatory processes are lacking and thus exacerbated Th1 cytokine profiles are prevail (226).

The lack of a fine-tuned Th1 immune response can cause an exacerbated reaction that precedes cytokine storm promoting the differentiation of Th2 cells that are related to a poor prognosis (227). In this regard, Gil-Etayo and colleagues observed in COVID-19 patients a significant reduction in the percentage of Th1 and Th17 cells whereas a higher frequency of activated Th2 cells. Moreover, a higher number of senescent Th2 cells together with higher levels of IL-15 were observed in patients with a fatal outcome (227).

In addition, Th17 cells are strongly activated in severe COVID-19, thus favoring cell-mediated immunopathology through the production of IL-17 and GM-CSF (44). IL-17 released by Th17 cells induces the activation of monocytes/macrophages, DCs, and neutrophils which, in turn, increases the release of cytokines (IL-1, IL-6, IL-8, IL-21, TNF-α, and MCP-1), thus promoting the cytokine storm (44).

It has been reported that the polarization of $CD4^+$ T cells toward Th17 instead of Th1 can be promoted by neutrophils as well as by the up-regulation of pro-inflammatory cytokines IL-1β, IL-6 and IL-23 (228).

Tfh cells are localized within the germinal centers of the secondary lymphoid organs and they are primarily involved in the activation and proliferation of B cells, and the production of high affinity antibodies (59) as well as in the assistance of $CD8^+$ T cell functions (229).

In rhesus macaques $CD8^+$ T cells are crucial for viral clearance especially when a reduced humoral response is present (230). In this regard, a weak $CD8^+$ T cell response has been associated with a poor prognosis (45, 231). Indeed, a delayed or lacking $CD8^+$ T cell response was found in patients with severe or fatal outcomes probably due to the inability of T cells to rapidly limit viral replication (59).

Besides $CD4^+$ and $CD8^+$ T cells, regulatory T cells (Treg) have been shown to play a critical role in SARS-CoV-2 infection, particularly as regulators of the inflammatory response. Perturbations in Treg phenotype, such as the reduced expression of Foxp3 and cytokines including IL-10 and TGF-β, have been associated with disease severity (232).

Quantitative and/or functional deficiency of T cells is associated with pathological processes responsible for tissue damage. Indeed, a characteristic hallmark of severe COVID-19 is the peripheral lymphopenia accompanied by a reduced count of monocytes, eosinophils, basophils, but not neutrophils (46). Possible explanations for T cell depletion is the SARS-CoV-2 infection of T cells through the binding of the spike protein to the CD147 or CD26 expressed on cell surface (233), their recruitment to infected site, or their apoptosis *via* Fas/Fas ligand or TNF (234–237). Furthermore, increased levels of IL-6, IL-10 and TNF-α may contribute to lymphopenia (47, 238). The prolonged peripheral lymphocytopenia increases the risk of secondary bacterial infections (88). Also, an immunosuppression following hyperinflammation in COVID-19 disease has been described, in particular NLRs and TLRs were shown to be associated to immunosuppression (239).

A reduced number of peripheral Treg cells has also been observed in severe cases of COVID-19, likely leading to the development of lung pathology (232).

As COVID-19 progresses, a different T cell functionality has also been observed. Early during the acute phase of SARS-CoV-2

infection, T lymphocytes are characterized by a highly activated cytotoxic phenotype, whereas in convalescent individuals they show a polyfunctional and memory phenotype (41, 44, 47, 240). $CD8^+$ T cells expressing markers of exhaustion such as $PD\text{-}1^+$ $TIM3^+$ increase over the infection and this scenario seems to be related to IL-10 blood levels. The hyper-activation of T cells along with the dysfunctionality of DCs and Tregs may increase the overwhelming alveoli inflammation and cytokine storm in COVID-19 (241).

In light of what is reported in literature, an efficient T cell response is fundamental for viral clearance.

Antibody response to SARS-CoV-2

The antibody response usually appears by 1-2 weeks later than SARS-CoV-2 specific T cell response that is detectable 5-6 days post-infection (4, 5) (Figure 1). Within few days post-infection, B cells are rapidly activated in extrafollicular foci to differentiate in short-lived plasma cells that predominantly produce IgM antibodies but also IgG or IgA-switched to initially stem viral infection, while waiting for the production of antibodies with higher affinity. The first IgM, IgA and IgG are measurable in the sera between 8 and 12 days after symptom onset (48). Subsequently, within the germinal centers in the secondary lymphoid organs, antigen-specific B cells undergo somatic hypermutation and isotype-switching resulting in the production of high-affinity IgG antibodies that mainly recognize nucleocapsid and spike proteins (242, 243). Cross-sectional and longitudinal studies showed that Enzyme-linked immunosorbent assay (ELISA) titers and neutralizing antibodies are detectable around 14 days after symptom onset, peak in 3 to 4 weeks, and decline subsequently causing a reduction of protection and increasing the risk of SARS-CoV-2 re-infection (9, 49, 50, 244).

However, it has been observed that anti-RBD antibodies, neutralizing activity and RBD-specific memory B cells are mostly stable between 6 and 12 months after infection (245, 246), likely owing to the presence of a long-lived plasma cell compartment located in the bone marrow (247–249).

The protective role of the antibodies is limited to those specific for the viral spike protein because they neutralize the virus by hindering the binding between spike and ACE2 receptor and thus blocking its entry, and by promoting effector functions *via* the binding to the complement and Fc receptors (250).

In the case of neutralizing antibodies, the engagement of Fc receptors can potentiate neutralization (251, 252). Non-neutralizing antibodies may promote antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). In this regard, high ADCC activities are detected mainly in hospitalized patients and showed a kinetic similar to antibody titers with a peak at 2-4 weeks post-infection followed by a gradual decline (253–255).

Most of the antibodies are directed against epitopes localized in the receptor-binding motif (RBM) within the RBD of spike, whereas a minority is directed against the N-terminal domain (NTD) (256–258). Anti-NTD antibodies have less neutralizing activity than anti-RBD antibodies and they may act by interfering with the conformational changes necessary for fusion or binding to receptors such as transmembrane lectins DC-SIGN, L-SIGN and SIGLEC1 (259, 260).

The antibody response, either qualitative and quantitative, is dependent on the amount of the antigen and on the activity of the germinal centers. In this regard, patients with severe COVID-19 show higher titers of total and neutralizing antibodies than mild or asymptomatic patients, likely due to the stronger antigen response (261, 262). On the other hand, individuals undergoing B-cell depleting therapies, such as anti-CD20, show an impaired antibody response that is associated with a more severe course of COVID-19 (263).

While circulating antibodies may help to control viral dissemination within the host, mucosal antibodies such as the dimeric form of IgA that is secreted in the upper respiratory tract, play an important role in preventing the transmission of SARS-CoV-2, present a stronger neutralizing activity than circulating antibodies, and contribute to protection against re-infection (51, 264). Indeed, SARS-CoV-2 specific IgA have been found in saliva samples collected from infected individuals (249).

During SARS-CoV-2 infection, also autoantibodies targeting self-antigens, including type I IFN, were identified in some COVID-19 patients, particularly in those with a severe disease that are characterized by a reduced IFN production, as mentioned above (32, 70, 71). COVID-19 patients are also characterized by changes in B-cell subpopulations. In particular, increased number of proliferating, metabolically hyperactive plasma blasts and reduction of memory B cells have been found in patients with severe disease, whereas they disappeared with convalescence (261, 265, 266).

Nonetheless, SARS-CoV-2 has evolved different strategies to escape the immune response. Unlike bacteria such as Mtb, RNA viruses are usually characterized by high mutation rates. SARS-CoV-2 exploits this ability to accumulate mutations in the spike protein in order to avoid the immune recognition by neutralizing antibodies and to increase its transmissibility (69). In particular, the emerging VOCs has accumulated mutations mainly located in the RBM, in part due to the pressure exerted by the host immune system. It has been proposed that the concurrent onset of multiple mutations in the spike protein might occur during the prolonged infection in immunocompromised patients resulting in the emergence of variant strains (72, 73). These mutations increased affinity of the virus for the ACE2 receptor and improved its ability to evade the neutralizing antibody response induced by natural infection or following vaccination with the spike protein derived from the ancestral strain (74, 75).

Altogether, the humoral response has been shown to play a crucial role in the host immune protection against SARS-CoV-2 together with the T cell response.

T cell response to M. tuberculosis

The infected monocytes, macrophages and DCs are thought to be key elements leading to Mtb dissemination and granuloma formation (39, 267). The infected professional antigen-presenting DCs travel to the lung draining lymph nodes where priming of

naïve $CD4^+$ and $CD8^+$ T cells is initiated (52, 64, 190, 191, 268). Priming is a critical step for the initiation of the adaptive immunity that is crucial to hinder bacilli dissemination and control the infection. However, the adaptive (T cell) response takes longer to appear in infected hosts because Mtb or its antigens are transported late into the lymph nodes for T cell priming (269). In mice this occurs within 2-3 weeks post-infection (64, 65, 270), but in humans and non-human primates Mtb-specific T cell response in the periphery, measured as a response to TST, or IGRA, is usually not detectable until 4–6 weeks post-infection (6, 7).

It was found that Mtb-infected DCs in the lymph node are capable to release soluble and intact Mtb antigens that can be caught by uninfected DCs and efficiently presented to naïve $CD4^+$ T cells to optimize $CD4^+$ T cell priming and to initiate the adaptive immune response (271). Surprisingly, the capacity of Mtb-infected DCs in activation and proliferation of naïve Mtb-specific $CD4^+$ T cells in the murine lymph node was found to be impaired likely due to lower MHC class II-peptide presentation by these infected APCs (189).

The primed T (and likely B) lymphocytes can then move to the site of infection and contribute to the formation of the organized granuloma that consists of modified macrophages as epithelioid cells and multinucleated giant cells accompanied by neutrophils and DCs in the center, infiltrated immune cells including granulocytes, antigen-specific T cells and few B cells in the periphery, with variable degrees of fibrosis or central caseous necrosis (Figure 2) (272, 273). Although the mechanisms driving protection and pathology within the granuloma microenvironments are still poorly understood, such mechanisms can be very important for the prognosis, and outcome of the disease (52).

Notably, granuloma structure and function protect the host from the dissemination of the infection, but it is also a way to facilitate the persistency of the infection (274). In fact, sterilizing immunity following Mtb infection is rare and even in the presence of a robust adaptive immune response to Mtb, the nature of the granulomas as well as the immune escape mechanisms of Mtb can restrict the host immune response to reliably eliminate the infection. This leads to develop a controlled infection, traditionally called latent infection, in most infected individuals. Mtb can survive in a dormant (non-replicating) state favored by hypoxic conditions inside solid granulomas that makes it difficult to be detected by the immune system (53, 275).

Within the granuloma, Mtb antigens persistently stimulate immune cells leading to immune activation, chronic inflammation, and finally cell exhaustion (54). Different T cell types and functions can exert a beneficial or even detrimental role. The peripheral localization of T cells restricts their access to the central core of the granuloma, where Mtb-infected macrophages reside, and this can limit the interactions between macrophages and lymphocytes. Moreover, a Mtb-induced immunosuppressive environment has been indicated in the granuloma in which IL-10 impairs Th1 activity and lysis of infected macrophages (276).

The important role for T-cell immunity and particularly IFN-γ-producing Th1 in controlling Mtb infection has been demonstrated in humans (10) and animal models (277, 278). IFN-γ is a key factor involved in $CD4^+$ T cell-mediated protection by increasing autophagy and promoting phagosome maturation in macrophages (79) inducing the production of antimicrobial peptides (279), and limits the accumulation of non-protective $CD4^+$ T cells in the lung vasculature (280).

In humans, HIV infection appears to be an important risk factor for TB disease progression likely due to $CD4^+$ T cell depletion (10, 90). Also, depletion of $CD4^+$ T cells in cynomolgus macaques with acute Mtb infection leads to exacerbated disease in most animals (278). Moreover, TB disease increases HIV replication, *in vivo* and *in vitro* through a mechanism of immune activation (281, 282).

The activation and proliferation of antigen-specific naïve $CD4^+$ T cell subsets strongly depends on the cytokine milieu released by APCs. Particularly, macrophages are the main source of IL-1β, IL-6, IL-18, TNF-α, IL-10, and TGF-β, while DCs are the main producers of IL-12, IL-23, IL-27 and IFN-β (39). For instance, IL-12 produced by DCs differentiates naïve $CD4^+$ T cells to Th1 which promote activation of the cell-mediated immunity needed to counteract intracellular pathogens (55). These cells secrete pro-inflammatory cytokines such as IL-2, IFN-γ and TNF-α to activate macrophages and cytotoxic $CD8^+$ T cells (283). TNF-α is known to be necessary for the formation of a well-organized granuloma and host protection, as confirmed by the higher risk of developing TB disease and disseminated infection in subjects who underwent anti-TNF-α treatment (284, 285).

Activated cytotoxic $CD8^+$ T cells and macrophages kill and eliminate pathogens and infected host cells by cytotoxic effector molecules such as perforin, granzymes and granulysin and by death receptor/ligand ligation (286).

Furthermore, IL-23 produced by DCs drives differentiation and functionality of Th17 cells that produce IL-17 which is a cytokine involved in neutrophil recruitment (287). IL-17 signaling appears to be essential for recruiting neutrophils to the site of infection early after Mtb infection in murine models (288), but a dysregulated production of this cytokine was also found to be associated with immunopathology driven by excess neutrophil recruitment and inflammation (289, 290).

Although inflammation is required for an effective immune response against harmful pathogens, the balance between pro- and anti-inflammatory cytokines is critical to control the disease and lung damage during Mtb infection (56, 291). Anti-inflammatory cytokines such as IL-4, IL-5, IL-13 released by Th2 cells and IL-10 and TGF-β by regulatory T cells are needed to suppress inflammation during immune response. However, these cells may promote long-term persistence of Mtb by favoring active immunosuppression rather than the expected tissue repair response (292).

In patients with TB disease, TST positive, *in vitro* PPD stimulation induced the production of IL-10, IFN-γ, and cell proliferation, whereas in those TST-negative PPD induced IL-10 but not IFN-γ release, without cell proliferation (293).

Altogether, a better understanding of the dynamically balanced immune response is fundamental for therapeutic strategies and subsequently for vaccine development.

The role of B cells and antibodies in TB

Although Mtb infection induces strong antibody responses, the role of antibodies and B cells in TB has not been fully elucidated. Previous studies on B cell depletion have failed to definitively establish a role for these cells or antibodies in Mtb infection and control, although recent studies have demonstrated potentially protective roles of antibodies in humans and non-human primates (NHPs) after intravenous bacille Calmette-Guérin (BCG) vaccination (294, 295).

It has been shown that TB disease is associated with decreased B cell count and function compared with individuals who are infected with Mtb but without any clinical symptoms, suggesting that TB patients may be less able to develop successful antibody responses against Mtb (296–298).

Moreover, distinct glycosylation patterns on the Fc part of the antibodies (296), and isotype skewing to less potently immune-activating variants like IgG4 have been considered for this altered functional response (298, 299).

Surprisingly, heavily Mtb-exposed individuals who "resisted" to infection showed higher antibody functionality compared to those with TB infection, indicating an important role of antibodies in early protective immunity (300, 301).

Studies have shown that the interaction of Mtb with macrophages can be affected by antibodies in a variety of ways (57, 58). For instance, bacterial opsonization may alter vesicular trafficking and macrophage signaling. Moreover, the binding of antibodies to Fc receptors (activator or inhibitory) on macrophages can modulate their function (58).

Together, data suggest that B cells and antibodies may play an important role in protective immunity against mycobacterial infections; however, the diversity of antibody functions, the heterogeneity of the humoral immune response to Mtb, as well as the complexity of the interactions between B cells and other immune cells have been indicated as the major challenges to understand the impact of the humoral immune system in the immune protection at each stage of Mtb infection (58).

M. tuberculosis and SARS-CoV-2 co-infection

Information on TB-COVID-19 co-infection in humans is still limited. Co-infection was reported around 1% in the Philippines (302), 5% in South Africa (303), and between 0.37% and 4.47% in China (304). Recent works suggest that TB-COVID-19 co-infection is associated with elevated risk of unfavorable clinical outcome, with a longer time to recovery, treatment failure, loss to follow-up rates, and higher rates of mortality compared to patients with COVID-19 alone (89, 305–308).

However, mechanistic studies are needed to understand the interactions during Mtb and SARS-CoV-2 dual infections, their effect on the host immune response and clinical outcomes. Understanding the early events and pathophysiology of TB-COVID-19 co-infection is warranted to find better ways to manage such cases, particularly in the high TB endemic areas. The dysregulated immune response induced by each pathogen can lead to an unbalanced inflammatory response, which can promote the progression and worsening of both diseases.

To date, the immune response for each pathogen has been well studied, whereas the impact of Mtb and SARS-CoV-2 co-infection on the innate and adaptive immune response, their crosstalk and cumulative impact on disease outcome in humans still need to be delineated (309–313).

In fact, the studies available have mostly focused on the clinical features of co-infected patients, characterizing a marked lymphopenia and increased levels of some markers of inflammation, such as C-reactive protein (CRP), D-dimer, ferritin, and describing the lung tissue damages (308, 312, 314, 315).

There are few published studies either *in vitro*, ex-vivo using human samples from co-infected individuals or animal models evaluating the immune response and immunopathology in the context of co-infection (Table 2).

In vitro studies were recently performed by Sheerin and colleagues using a single-cell RNA-seq (scRNA-seq) approach to analyze the results from a co-infection performed using a whole blood platform (24 or 96 hours) from healthy adults. The authors characterized different and overlapping immunological responses generated by SARS-CoV-2 (ancestral strain) and Mtb (lineage 4 laboratory strain H37Rv) when a single infection or co-infection occurs. Based on marker gene expression, they identified 13 distinct clusters of cells showing diverse proportions of monocytes, T cells and neutrophils between different conditions and timepoints. The co-infected condition showed the major immune activation effect early (24h) post-infection with 238 immunological pathways uniquely enriched, including IFN-γ and TNF production, while 182 shared pathways were overlapping at 96h post-infection among different conditions. In contrast to SARS-CoV-2-only infection that caused extensive cell death by 96h post-infection, Mtb-only and co-infected conditions maintained monocyte, T cell and NK cell signatures, and negative regulation of the signaling of extrinsic apoptosis (316).

Interesting animal studies evaluating the impact of aerosol Mtb and SARS-CoV-2 co-infection in transgenic (K18-hACE2) C57BL/6 mice showed that pre-infection with Mtb resulted in lower SARS-CoV-2 viral loads at the lung tissue level, likely mediated by the heightened immune microenvironment of the lungs. In addition, after SARS-CoV-2 superinfection, increased bacterial loads in Mtb-infected tissues and decreased histiocytic inflammation were found. Moreover, SARS-CoV-2 caused a decreasing trend in type 1 (IFN-γ and TNF-α) and an increasing trend in type 2 (IL-4 and IL-13) cytokine transcript levels in Mtb-infected mice. These findings, which are usually associated with disseminated Mtb infection, suggest that SARS-CoV-2 may have a deleterious effect on TB outcome (317) through the immune dysregulation, potentially resulting in granuloma collapse and the subsequent Mtb dissemination (311).

Using two concomitant murine models of COVID-19 (SARS-CoV-2 infection of K18-hACE2 mice and mouse-adapted SARS-

TABLE 2 Studies that evaluated the immunopathology and the immune response in the context of M. tuberculosis and SARS-CoV-2 co-infection.

Study (ref)	Model	Immunological findings
Sheerin et al., 2023 (316)	*In vitro* model of infection with Mtb and SARS-COV-2 using human cells from HC	Characterizing distinct and overlapping immunological responses generated by SARS-CoV-2, Mtb, or during co-infection.
Hildebrand et al., 2022 (317)	*In vivo* animal model (mice) infected with Mtb and/or SARS-CoV-2; Uninfected controls	In lungs and spleen of co-infected mice: ↓ type 1 (IFN-γ, TNF-α), ↑ type 2 (IL-4 and IL-13) transcripts
Rosas Mejia et al., 2022 (318)	*In vivo* animal model (mice) infected with Mtb and/or SARS-CoV-2; Uninfected controls	In lungs of co-infected mice: ↓ IFN-γ, IL-6, IL-1β, and transcripts of IFN-γ, TNF-α, ↑ IL-10.
Rajamanickama et al., 2021 (319)	*In vitro* model using human cells from asymptomatic COVID-19 and TBI- asymptomatic COVID-19	In TBI+/SARS-CoV-2 IgG+: ↑ IgM, IgG, IgA, neutralizing antibodies against SARS-CoV-2 compared to TBI-/IgG+. ↑ proinflammatory cytokine/chemokines (IFN-γ, IL-2, TNF-α, IL-1α, IL-1β, IFN-α, IFN-β, IL-6, IL-12, IL-17, GM-CSF, CCL3, CXCL10) and anti-inflammatory cytokines (IL-4, IL-10, IL-25, and IL-33) compared to TBI-/IgG+.
Rajamanickam et al., 2022 (320)	*In vitro* model using human cells from asymptomatic COVID-19 with or without TBI	In TBI+/SARS-CoV-2 IgG+: ↑ baseline and Mtb-induced (but not mitogen) levels of IFN-γ, IL-2, TNF-α, IL-17A, IL-1β, IL-6, IL-12, CCL1, CXCL1, CXCL9, CXCL10, IL-4, IL-13. ↓ levels of IL-5 and IL-10 compared to TBI-/IgG+.
Musso et al., 2021 (315)	*In vitro* model using human cells from TB-COVID-19	Cell anergy in response to Mtb antigens and mitogen stimulation.
Petrone et al., 2021 (310)	*In vitro* model using human cells from COVID-19; TB-COVID-19; TBI-COVID-19; NO COVID-19	In TB-COVID-19 co-infected patients: ↓ specific IFN-γ response to SARS-CoV-2 compared to TBI-COVID-19 and COVID-19-only.
Najafi-Fard et al., 2023 (313)	*In vitro* model using human cells from TB-COVID-19; COVID-19; TB; HC	In co-infected patients: ↑ TNF-α, MIP-1β, and IL-9 compared with COVID-19-only. ↑ TNF-α, IL-1β, IL-17A, IL-5, FGF-basic, and GM-CSF compared with TB-only. ↓ specific response to SARS-CoV-2 and Mtb.
Riou et al., 2021 (311)	*In vitro* model using human cells from patients with or without COVID-19 co-infected or not with TB	In co-infected patients: ↓ SARS-CoV-2-specific and Mtb-specific CD4+ T cell responses with poor polyfunctional cell potentials.
du Bruyn et al., 2023 (314)	*In vitro* model using human cells from patients with or without COVID-19 co-infected or not with TB and/or HIV-1; HC	Comparable frequency of SARS-CoV-2-specific CD8+ T cell response between TB-COVID-19 co-infected and COVID-19-only patients.

COVID-19, CoronaVirus Disease 19; TB, tuberculosis; HC, healthy control; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; Mtb, Mycobacterium tuberculosis; IFN, interferon, TNF, tumor necrosis factor; IL, interleukin; MIP, macrophage inflammatory protein; FGF, fibroblast growth factor; GM-CSF, granulocyte-macrophage-colony-stimulating factor; TBI, tuberculosis infection; Ig, immunoglobulin; CCL, Chemokine (C-C motif) ligand; Chemokine (C-X-C motif) ligand.

CoV-2 [MACoV2] infection of C57BL/6 mice) it was shown that chronically Mtb H37Rv-infected mice were resistant to the pathological consequences of secondary SARS-CoV-2 infection, and SARS-CoV-2 infection did not affect Mtb burdens. Single-cell RNA sequencing of the lungs of the co-infected animals showed that resistance could be due to T and B cells expansion upon viral challenge. Interestingly, lower lung protein levels of IFN-γ, IL-6 and IL-1β as well as mRNA levels of IFN-γ and TNF-α and higher levels of IL-10 were found in co-infection than in Mtb-monoinfection at the 30 days post-infection (318), similar to Hildebrand and colleagues (317)

Regarding the evaluation of the immune responses in co-infected humans, two studies have demonstrated that Mtb infection can modulate humoral (antibody) and cytokine responses to SARS-CoV-2 infection (319) and *vice versa* (320) in investigations conducted in TB endemic countries. Rajamanickam and colleagues demonstrated that individuals seropositive (IgG^+) for SARS-CoV-2 infection and with TB infection (TBI^+/SARS-CoV-2 IgG^+) were characterized by higher levels of specific antibodies (IgM, IgG and IgA) and neutralizing antibodies against SARS-CoV-2 compared to individuals with only SARS-CoV-2 infection. Moreover, elevated plasma levels of proinflammatory cytokine/chemokine responses including IFN-γ, IL-2, TNF-α, IL-1α, IL-1β, IFN-α, IFN-β, IL-6, IL-12, IL-17, GM-CSF, CCL3, CXCL10 and anti-inflammatory cytokines such as IL-4, IL-10, IL-25 and IL-33 were found in TBI^+/SARS-CoV-2 IgG^+ subjects. These results show that Mtb infection can modulate the immune responses in asymptomatic SARS-CoV-2-infected individuals (319). In an additional study, it was shown that TBI^+/SARS-CoV-2 IgG^+ individuals have higher baseline and Mtb-induced (but not

mitogen) levels of several pro- and anti-inflammatory cytokines/chemokines including IFN-γ, IL-2, TNF-α, IL-17A, IL-1β, IL-6, IL-12, CCL1, CXCL1, CXCL9, CXCL10, IL-4, IL-13 and reduced levels of IL-5 and IL-10 compared to TBI^-/SARS-CoV-2 IgG^+ individuals. These findings suggest modulating effects of SARS-CoV-2 infection on the immune responses of individuals with Mtb infection (320). However, these results were obtained in TB-infected individuals with only asymptomatic SARS-CoV-2 infection and the influence of each pathogen on the disease severity and the outcome of each infection were not evaluated.

Differently, clinical outcome was assessed in a case of multidrug-resistant (MDR)/TB-COVID-19 co-infected patient affected by bilateral cavitary pulmonary TB, that subsequently developed COVID-19-associated pneumonia which led to a fatal outcome. Death was probably due to the immuno-suppressed state of the patient, as shown by the low lymphocyte count and by the lack of response to Mtb antigens and mitogen (315).

In addition, a cohort of TB-COVID-19 co-infected patients with different severity of COVID-19 showed a reduced ability to mount a specific immune response to SARS-CoV-2 stimulation compared to patients with TBI and COVID-19 (TBI-COVID-19) or with COVID-19 only (310). In particular, in TB-COVID-19 co-infected patients TNF-α, MIP-1β, and IL-9 showed significant elevated levels compared to COVID-19 only, and TNF-α had the highest discriminant power. Moreover, TNF-α, IL-1β, IL-17A, IL-5, FGF-basic, and GM-CSF were increased in co-infected compared to patients with TB-only. Importantly, co-infection was associated with an impairment of SARS-CoV-2-specific and a reduced Mtb-specific immune response (313).

In agreement with these results, Riou and colleagues demonstrated in TB-COVID-19 co-infection impaired SARS-CoV-2-specific and Mtb-specific $CD4^+$ T cells with reduced polyfunctional cell potentials, proliferation cell capacity, and augmented cell activation markers (311). However, the frequency of SARS-CoV-2 specific $CD8^+$ T cell response to peptides spanning the M, N and S sequences in TB-COVID-19 co-infected patients was found to be comparable with patients with COVID-19 only (314).

Furthermore, several recent case studies have raised concerns regarding the Mtb reactivation in TB-infected subjects following SARS-CoV-2 co-infection. These reports suggest that since the control of both Mtb and SARS-CoV-2 replication depends on cellular immunity, it is possible that the immune dysregulation caused by SARS-CoV-2 or the immunomodulatory therapies used for COVID-19 treatment may increase the risk for TB reactivation (321–326).

Both SARS-CoV-2 and Mtb have immunomodulating potentials to change the outcome of the course of each disease in co-infected patients: SARS-CoV-2 may cause immunosuppression and cytokine storm, which can contribute to the Mtb reactivation (327) and lung tissue damage; Mtb may cause T-cell exhaustion and uncontrolled release of proinflammatory cytokines resulting in lung damage (328, 329), thus potentially contributing to the susceptibility to SARS-CoV-2 infection and to a more severe COVID-19.

In TB-infected individuals, T cells are responsible for Mtb control *via* the granuloma formation. Co-infection with SARS-CoV-2 in these individuals may negatively affect immune regulation in the granuloma leading to Mtb reactivation (322, 330). This alteration of the immune system has been reported using a large-scale meta-analysis of transcriptomic data showing that some immune genes are enriched in COVID-19 and TB diseases (309). The findings from case reports indicate the presence of similarities in the immunopathogenesis of the two diseases, which may exacerbate disease severity during co-infection. Subclinical and clinical TB disease may increase the risk of severe COVID-19 disease and also SARS-CoV-2 co-infection may induce the progression to TB disease (309), as reported above (321–326). In this regard, IFN-I which is strongly induced by viral infection may be detrimental in the context of Mtb by inhibiting B cell responses, inducing the release of immunosuppressive molecules or reducing the macrophagic activation induced by IFN-γ (145), Also, the hyperinflammatory milieu caused by Mtb may raise the risk of severe COVID-19 and *vice versa* (331). Mtb spread or reactivation might be favored by inflammatory molecules released from the SARS-CoV-2-induced necroptosis, whereas the apoptosis might mitigate it (332). Moreover, while COVID-19 therapies targeting pro-inflammatory cytokines may limit the acute immunopathology, they may also repress the responses needed to control Mtb containment (308).

Altogether, these studies suggest that co-infection alters the capacity of the host to respond to and control Mtb and/or SARS-CoV-2, indicating the need for further investigation of the underlying immunological pathways.

Final remarks

SARS-CoV-2 and Mtb are currently the two deadliest infectious diseases in humans. While the route of infection and the target organ are similar, the time to disease manifestation and the pathways driving immunopathology differ significantly (Figure 3).

Evidence reported here show that both innate and adaptive immune response are critical components for the protection against SARS-CoV-2 and Mtb. The immune response to both SARS-CoV-2 and Mtb is complex and multifaceted, and there are still many aspects that are not well understood. However, it is known that an appropriate activation of the innate immunity in the early stages of infection followed by adaptive immunity is necessary to curb the pathogen dissemination in the host.

The comparison of these two pathogens highlights how the innate immune response induced after exposure to SARS-CoV-2 or Mtb share the production of some pro-inflammatory cytokines including IL-1β and TNF-α. Similar results were found in Mtb/SARS-CoV-2 co-infection. For SARS-CoV-2 infection, the early and robust IFN-I production as well as neutralizing antibodies have an outmost importance for guarantee an efficient control of viral spread and to determine the clinical outcome of COVID-19. On the other hand, in Mtb infection a central role is played by the alveolar macrophages and the cytokines they release as TNF-α and IL-1β.

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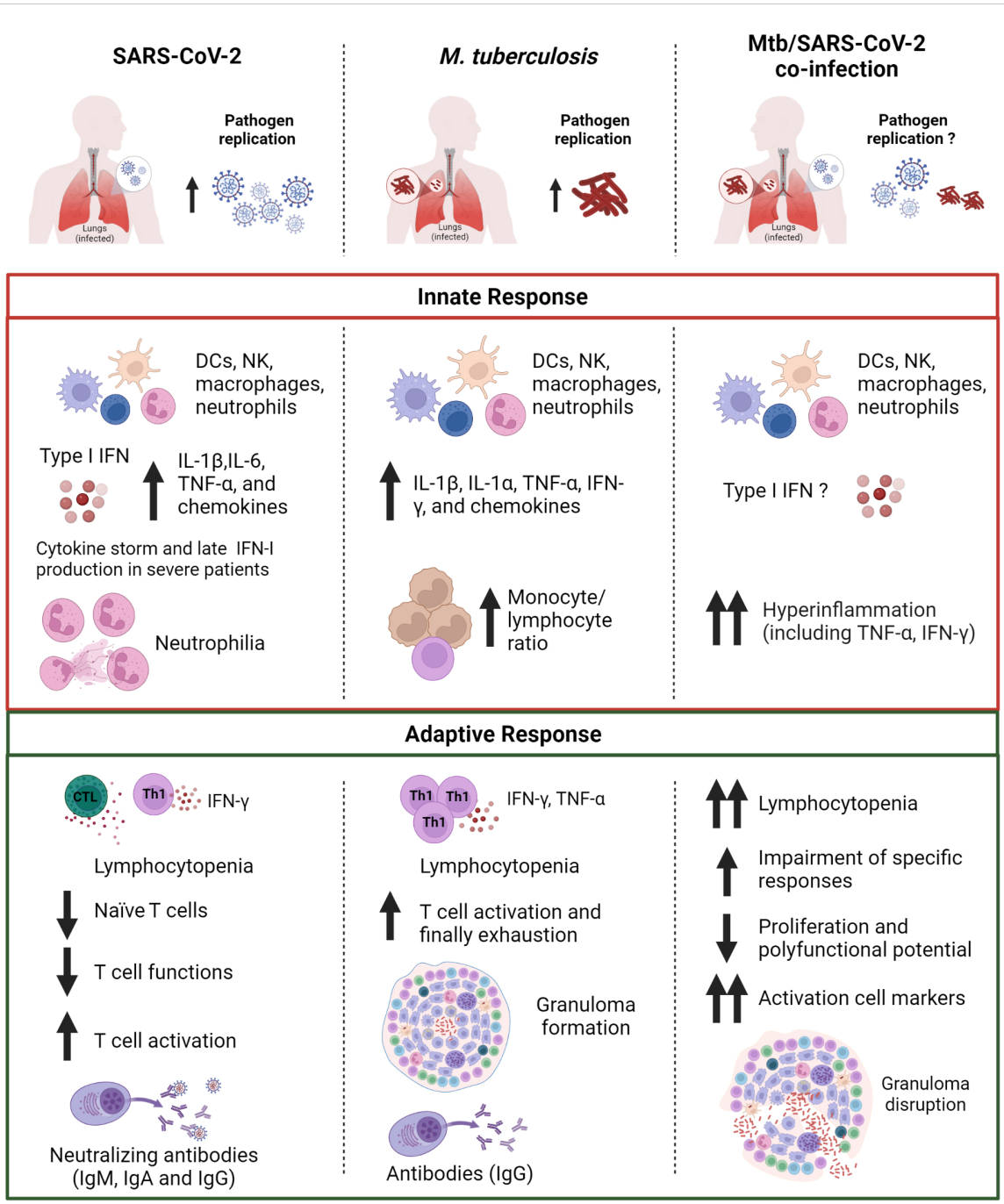

FIGURE 3
Comparison of the immune response in SARS-CoV-2, Mtb or Mtb/SARS-CoV-2 infection. The innate immune response induced after exposure to SARS-CoV-2 or Mtb is characterized by the production of pro-inflammatory cytokines including IL-1β and TNF-α. In Mtb/SARS-CoV-2 co-infection there is an overproduction of pro-inflammatory cytokines. SARS-CoV-2 infection presents also an early type I IFN production, which is absent or delayed in severe COVID-19 patients. SARS-CoV-2 infection is also characterized by a higher neutrophil count, whereas a higher monocyte/ lymphocyte ratio is observed in Mtb-infected patients. Both SARS-CoV-2 and Mtb infected subjects show lymphocytopenia and T cell activation, which are even more prominent in case of co-infection. In co-infected individuals a major impairment of antigen-specific response to Mtb and SARS-CoV-2, and granuloma disruption is present. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Mtb, *Mycobacterium tuberculosis*; IFNs, interferons; DCs, dendritic cells; NK, natural killer; Th, T helper; Ig, immunoglobulin. Created with BioRender.com.

Although the infections caused by the individual pathogens have been intensively studied, there are still many unanswered questions about the influence of these pathogens on each other, the immune response, and clinical outcome in the context of co-infection. Recent data has raised concerns regarding the Mtb reactivation following SARS-CoV-2 infection likely due to immune dysregulation caused by SARS-CoV-2 or immunomodulatory COVID-19 therapies. Further clinical and scientific research is needed to better understand the interaction and outcome of the co-infection.

Author contributions

AA contributed to the writing of introduction, immune response to SARS-CoV-2, final remarks and created the figures. SN-F was responsible for immune response to *M. tuberculosis*, co-infection and tables. DG conceived the review, contributed to the first draft and revised the whole manuscript. All the authors approved the final version of the manuscript.

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Conflict of interest

Author DG has been a member of the advisory board of Biomerieux and Eli Lilly in 2020 and 2021 and is currently scientific advisor of PDB Biotec. She received fees for educational training or consultancy from Almirall, Biogen, Celgene, Diasorin, Janssen, Qiagen and Quidel.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. WHO. *Coronavirus disease (COVID-19) – World Health Organization* (2023). Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (Accessed June 13, 2023).

2. Global tuberculosis report 2022 . Available at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022 (Accessed June 19, 2023).

3. Najafi Fard S, Petrone L, Petruccioli E, Alonzi T, Matusali G, Colavita F, et al. *In vitro* models for studying entry, tissue tropism, and therapeutic approaches of highly pathogenic coronaviruses. *BioMed Res Int* (2021) 2021:8856018. doi: 10.1155/2021/8856018

4. Chandran A, Rosenheim J, Nageswaran G, Swadling L, Pollara G, Gupta RK, et al. Rapid synchronous type 1 IFN and virus-specific T cell responses characterize first wave non-severe SARS-CoV-2 infections. *Cell Rep Med* (2022) 3:100557. doi: 10.1016/j.xcrm.2022.100557

5. Aiello A, Grossi A, Meschi S, Meledandri M, Vanini V, Petrone L, et al. Coordinated innate and T-cell immune responses in mild COVID-19 patients from household contacts of COVID-19 cases during the first pandemic wave. *Front Immunol* (2022) 13:920227. doi: 10.3389/fimmu.2022.920227

6. Capuano SV, Croix DA, Pawar S, Zinovik A, Myers A, Lin PL, et al. Experimental Mycobacterium tuberculosis infection of cynomolgus macaques closely resembles the various manifestations of human M. tuberculosis infection. *Infect Immun* (2003) 71:5831–44. doi: 10.1128/IAI.71.10.5831-5844.2003

7. Lin PL, Rodgers M, Smith L, Bigbee M, Myers A, Bigbee C, et al. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. *Infect Immun* (2009) 77:4631–42. doi: 10.1128/IAI.00592-09

8. Goldblatt D, Alter G, Crotty S, Plotkin SA. Correlates of protection against SARS-CoV-2 infection and COVID-19 disease. *Immunol Rev* (2022) 310:6–26. doi: 10.1111/imr.13091

9. Regev-Yochay G, Lustig Y, Joseph G, Gilboa M, Barda N, Gens I, et al. Correlates of protection against COVID-19 infection and intensity of symptomatic disease in vaccinated individuals exposed to SARS-CoV-2 in households in Israel (ICoFS): a prospective cohort study. *Lancet Microbe* (2023) 4:e309–18. doi: 10.1016/S2666-5247(23)00012-5

10. Lawn SD, Myer L, Edwards D, Bekker L-G, Wood R. Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa. *AIDS* (2009) 23:1717–25. doi: 10.1097/QAD.0b013e32832d3b6d

11. Wang J, Du G. COVID-19 may transmit through aerosol. *Ir J Med Sci* (2020) 189:1143–4. doi: 10.1007/s11845-020-02218-2

12. Zhou L, Ayeh SK, Chidambaram V, Karakousis PC. Modes of transmission of SARS-CoV-2 and evidence for preventive behavioral interventions. *BMC Infect Dis* (2021) 21:496. doi: 10.1186/s12879-021-06222-4

13. Baker CA, Gibson KE. Persistence of SARS-CoV-2 on surfaces and relevance to the food industry. *Curr Opin Food Sci* (2022) 47:100875. doi: 10.1016/j.cofs.2022.100875

14. Chin AWH, Chu JTS, Perera MRA, Hui KPY, Yen H-L, Chan MCW, et al. Stability of SARS-CoV-2 in different environmental conditions. *Lancet Microbe* (2020) 1:e10. doi: 10.1016/S2666-5247(20)30003-3

15. Hobby GL, Holman AP, Iseman MD, Jones JM. Enumeration of tubercle bacilli in sputum of patients with pulmonary tuberculosis. *Antimicrob Agents Chemother* (1973) 4:94–104. doi: 10.1128/AAC.4.2.94

16. Patterson B, Bryden W, Call C, McKerry A, Leonard B, Seldon R, et al. Cough-independent production of viable Mycobacterium tuberculosis in bioaerosol. *Tuberculosis* (2021) 126:102038. doi: 10.1016/j.tube.2020.102038

17. Liu J, Li Y, Liu Q, Yao Q, Wang X, Zhang H, et al. SARS-CoV-2 cell tropism and multiorgan infection. *Cell Discovery* (2021) 7:1–4. doi: 10.1038/s41421-021-00249-2

18. Sungnak W, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med* (2020) 26:681–7. doi: 10.1038/s41591-020-0868-6

19. Scordo JM, Knoell DL, Torrelles JB. Alveolar epithelial cells in mycobacterium tuberculosis infection: active players or innocent bystanders? *J Innate Immun* (2016) 8:3–14. doi: 10.1159/000439275

20. Corleis B, Dorhoi A. Early dynamics of innate immunity during pulmonary tuberculosis. *Immunol Lett* (2020) 221:56–60. doi: 10.1016/j.imlet.2020.02.010

21. de Waal AM, Hiemstra PS, Ottenhoff TH, Joosten SA, van der Does AM. Lung epithelial cells interact with immune cells and bacteria to shape the microenvironment in tuberculosis. *Thorax* (2022) 77:408–16. doi: 10.1136/thoraxjnl-2021-217997

22. Behr MA, Waters WR. Is tuberculosis a lymphatic disease with a pulmonary portal? *Lancet Infect Dis* (2014) 14:250–5. doi: 10.1016/S1473-3099(13)70253-6

23. Lerner TR, de Souza Carvalho-Wodarz C, Repnik U, Russell MRG, Borel S, Diedrich CR, et al. Lymphatic endothelial cells are a replicative niche for Mycobacterium tuberculosis. *J Clin Invest* (2016) 126:1093–108. doi: 10.1172/JCI83379

24. Beigier-Bompadre M, Montagna GN, Kühl AA, Lozza L, Iii JW, Kupz A, et al. Mycobacterium tuberculosis infection modulates adipose tissue biology. *PloS Pathog* (2017) 13:e1006676. doi: 10.1371/journal.ppat.1006676

25. Mayito J, Andia I, Belay M, Jolliffe DA, Kateete DP, Reece ST, et al. Anatomic and cellular niches for mycobacterium tuberculosis in latent tuberculosis infection. *J Infect Dis* (2019) 219:685–94. doi: 10.1093/infdis/jiy579

26. Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the spike protein of SARS-coV-2 is essential for infection of human lung cells. *Mol Cell* (2020) 78:779–784.e5. doi: 10.1016/j.molcel.2020.04.022

27. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. *Nat Rev Mol Cell Biol* (2022) 23:3–20. doi: 10.1038/s41580-021-00418-x

28. Koch J, Uckeley ZM, Doldan P, Stanifer M, Boulant S, Lozach P-Y. TMPRSS2 expression dictates the entry route used by SARS-CoV-2 to infect host cells. *EMBO J* (2021) 40:e107821. doi: 10.15252/embj.2021107821

29. Schäfer G, Jacobs M, Wilkinson RJ, Brown GD. Non-opsonic recognition of Mycobacterium tuberculosis by phagocytes. *J Innate Immun* (2009) 1:231–43. doi: 10.1159/000173703

30. Chatterjee S, Kundapura SV, Basak AJ, Mukherjee D, Dash S, Ganguli N, et al. High-resolution crystal structure of LpqH, an immunomodulatory surface lipoprotein

of Mycobacterium tuberculosis reveals a distinct fold and a conserved cleft on its surface. *Int J Biol Macromol* (2022) 210:494–503. doi: 10.1016/j.ijbiomac.2022.04.196

31. Eslami N, Aghbash PS, Shamekh A, Entezari-Maleki T, Nahand JS, Sales AJ, et al. SARS-coV-2: receptor and co-receptor tropism probability. *Curr Microbiol* (2022) 79:133. doi: 10.1007/s00284-022-02807-7

32. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, Uhl S, Hoagland D, Møller R, et al. Imbalanced host response to SARS-coV-2 drives development of COVID-19. *Cell* (2020) 181:1036–1045.e9. doi: 10.1016/j.cell.2020.04.026

33. Del Valle DM, Kim-Schulze S, Huang H-H, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med* (2020) 26:1636–43. doi: 10.1038/s41591-020-1051-9

34. Severa M, Diotti RA, Etna MP, Rizzo F, Fiore S, Ricci D, et al. Differential plasmacytoid dendritic cell phenotype and type I Interferon response in asymptomatic and severe COVID-19 infection. *PloS Pathog* (2021) 17:e1009878. doi: 10.1371/journal.ppat.1009878

35. Parackova Z, Zentsova I, Bloomfield M, Vrabcova P, Smetanova J, Klocperk A, et al. Disharmonic inflammatory signatures in COVID-19: augmented neutrophils' but impaired monocytes' and dendritic cells' Responsiveness. *Cells* (2020) 9:2206. doi: 10.3390/cells9102206

36. Al-Kuraishy HM, Al-Gareeb AI, Al-Hussaniy HA, Al-Harcan NAH, Alexiou A, Batiha GE-S. Neutrophil Extracellular Traps (NETs) and Covid-19: A new frontiers for therapeutic modality. *Int Immunopharmacol* (2022) 104:108516. doi: 10.1016/j.intimp.2021.108516

37. Veras FP, Gomes GF, Silva BMS, Caetité DB, Almeida CJLR, Silva CMS, et al. Targeting neutrophils extracellular traps (NETs) reduces multiple organ injury in a COVID-19 mouse model. *Respir Res* (2023) 24:66. doi: 10.1186/s12931-023-02336-2

38. Masso-Silva JA, Moshensky A, Lam MTY, Odish MF, Patel A, Xu L, et al. Increased peripheral blood neutrophil activation phenotypes and neutrophil extracellular trap formation in critically ill coronavirus disease 2019 (COVID-19) patients: A case series and review of the literature. *Clin Infect Dis* (2022) 74:479–89. doi: 10.1093/cid/ciab437

39. Etna MP, Giacomini E, Severa M, Coccia EM. Pro- and anti-inflammatory cytokines in tuberculosis: a two-edged sword in TB pathogenesis. *Semin Immunol* (2014) 26:543–51. doi: 10.1016/j.smim.2014.09.011

40. Adane T, Melku M, Ayalew G, Bewket G, Aynalem M, Getawa S. Accuracy of monocyte to lymphocyte ratio for tuberculosis diagnosis and its role in monitoring anti-tuberculosis treatment: Systematic review and meta-analysis. *Med (Baltimore)* (2022) 101:e31539. doi: 10.1097/MD.0000000000031539

41. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin J-B, Olsson A, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell* (2020) 183:158–168.e14. doi: 10.1016/j.cell.2020.08.017

42. Notarbartolo S, Ranzani V, Bandera A, Gruarin P, Bevilacqua V, Putignano AR, et al. Integrated longitudinal immunophenotypic, transcriptional and repertoire analyses delineate immune responses in COVID-19 patients. *Sci Immunol* (2021) 6: eabg5021. doi: 10.1126/sciimmunol.abg5021

43. Oja AE, Saris A, Ghandour CA, Kragten NAM, Hogema BM, Nossent EJ, et al. Divergent SARS-CoV-2-specific T- and B-cell responses in severe but not mild COVID-19 patients. *Eur J Immunol* (2020) 50:1998–2012. doi: 10.1002/eji.202048908

44. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* (2020) 8:420–2. doi: 10.1016/S2213-2600(20)30076-X

45. Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, et al. Antigen-specific adaptive immunity to SARS-coV-2 in acute COVID-19 and associations with age and disease severity. *Cell* (2020) 183:996–1012.e19. doi: 10.1016/j.cell.2020.09.038

46. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in wuhan, China. *Clin Infect Dis* (2020) 71:762–8. doi: 10.1093/cid/ciaa248

47. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis* (2020) 221:1762–9. doi: 10.1093/infdis/jiaa150

48. Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* (2020) 26:845–8. doi: 10.1038/s41591-020-0897-1

49. Seow J, Graham C, Merrick B, Acors S, Pickering S, Steel KJA, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat Microbiol* (2020) 5:1598–607. doi: 10.1038/s41564-020-00813-8

50. Wang K, Long Q-X, Deng H-J, Hu J, Gao Q-Z, Zhang G-J, et al. Longitudinal dynamics of the neutralizing antibody response to severe acute respiratory syndrome coronavirus 2 (SARS-coV-2) infection. *Clin Infect Dis* (2021) 73:e531–9. doi: 10.1093/cid/ciaa1143

51. Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Viant C, Gaebler C, et al. Enhanced SARS-CoV-2 neutralization by dimeric IgA. *Sci Transl Med* (2021) 13:eabf1555. doi: 10.1126/scitranslmed.abf1555

52. Flynn JL, Chan J. Immune cell interactions in tuberculosis. *Cell* (2022) 185:4682–702. doi: 10.1016/j.cell.2022.10.025

53. Ehlers S, Schaible UE. The granuloma in tuberculosis: dynamics of a host-pathogen collusion. *Front Immunol* (2012) 3:411. doi: 10.3389/fimmu.2012.00411

54. Ashenafi S, Brighenti S. Reinventing the human tuberculosis (TB) granuloma: Learning from the cancer field. *Front Immunol* (2022) 13:1059725. doi: 10.3389/fimmu.2022.1059725

55. Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology* (2008) 123:326–38. doi: 10.1111/j.1365-2567.2007.02719.x

56. Gideon HP, Phuah J, Myers AJ, Bryson BD, Rodgers MA, Coleman MT, et al. Variability in tuberculosis granuloma T cell responses exists, but a balance of pro- and anti-inflammatory cytokines is associated with sterilization. *PloS Pathog* (2015) 11: e1004603. doi: 10.1371/journal.ppat.1004603

57. Chan J, Mehta S, Bharrhan S, Chen Y, Achkar JM, Casadevall A, et al. The role of B cells and humoral immunity in Mycobacterium tuberculosis infection. *Semin Immunol* (2014) 26:588–600. doi: 10.1016/j.smim.2014.10.005

58. Achkar JM, Chan J, Casadevall A. B cells and antibodies in the defense against Mycobacterium tuberculosis infection. *Immunol Rev* (2015) 264:167–81. doi: 10.1111/imr.12276

59. Sette A, Crotty S. Adaptive immunity to SARS-coV-2 and COVID-19. *Cell* (2021) 184:861–80. doi: 10.1016/j.cell.2021.01.007

60. Petrone L, Petruccioli E, Vanini V, Cuzzi G, Najafi Fard S, Alonzi T, et al. A whole blood test to measure SARS-CoV-2-specific response in COVID-19 patients. *Clin Microbiol Infect* (2021) 27:286.e7–286.e13. doi: 10.1016/j.cmi.2020.09.051

61. Aiello A, Najafi Fard S, Petruccioli E, Petrone L, Vanini V, Farroni C, et al. Spike is the most recognized antigen in the whole-blood platform in both acute and convalescent COVID-19 patients. *Int J Infect Dis* (2021) 106:338–47. doi: 10.1016/j.ijid.2021.04.034

62. Petruccioli E, Najafi Fard S, Navarra A, Petrone L, Vanini V, Cuzzi G, et al. Exploratory analysis to identify the best antigen and the best immune biomarkers to study SARS-CoV-2 infection. *J Transl Med* (2021) 19:272. doi: 10.1186/s12967-021-02938-8

63. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T cell responses to SARS-coV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* (2020) 181:1489–1501.e15. doi: 10.1016/j.cell.2020.05.015

64. Wolf AJ, Desvignes L, Linas B, Banaiee N, Tamura T, Takatsu K, et al. Initiation of the adaptive immune response to Mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs. *J Exp Med* (2008) 205:105–15. doi: 10.1084/jem.20071367

65. Reiley WW, Calayag MD, Wittmer ST, Huntington JL, Pearl JE, Fountain JJ, et al. ESAT-6-specific CD4 T cell responses to aerosol Mycobacterium tuberculosis infection are initiated in the mediastinal lymph nodes. *Proc Natl Acad Sci U.S.A.* (2008) 105:10961–6. doi: 10.1073/pnas.0801496105

66. Kauffman KD, Sallin MA, Hoft SG, Sakai S, Moore R, Wilder-Kofie T, et al. Limited Pulmonary Mucosal-Associated Invariant T Cell Accumulation and Activation during Mycobacterium tuberculosis Infection in Rhesus Macaques. *Infect Immun* (2018) 86:e00431–18. doi: 10.1128/IAI.00431-18

67. Goletti D, Butera O, Vanini V, Lauria FN, Lange C, Franken KLMC, et al. Response to Rv2628 latency antigen associates with cured tuberculosis and remote infection. *Eur Respir J* (2010) 36:135–42. doi: 10.1183/09031936.00140009

68. Sali M, Buonsenso D, D'Alfonso P, De Maio F, Ceccarelli M, Battah B, et al. Combined use of Quantiferon and HBHA-based IGRA supports tuberculosis diagnosis and therapy management in children. *J Infect* (2018) 77:526–33. doi: 10.1016/j.jinf.2018.09.011

69. Bálint G, Vörös-Horváth B, Széchenyi A. Omicron: increased transmissibility and decreased pathogenicity. *Sig Transduct Target Ther* (2022) 7:1–3. doi: 10.1038/s41392-022-01009-8

70. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* (2020) 369:718–24. doi: 10.1126/science.abc6027

71. Galani I-E, Rovina N, Lampropoulou V, Triantafyllia V, Manioudaki M, Pavlos E, et al. Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. *Nat Immunol* (2021) 22:32–40. doi: 10.1038/s41590-020-00840-x

72. McCarthy KR, Rennick LJ, Nambulli S, Robinson-McCarthy LR, Bain WG, Haidar G, et al. Recurrent deletions in the SARS-CoV-2 spike glycoprotein drive antibody escape. *Science* (2021) 371:1139–42. doi: 10.1126/science.abf6950

73. Kemp SA, Collier DA, Datir RP, Ferreira IATM, Gayed S, Jahun A, et al. SARS-CoV-2 evolution during treatment of chronic infection. *Nature* (2021) 592:277–82. doi: 10.1038/s41586-021-03291-y

74. Collier DA, De Marco A, Ferreira IATM, Meng B, Datir RP, Walls AC, et al. Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. *Nature* (2021) 593:136–41. doi: 10.1038/s41586-021-03412-7

75. Zhou D, Dejnirattisai W, Supasa P, Liu C, Mentzer AJ, Ginn HM, et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell* (2021) 184:2348–2361.e6. doi: 10.1016/j.cell.2021.02.037

76. Lu H, Liu Z, Deng X, Chen S, Zhou R, Zhao R, et al. Potent NKT cell ligands overcome SARS-CoV-2 immune evasion to mitigate viral pathogenesis in mouse models. *PloS Pathog* (2023) 19:e1011240. doi: 10.1371/journal.ppat.1011240

77. Carranza C, Chavez-Galan L. Several routes to the same destination: inhibition of phagosome-lysosome fusion by mycobacterium tuberculosis. *Am J Med Sci* (2019) 357:184–94. doi: 10.1016/j.amjms.2018.12.003

78. Hayakawa E, Tokumasu F, Nardone GA, Jin AJ, Hackley VA, Dvorak JA. A Mycobacterium tuberculosis-derived lipid inhibits membrane fusion by modulating lipid membrane domains. *Biophys J* (2007) 93:4018–30. doi: 10.1529/biophysj.107.104075

79. Schnettger L, Rodgers A, Repnik U, Lai RP, Pei G, Verdoes M, et al. A rab20-dependent membrane trafficking pathway controls M. tuberculosis replication by regulating phagosome spaciousness and integrity. *Cell Host Microbe* (2017) 21:619–628.e5. doi: 10.1016/j.chom.2017.04.004

80. Jayachandran R, Sundaramurthy V, Combaluzier B, Mueller P, Korf H, Huygen K, et al. Survival of mycobacteria in macrophages is mediated by coronin 1-dependent activation of calcineurin. *Cell* (2007) 130:37–50. doi: 10.1016/j.cell.2007.04.043

81. Braian C, Hogea V, Stendahl O. Mycobacterium tuberculosis- induced neutrophil extracellular traps activate human macrophages. *J Innate Immun* (2013) 5:591–602. doi: 10.1159/000348676

82. Chowdhury CS, Kinsella RL, Nehls EM, Naik SK, Lane DS, Talukdar P, et al. Type I IFN signaling mediates NET release to promote Mycobacterium tuberculosis replication and granuloma caseation. (2022) 2022:. doi: 10.1101/2022.11.29.518376

83. Blanc L, Gilleron M, Prandi J, Song O-R, Jang M-S, Gicquel B, et al. Mycobacterium tuberculosis inhibits human innate immune responses *via* the production of TLR2 antagonist glycolipids. *Proc Natl Acad Sci U.S.A.* (2017) 114:11205–10. doi: 10.1073/pnas.1707840114

84. Domingo-Gonzalez R, Prince O, Cooper A, Khader SA. Cytokines and chemokines in mycobacterium tuberculosis infection. *Microbiol Spectr* (2016) 4. doi: 10.1128/microbiolspec.TBTB2-0018-2016

85. CDC. COVID-19 and your health, in: *Centers for disease control and prevention* (2020). Available at: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms (Accessed June 13, 2023).

86. *Fact sheets | General | Tuberculosis: general information | TB | CDC* (2022). Available at: https://www.cdc.gov/tb/publications/factsheets/general/tb.htm (Accessed June 13, 2023).

87. Grifoni A, Alonzi T, Alter G, Noonan DM, Landay AL, Albini A, et al. Impact of aging on immunity in the context of COVID-19, HIV, and tuberculosis. *Front Immunol* (2023) 14:1146704. doi: 10.3389/fimmu.2023.1146704

88. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3

89. Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases, South Africa. Risk factors for coronavirus disease 2019 (COVID-19) death in a population cohort study from the western cape province, South Africa. *Clin Infect Dis* (2021) 73:e2005–15. doi: 10.1093/cid/ciaa1198

90. Goletti D, Pisapia R, Fusco FM, Aiello A, Van Crevel R. Epidemiology, pathogenesis, clinical presentation and management of TB in patients with HIV and diabetes. *Int J Tuberc Lung Dis* (2023) 27:284–90. doi: 10.5588/ijtld.22.0685

91. ISS. Test di laboratorio per SARS-CoV-2 e loro uso in sanità pubblica, online la nota tecnica ad interim . Available at: https://www.inail.it/cs/internet/comunicazione/news-ed-eventi/news/news-nota-tecnica-test-sars-cov-2-2020.html (Accessed June 15, 2023).

92. Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, et al. Official american thoracic society/infectious diseases society of america/centers for disease control and prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. *Clin Infect Dis* (2017) 64:111–5. doi: 10.1093/cid/ciw778

93. Diriba K, Churiso G. The prevalence of Mycobacterium tuberculosis using Gene Xpert among tuberculosis suspected patients in Gedeo Zone, Southern Ethiopia. *Eur J Med Res* (2022) 27:24. doi: 10.1186/s40001-022-00650-x

94. Liu X, Mostafavi H, Ng WH, Freitas JR, King NJC, Zaid A, et al. The delta SARS-coV-2 variant of concern induces distinct pathogenic patterns of respiratory disease in K18-hACE2 transgenic mice compared to the ancestral strain from wuhan. *mBio* (2022) 13:e0068322. doi: 10.1128/mbio.00683-22

95. Lyngse FP, Mortensen LH, Denwood MJ, Christiansen LE, Møller CH, Skov RL, et al. Household transmission of the SARS-CoV-2 Omicron variant in Denmark. *Nat Commun* (2022) 13:5573. doi: 10.1038/s41467-022-33328-3

96. Flavin RJ, Gibbons N, O'Briain DS. Mycobacterium tuberculosis at autopsy–exposure and protection: an old adversary revisited. *J Clin Pathol* (2007) 60:487–91. doi: 10.1136/jcp.2005.032276

97. Grosset J. Mycobacterium tuberculosis in the extracellular compartment: an underestimated adversary. *Antimicrob Agents Chemother* (2003) 47:833–6. doi: 10.1128/AAC.47.3.833-836.2003

98. Canetti G DM. Thé Tubercle bacillus in the pulmonary lesion of man, in: *Thé Tubercle bacillus in the pulmonary lesion of man* (1955). Available at: https://www.cabdirect.org/cabdirect/abstract/19562700285 (Accessed June 19, 2023).

99. Opie, Aronson. Tubercle bacilli in latent tuberculous lesions in lung tissue without tuberculous lesions. *Am J Med Sci* (1928) 176:460. doi: 10.1097/00000441-192809000-00064

100. Silva MJA, Ribeiro LR, Gouveia MIM, Marcelino B dos R, dos Santos CS, Lima KVB, et al. Hyperinflammatory response in COVID-19: A systematic review. *Viruses* (2023) 15:553. doi: 10.3390/v15020553

101. Valdebenito S, Bessis S, Annane D, Lorin de la Grandmaison G, Cramer–Bordé E, Prideaux B, et al. COVID-19 lung pathogenesis in SARS-coV-2 autopsy cases(2021) (Accessed July 27, 2023).

102. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MPR. The immune response in tuberculosis. *Annu Rev Immunol* (2013) 31:475–527. doi: 10.1146/annurev-immunol-032712-095939

103. Cooper AM. Cell-mediated immune responses in tuberculosis. *Annu Rev Immunol* (2009) 27:393–422. doi: 10.1146/annurev.immunol.021908.132703

104. Kroon EE, Kinnear CJ, Orlova M, Fischinger S, Shin S, Boolay S, et al. An observational study identifying highly tuberculosis-exposed, HIV-1-positive but persistently TB, tuberculin and IGRA negative persons with M. tuberculosis specific antibodies in Cape Town, South Africa. *EBioMedicine* (2020) 61:103053. doi: 10.1016/j.ebiom.2020.103053

105. Bost P, Giladi A, Liu Y, Bendjelal Y, Xu G, David E, et al. Host-viral infection maps reveal signatures of severe COVID-19 patients. *Cell* (2020) 181:1475–1488.e12. doi: 10.1016/j.cell.2020.05.006

106. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-coV-2 spike glycoprotein. *Cell* (2020) 181:281–292.e6. doi: 10.1016/j.cell.2020.02.058

107. Yu S, Hu H, Ai Q, Bai R, Ma K, Zhou M, et al. SARS-coV-2 spike-mediated entry and its regulation by host innate immunity. *Viruses* (2023) 15:639. doi: 10.3390/v15030639

108. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* (2020) 581:215–20. doi: 10.1038/s41586-020-2180-5

109. Benton DJ, Wrobel AG, Xu P, Roustan C, Martin SR, Rosenthal PB, et al. Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion. *Nature* (2020) 588:327–30. doi: 10.1038/s41586-020-2772-0

110. Yu S, Zheng X, Zhou B, Li J, Chen M, Deng R, et al. SARS-CoV-2 spike engagement of ACE2 primes S2' site cleavage and fusion initiation. *Proc Natl Acad Sci U.S.A.* (2022) 119:e2111199119. doi: 10.1073/pnas.2111199119

111. Wettstein L, Kirchhoff F, Münch J. The transmembrane protease TMPRSS2 as a therapeutic target for COVID-19 treatment. *Int J Mol Sci* (2022) 23:1351. doi: 10.3390/ijms23031351

112. Vaarala MH, Porvari KS, Kellokumpu S, Kyllönen AP, Vihko PT. Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. *J Pathol* (2001) 193:134–40. doi: 10.1002/1096-9896(2000)9999:9999<::AID-PATH743>3.0.CO;2-T

113. Liu Y, Qu H-Q, Qu J, Tian L, Hakonarson H. Expression pattern of the SARS-coV-2 entry genes ACE2 and TMPRSS2 in the respiratory tract. *Viruses* (2020) 12:1174. doi: 10.3390/v12101174

114. Yang H, Yuan H, Zhao X, Xun M, Guo S, Wang N, et al. Cytoplasmic domain and enzymatic activity of ACE2 are not required for PI4KB dependent endocytosis entry of SARS-CoV-2 into host cells. *Virol Sin* (2022) 37:380–9. doi: 10.1016/j.virs.2022.03.003

115. Lau S-Y, Wang P, Mok BW-Y, Zhang AJ, Chu H, Lee AC-Y, et al. Attenuated SARS-CoV-2 variants with deletions at the S1/S2 junction. *Emerg Microbes Infect* (2020) 9:837–42. doi: 10.1080/22221751.2020.1756700

116. Hui KPY, Ho JCW, Cheung M-C, Ng K-C, Ching RHH, Lai K-L, et al. SARS-CoV-2 Omicron variant replication in human bronchus and lung ex vivo. *Nature* (2022) 603:715–20. doi: 10.1038/s41586-022-04479-6

117. Meng B, Abdullahi A, Ferreira IATM, Goonawardane N, Saito A, Kimura I, et al. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. *Nature* (2022) 603:706–14. doi: 10.1038/s41586-022-04474-x

118. Alonzi T, Aiello A, Repele F, Falasca L, Francalancia M, Garbuglia AR, et al. Cysteamine exerts in *vitro* antiviral activity against the SARS-CoV-2 Delta and Omicron variants. *Cell Death Discovery* (2022) 8:288. doi: 10.1038/s41420-022-01080-8

119. Halfmann PJ, Iida S, Iwatsuki-Horimoto K, Maemura T, Kiso M, Scheaffer SM, et al. SARS-CoV-2 Omicron virus causes attenuated disease in mice and hamsters. *Nature* (2022) 603:687–92. doi: 10.1038/s41586-022-04441-6

120. Suzuki R, Yamasoba D, Kimura I, Wang L, Kishimoto M, Ito J, et al. Attenuated fusogenicity and pathogenicity of SARS-CoV-2 Omicron variant. *Nature* (2022) 603:700–5. doi: 10.1038/s41586-022-04462-1

121. Zang R, Gomez Castro MF, McCune BT, Zeng Q, Rothlauf PW, Sonnek NM, et al. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci Immunol* (2020) 5:eabc3582. doi: 10.1126/sciimmunol.abc3582

122. Jocher G, Grass V, Tschirner SK, Riepler L, Breimann S, Kaya T, et al. ADAM10 and ADAM17 promote SARS-CoV-2 cell entry and spike protein-mediated lung cell fusion. *EMBO Rep* (2022) 23:e54305. doi: 10.15252/embr.202154305

123. Harte JV, Wakerlin SL, Lindsay AJ, McCarthy JV, Coleman-Vaughan C. Metalloprotease-dependent S2'-activation promotes cell-cell fusion and syncytiation of SARS-coV-2. *Viruses* (2022) 14:2094. doi: 10.3390/v14102094

124. Yamamoto M, Gohda J, Kobayashi A, Tomita K, Hirayama Y, Koshikawa N, et al. Metalloproteinase-dependent and TMPRSS2-independent cell surface entry pathway of SARS-coV-2 requires the furin cleavage site and the S2 domain of spike protein. *mBio* (2022) 13:e0051922. doi: 10.1128/mbio.00519-22

125. Kastenhuber ER, Mercadante M, Nilsson-Payant B, Johnson JL, Jaimes JA, Muecksch F, et al. Coagulation factors directly cleave SARS-CoV-2 spike and enhance viral entry. *Elife* (2022) 11:e77444. doi: 10.7554/eLife.77444

126. Wettstein L, Immenschuh P, Weil T, Conzelmann C, Almeida-Hernández Y, Hoffmann M, et al. Native and activated antithrombin inhibits TMPRSS2 activity and SARS-CoV-2 infection. *J Med Virol* (2023) 95:e28124. doi: 10.1002/jmv.28124

127. Buchrieser J, Dufloo J, Hubert M, Monel B, Planas D, Rajah MM, et al. Syncytia formation by SARS-CoV-2-infected cells. *EMBO J* (2021) 40:e107405. doi: 10.15252/embj.2020107405

128. Rajah MM, Bernier A, Buchrieser J, Schwartz O. The mechanism and consequences of SARS-coV-2 spike-mediated fusion and syncytia formation. *J Mol Biol* (2022) 434:167280. doi: 10.1016/j.jmb.2021.167280

129. Zeng C, Evans JP, King T, Zheng Y-M, Oltz EM, Whelan SPJ, et al. SARS-CoV-2 spreads through cell-to-cell transmission. *Proc Natl Acad Sci* (2022) 119: e2111400119. doi: 10.1073/pnas.2111400119

130. Bussani R, Schneider E, Zentilin L, Collesi C, Ali H, Braga L, et al. Persistence of viral RNA, pneumocyte syncytia and thrombosis are hallmarks of advanced COVID-19 pathology. *EBioMedicine* (2020) 61:103104. doi: 10.1016/j.ebiom.2020.103104

131. Sanders DW, Jumper CC, Ackerman PJ, Bracha D, Donlic A, Kim H, et al. SARS-CoV-2 requires cholesterol for viral entry and pathological syncytia formation. *Elife* (2021) 10:e65962. doi: 10.7554/eLife.65962

132. Wang H, Guo S, Yang H. Rapid quantitative monitoring of SARS-CoV-2 spike protein-mediated syncytia formation using split NanoLuc. *J Med Virol* (2022) 94:6073–7. doi: 10.1002/jmv.28053

133. Zhang Z, Zheng Y, Niu Z, Zhang B, Wang C, Yao X, et al. SARS-CoV-2 spike protein dictates syncytium-mediated lymphocyte elimination. *Cell Death Differ* (2021) 28:2765–77. doi: 10.1038/s41418-021-00782-3

134. Mendonça L, Howe A, Gilchrist JB, Sheng Y, Sun D, Knight ML, et al. Correlative multi-scale cryo-imaging unveils SARS-CoV-2 assembly and egress. *Nat Commun* (2021) 12:4629. doi: 10.1038/s41467-021-24887-y

135. Fennelly KP, Jones-López EC. Quantity and quality of inhaled dose predicts immunopathology in tuberculosis. *Front Immunol* (2015) 6:313. doi: 10.3389/fimmu.2015.00313

136. Khan HS, Nair VR, Ruhl CR, Alvarez-Arguedas S, Galvan Rendiz JL, Franco LH, et al. Identification of scavenger receptor B1 as the airway microfold cell receptor for Mycobacterium tuberculosis. *eLife* (2020) 9:e52551. doi: 10.7554/eLife.52551

137. Nair VR, Franco LH, Zacharia VM, Khan HS, Stamm CE, You W, et al. Microfold cells actively translocate mycobacterium tuberculosis to initiate infection. *Cell Rep* (2016) 16:1253–8. doi: 10.1016/j.celrep.2016.06.080

138. Lovey A, Verma S, Kaipilyawar V, Ribeiro-Rodrigues R, Husain S, Palaci M, et al. Early alveolar macrophage response and IL-1R-dependent T cell priming determine transmissibility of Mycobacterium tuberculosis strains. *Nat Commun* (2022) 13:884. doi: 10.1038/s41467-022-28506-2

139. Cohen SB, Gern BH, Delahaye JL, Adams KN, Plumlee CR, Winkler JK, et al. Alveolar macrophages provide an early mycobacterium tuberculosis niche and initiate dissemination. *Cell Host Microbe* (2018) 24:439–446.e4. doi: 10.1016/j.chom.2018.08.001

140. Boechat JL, Chora I, Morais A, Delgado L. The immune response to SARS-CoV-2 and COVID-19 immunopathology - Current perspectives. *Pulmonology* (2021) 27:423–37. doi: 10.1016/j.pulmoe.2021.03.008

141. Schultze JL, Aschenbrenner AC. COVID-19 and the human innate immune system. *Cell* (2021) 184:1671–92. doi: 10.1016/j.cell.2021.02.029

142. Roukens AHE, Pothast CR, König M, Huisman W, Dalebout T, Tak T, et al. Prolonged activation of nasal immune cell populations and development of tissue-resident SARS-CoV-2-specific CD8+ T cell responses following COVID-19. *Nat Immunol* (2022) 23:23–32. doi: 10.1038/s41590-021-01095-w

143. Schiuma G, Beltrami S, Bortolotti D, Rizzo S, Rizzo R. Innate immune response in SARS-coV-2 infection. *Microorganisms* (2022) 10:501. doi: 10.3390/microorganisms10030501

144. Arunachalam PS, Wimmers F, Mok CKP, Perera RAPM, Scott M, Hagan T, et al. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. *Science* (2020) 369:1210–20. doi: 10.1126/science.abc6261

145. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. *Nat Rev Immunol* (2015) 15:87–103. doi: 10.1038/nri3787

146. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann H-H, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* (2020) 370:eabd4585. doi: 10.1126/science.abd4585

147. Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science* (2020) 370:eabd4570. doi: 10.1126/science.abd4570

148. Contoli M, Papi A, Tomassetti L, Rizzo P, Vieceli Dalla Sega F, Fortini F, et al. Blood interferon-α Levels and severity, outcomes, and inflammatory profiles in hospitalized COVID-19 patients. *Front Immunol* (2021) 12:648004. doi: 10.3389/fimmu.2021.648004

149. Venet M, Ribeiro MS, Décembre E, Bellomo A, Joshi G, Nuovo C, et al. Severe COVID-19 patients have impaired plasmacytoid dendritic cell-mediated control of SARS-CoV-2. *Nat Commun* (2023) 14:694. doi: 10.1038/s41467-023-36140-9

150. Zhou R, To KK-W, Wong Y-C, Liu L, Zhou B, Li X, et al. Acute SARS-coV-2 infection impairs dendritic cell and T cell responses. *Immunity* (2020) 53:864–877.e5. doi: 10.1016/j.immuni.2020.07.026

151. Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. *Emerg Microbes Infect* (2020) 9:761–70. doi: 10.1080/22221751.2020.1747363

152. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5

153. Wang J, Jiang M, Chen X, Montaner LJ. Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: Review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts. *J Leukocyte Biol* (2020) 108:17–41. doi: 10.1002/JLB.3COVR0520-272R

154. Wu D, Yang XO. TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib. *J Microbiology Immunol Infection* (2020) 53:368–70. doi: 10.1016/j.jmii.2020.03.005

155. Yang Y, Shen C, Li J, Yuan J, Wei J, Huang F, et al. Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. *J Allergy Clin Immunol* (2020) 146:119–127.e4. doi: 10.1016/j.jaci.2020.04.027

156. Chua RL, Lukassen S, Trump S, Hennig BP, Wendisch D, Pott F, et al. COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis. *Nat Biotechnol* (2020) 38:970–9. doi: 10.1038/s41587-020-0602-4

157. Pan P, Shen M, Yu Z, Ge W, Chen K, Tian M, et al. SARS-CoV-2 N protein promotes NLRP3 inflammasome activation to induce hyperinflammation. *Nat Commun* (2021) 12:4664. doi: 10.1038/s41467-021-25015-6

158. Sefik E, Qu R, Junqueira C, Kaffe E, Mirza H, Zhao J, et al. Inflammasome activation in infected macrophages drives COVID-19 pathology. *Nature* (2022) 606:585–93. doi: 10.1038/s41586-022-04802-1

159. Qin S, Jiang Y, Wei X, Liu X, Guan J, Chen Y, et al. Dynamic changes in monocytes subsets in COVID-19 patients. *Hum Immunol* (2021) 82:170–6. doi: 10.1016/j.humimm.2020.12.010

160. Toor D, Jain A, Kalhan S, Manocha H, Sharma VK, Jain P, et al. Tempering macrophage plasticity for controlling SARS-coV-2 infection for managing COVID-19 disease. *Front Pharmacol* (2020) 11:570698. doi: 10.3389/fphar.2020.570698

161. Lian Q, Zhang K, Zhang Z, Duan F, Guo L, Luo W, et al. Differential effects of macrophage subtypes on SARS-CoV-2 infection in a human pluripotent stem cell-derived model. *Nat Commun* (2022) 13:2028. doi: 10.1038/s41467-022-29731-5

162. Zhang D, Guo R, Lei L, Liu H, Wang Y, Wang Y, et al. Frontline Science: COVID-19 infection induces readily detectable morphologic and inflammation-related phenotypic changes in peripheral blood monocytes. *J Leukocyte Biol* (2021) 109:13–22. doi: 10.1002/JLB.4HI0720-470R

163. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med* (2020) 26:842–4. doi: 10.1038/s41591-020-0901-9

164. Björkström NK, Strunz B, Ljunggren H-G. Natural killer cells in antiviral immunity. *Nat Rev Immunol* (2022) 22:112–23. doi: 10.1038/s41577-021-00558-3

165. Witkowski M, Tizian C, Ferreira-Gomes M, Niemeyer D, Jones TC, Heinrich F, et al. Untimely TGFβ responses in COVID-19 limit antiviral functions of NK cells. *Nature* (2021) 600:295–301. doi: 10.1038/s41586-021-04142-6

166. Bi J. NK cell dysfunction in patients with COVID-19. *Cell Mol Immunol* (2022) 19:127–9. doi: 10.1038/s41423-021-00825-2

167. Krämer B, Knoll R, Bonaguro L, ToVinh M, Raabe J, Astaburuaga-García R, et al. Early IFN-α signatures and persistent dysfunction are distinguishing features of NK cells in severe COVID-19. *Immunity* (2021) 54:2650–2669.e14. doi: 10.1016/j.immuni.2021.09.002

168. Lenart M, Górecka M, Bochenek M, Barreto-Duran E, Szczepański A, Gałuszka-Bulaga A, et al. SARS-CoV-2 infection impairs NK cell functions *via* activation of the LLT1-CD161 axis. *Front Immunol* (2023) 14:1123155. doi: 10.3389/fimmu.2023.1123155

169. Crosby CM, Kronenberg M. Invariant natural killer T cells: front line fighters in the war against pathogenic microbes. *Immunogenetics* (2016) 68:639–48. doi: 10.1007/s00251-016-0933-y

170. Wang X, Guan F, Miller H, Byazrova MG, Candotti F, Benlagha K, et al. The role of dendritic cells in COVID-19 infection. *Emerg Microbes Infect* (2023) 12:2195019. doi: 10.1080/22221751.2023.2195019

171. Lerner TR, Borel S, Gutierrez MG. The innate immune response in human tuberculosis. *Cell Microbiol* (2015) 17:1277–85. doi: 10.1111/cmi.12480

172. Khan N, Vidyarthi A, Javed S, Agrewala JN. Innate Immunity Holding the Flanks until Reinforced by Adaptive Immunity against Mycobacterium tuberculosis Infection. *Front Microbiol* (2016) 7:328. doi: 10.3389/fmicb.2016.00328

173. Castillo EF, Dekonenko A, Arko-Mensah J, Mandell MA, Dupont N, Jiang S, et al. Autophagy protects against active tuberculosis by suppressing bacterial burden

and inflammation. *Proc Natl Acad Sci U.S.A.* (2012) 109:E3168–3176. doi: 10.1073/pnas.1210500109

174. Schön T, Elmberger G, Negesse Y, Pando RH, Sundqvist T, Britton S. Local production of nitric oxide in patients with tuberculosis. *Int J Tuberc Lung Dis* (2004) 8:1134–7.

175. Cadena AM, Flynn JL, Fortune SM. The importance of first impressions: early events in mycobacterium tuberculosis infection influence outcome. *mBio* (2016) 7: e00342–00316. doi: 10.1128/mBio.00342-16

176. Reuschl A-K, Edwards MR, Parker R, Connell DW, Hoang L, Halliday A, et al. Innate activation of human primary epithelial cells broadens the host response to Mycobacterium tuberculosis in the airways. *PloS Pathog* (2017) 13:e1006577. doi: 10.1371/journal.ppat.1006577

177. Mukundan S, Singh P, Shah A, Kumar R, O'Neill KC, Carter CL, et al. *In vitro* miniaturized tuberculosis spheroid model. *Biomedicines* (2021) 9:1209. doi: 10.3390/biomedicines9091209

178. Iakobachvili N, Leon-Icaza SA, Knoops K, Sachs N, Mazères S, Simeone R, et al. Mycobacteria-host interactions in human bronchiolar airway organoids. *Mol Microbiol* (2022) 117:682–92. doi: 10.1111/mmi.14824

179. Clay H, Davis JM, Beery D, Huttenlocher A, Lyons SE, Ramakrishnan L. Dichotomous role of the macrophage in early Mycobacterium marinum infection of the zebrafish. *Cell Host Microbe* (2007) 2:29–39. doi: 10.1016/j.chom.2007.06.004

180. Gupta UD, Katoch VM. Animal models of tuberculosis. *Tuberculosis (Edinb)* (2005) 85:277–93. doi: 10.1016/j.tube.2005.08.008

181. Kashino SS, Napolitano DR, Skobe Z, Campos-Neto A. Guinea pig model of Mycobacterium tuberculosis latent/dormant infection. *Microbes Infect* (2008) 10:1469–76. doi: 10.1016/j.micinf.2008.08.010

182. Subbian S, Tsenova L, O'Brien P, Yang G, Koo M-S, Peixoto B, et al. Phosphodiesterase-4 inhibition alters gene expression and improves isoniazid-mediated clearance of Mycobacterium tuberculosis in rabbit lungs. *PloS Pathog* (2011) 7:e1002262. doi: 10.1371/journal.ppat.1002262

183. Singhal A, Aliouat EM, Hervé M, Mathys V, Kiass M, Creusy C, et al. Experimental tuberculosis in the Wistar rat: a model for protective immunity and control of infection. *PloS One* (2011) 6:e18632. doi: 10.1371/journal.pone.0018632

184. Lin PL, Myers A, Smith L, Bigbee C, Bigbee M, Fuhrman C, et al. Tumor necrosis factor neutralization results in disseminated disease in acute and latent Mycobacterium tuberculosis infection with normal granuloma structure in a cynomolgus macaque model. *Arthritis Rheum* (2010) 62:340–50. doi: 10.1002/art.27271

185. Singh PP, Smith VL, Karakousis PC, Schorey JS. Exosomes isolated from mycobacteria-infected mice or cultured macrophages can recruit and activate immune cells in *vitro* and in *vivo*. *J Immunol* (2012) 189:777–85. doi: 10.4049/jimmunol.1103638

186. Alvarez-Jiménez VD, Leyva-Paredes K, García-Martínez M, Vázquez-Flores L, García-Paredes VG, Campillo-Navarro M, et al. Extracellular vesicles released from mycobacterium tuberculosis-infected neutrophils promote macrophage autophagy and decrease intracellular mycobacterial survival. *Front Immunol* (2018) 9:272. doi: 10.3389/fimmu.2018.00272

187. Rothchild AC, Olson GS, Nemeth J, Amon LM, Mai D, Gold ES, et al. Alveolar macrophages generate a noncanonical NRF2-driven transcriptional response to Mycobacterium tuberculosis in vivo. *Sci Immunol* (2019) 4:eaaw6693. doi: 10.1126/sciimmunol.aaw6693

188. Repasy T, Lee J, Marino S, Martinez N, Kirschner DE, Hendricks G, et al. Intracellular bacillary burden reflects a burst size for Mycobacterium tuberculosis in *vivo*. *PloS Pathog* (2013) 9:e1003190. doi: 10.1371/journal.ppat.1003190

189. Wolf AJ, Linas B, Trevejo-Nuñez GJ, Kincaid E, Tamura T, Takatsu K, et al. Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function in *vivo*. *J Immunol* (2007) 179:2509–19. doi: 10.4049/jimmunol.179.4.2509

190. Khader SA, Partida-Sanchez S, Bell G, Jelley-Gibbs DM, Swain S, Pearl JE, et al. Interleukin 12p40 is required for dendritic cell migration and T cell priming after Mycobacterium tuberculosis infection. *J Exp Med* (2006) 203:1805–15. doi: 10.1084/jem.20052545

191. Tian T, Woodworth J, Sköld M, Behar SM. *In vivo* depletion of CD11c+ cells delays the CD4+ T cell response to Mycobacterium tuberculosis and exacerbates the outcome of infection. *J Immunol* (2005) 175:3268–72. doi: 10.4049/jimmunol.175.5.3268

192. Samstein M, Schreiber HA, Leiner IM, Sušac B, Glickman MS, Pamer EG. Essential yet limited role for CCR2+ inflammatory monocytes during Mycobacterium tuberculosis-specific T cell priming. *eLife* (2013) 2:e01086. doi: 10.7554/eLife.01086

193. Blomgran R, Ernst JD. Lung neutrophils facilitate activation of naive antigen-specific CD4+ T cells during Mycobacterium tuberculosis infection. *J Immunol* (2011) 186:7110–9. doi: 10.4049/jimmunol.1100001

194. Zheng L, He M, Long M, Blomgran R, Stendahl O. Pathogen-induced apoptotic neutrophils express heat shock proteins and elicit activation of human macrophages. *J Immunol* (2004) 173:6319–26. doi: 10.4049/jimmunol.173.10.6319

195. Alemán M, de la Barrera S, Schierloh P, Yokobori N, Baldini M, Musella R, et al. Spontaneous or Mycobacterium tuberculosis-induced apoptotic neutrophils exert opposite effects on the dendritic cell-mediated immune response. *Eur J Immunol* (2007) 37:1524–37. doi: 10.1002/eji.200636771

196. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* (2012) 491:717–23. doi: 10.1038/nature11605

197. Sia JK, Georgieva M, Rengarajan J. Innate Immune Defenses in Human Tuberculosis: An Overview of the Interactions between Mycobacterium tuberculosis and Innate Immune Cells. *J Immunol Res* (2015) 2015:747543. doi: 10.1155/2015/747543

198. Portevin D, Via LE, Eum S, Young D. Natural killer cells are recruited during pulmonary tuberculosis and their ex vivo responses to mycobacteria vary between healthy human donors in association with KIR haplotype. *Cell Microbiol* (2012) 14:1734–44. doi: 10.1111/j.1462-5822.2012.01834.x

199. Abebe F. Immunological basis of early clearance of Mycobacterium tuberculosis infection: the role of natural killer cells. *Clin Exp Immunol* (2021) 204:32–40. doi: 10.1111/cei.13565

200. Divangahi M, Chen M, Gan H, Desjardins D, Hickman TT, Lee DM, et al. Mycobacterium tuberculosis evades macrophage defenses by inhibiting plasma membrane repair. *Nat Immunol* (2009) 10:899–906. doi: 10.1038/ni.1758

201. Mohareer K, Asalla S, Banerjee S. Cell death at the cross roads of host-pathogen interaction in Mycobacterium tuberculosis infection. *Tuberculosis (Edinb)* (2018) 113:99–121. doi: 10.1016/j.tube.2018.09.007

202. Filio-Rodríguez G, Estrada-García I, Arce-Paredes P, Moreno-AltamIrano MM, Islas-Trujillo S, Ponce-Regalado MD, et al. *In vivo* induction of neutrophil extracellular traps by Mycobacterium tuberculosis in a Guinea pig model. *Innate Immun* (2017) 23:625–37. doi: 10.1177/1753425917732406

203. Beckwith KS, Beckwith MS, Ullmann S, Sætra RS, Kim H, Marstad A, et al. Plasma membrane damage causes NLRP3 activation and pyroptosis during Mycobacterium tuberculosis infection. *Nat Commun* (2020) 11:2270. doi: 10.1038/s41467-020-16143-6

204. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol* (2020) 20:363–74. doi: 10.1038/s41577-020-0311-8

205. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol* (2020) 20:355–62. doi: 10.1038/s41577-020-0331-4

206. Wong L-YR, Perlman S. Author Correction: Immune dysregulation and immunopathology induced by SARS-CoV-2 and related coronaviruses - are we our own worst enemy? *Nat Rev Immunol* (2022) 22:200. doi: 10.1038/s41577-021-00673-1

207. Zhang Q, Bastard PCOVID Human Genetic Effor, , Cobat A, Casanova J-L. Human genetic and immunological determinants of critical COVID-19 pneumonia. *Nature* (2022) 603:587–98. doi: 10.1038/s41586-022-04447-0

208. Kudlay D, Kofiadi I, Khaitov M. Peculiarities of the T cell immune response in COVID-19. *Vaccines (Basel)* (2022) 10:242. doi: 10.3390/vaccines10020242

209. Snyder ME, Farber DL. Human lung tissue resident memory T cells in health and disease. *Curr Opin Immunol* (2019) 59:101–8. doi: 10.1016/j.coi.2019.05.011

210. Smith VL, Cheng Y, Bryant BR, Schorey JS. Exosomes function in antigen presentation during an in *vivo* Mycobacterium tuberculosis infection. *Sci Rep* (2017) 7:43578. doi: 10.1038/srep43578

211. Le Bert N, Clapham HE, Tan AT, Chia WN, Tham CYL, Lim JM, et al. Highly functional virus-specific cellular immune response in asymptomatic SARS-CoV-2 infection. *J Exp Med* (2021) 218:e20202617. doi: 10.1084/jem.20202617

212. Boyton RJ, Altmann DM. The immunology of asymptomatic SARS-CoV-2 infection: what are the key questions? *Nat Rev Immunol* (2021) 21:762–8. doi: 10.1038/s41577-021-00631-x

213. Reynolds CJ, Swadling L, Gibbons JM, Pade C, Jensen MP, Diniz MO, et al. Discordant neutralizing antibody and T cell responses in asymptomatic and mild SARS-CoV-2 infection. *Sci Immunol* (2020) 5:eabf3698. doi: 10.1126/sciimmunol.abf3698

214. Cao Y, Wang J, Jian F, Xiao T, Song W, Yisimayi A, et al. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature* (2022) 602:657–63. doi: 10.1038/s41586-021-04385-3

215. van Dorp L, Houldcroft CJ, Richard D, Balloux F. COVID-19, the first pandemic in the post-genomic era. *Curr Opin Virol* (2021) 50:40–8. doi: 10.1016/j.coviro.2021.07.002

216. Liu L, Iketani S, Guo Y, Chan JF-W, Wang M, Liu L, et al. Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature* (2022) 602:676–81. doi: 10.1038/s41586-021-04388-0

217. De Marco L, D'Orso S, Pirronello M, Verdiani A, Termine A, Fabrizio C, et al. Assessment of T-cell reactivity to the SARS-coV-2 omicron variant by immunized individuals. *JAMA Netw Open* (2022) 5:e2210871. doi: 10.1001/jamanetworkopen.2022.10871

218. Gao Y, Cai C, Grifoni A, Müller TR, Niessl J, Olofsson A, et al. Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. *Nat Med* (2022) 28:472–6. doi: 10.1038/s41591-022-01700-x

219. Tarke A, Coelho CH, Zhang Z, Dan JM, Yu ED, Methot N, et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. *Cell* (2022) 185:847–859.e11. doi: 10.1016/j.cell.2022.01.015

220. Petrone L, Picchianti-Diamanti A, Sebastiani GD, Aiello A, Laganà B, Cuzzi G, et al. Humoral and cellular responses to spike of δ SARS-CoV-2 variant in vaccinated

patients with immune-mediated inflammatory diseases. *Int J Infect Dis* (2022) 121:24–30. doi: 10.1016/j.ijid.2022.04.027

221. Petrone L, Tortorella C, Aiello A, Farroni C, Ruggieri S, Castilletti C, et al. Humoral and cellular response to spike of delta SARS-coV-2 variant in vaccinated patients with multiple sclerosis. *Front Neurol* (2022) 13:881988. doi: 10.3389/fneur.2022.881988

222. Tan AT, Linster M, Tan CW, Le Bert N, Chia WN, Kunasegaran K, et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. *Cell Rep* (2021) 34:108728. doi: 10.1016/j.celrep.2021.108728

223. Bergamaschi L, Mescia F, Turner L, Hanson AL, Kotagiri P, Dunmore BJ, et al. Longitudinal analysis reveals that delayed bystander CD8+ T cell activation and early immune pathology distinguish severe COVID-19 from mild disease. *Immunity* (2021) 54:1257–1275.e8. doi: 10.1016/j.immuni.2021.05.010

224. Tarke A, Potesta M, Varchetta S, Fenoglio D, Iannetta M, Sarmati L, et al. Early and polyantigenic CD4 T cell responses correlate with mild disease in acute COVID-19 donors. *Int J Mol Sci* (2022) 23:7155. doi: 10.3390/ijms23137155

225. Chauss D, Freiwald T, McGregor R, Yan B, Wang L, Nova-Lamperti E, et al. Autocrine vitamin D signaling switches off pro-inflammatory programs of TH1 cells. *Nat Immunol* (2022) 23:62–74. doi: 10.1038/s41590-021-01080-3

226. Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* (2020) 584:463–9. doi: 10.1038/s41586-020-2588-y

227. Gil-Etayo FJ, Suàrez-Fernández P, Cabrera-Marante O, Arroyo D, Garcinuño S, Naranjo L, et al. T-helper cell subset response is a determining factor in COVID-19 progression. *Front Cell Infect Microbiol* (2021) 11:624483. doi: 10.3389/fcimb.2021.624483

228. Pourgholaminejad A, Aghdami N, Baharvand H, Moazzeni SM. Is TGFβ as an anti-inflammatory cytokine required for differentiation of inflammatory TH17 cells? *J Immunotoxicol* (2016) 13:775–83. doi: 10.1080/1547691X.2016.1193574

229. Zander R, Kasmani MY, Chen Y, Topchyan P, Shen J, Zheng S, et al. Tfh-cell-derived interleukin 21 sustains effector CD8+ T cell responses during chronic viral infection. *Immunity* (2022) 55:475–493.e5. doi: 10.1016/j.immuni.2022.01.018

230. McMahan K, Yu J, Mercado NB, Loos C, Tostanoski LH, Chandrashekar A, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature* (2021) 590:630–4. doi: 10.1038/s41586-020-03041-6

231. Mallajosyula V, Ganjavi C, Chakraborty S, McSween AM, Pavlovitch-Bedzyk AJ, Wilhelmy J, et al. CD8+ T cells specific for conserved coronavirus epitopes correlate with milder disease in COVID-19 patients. *Sci Immunol* (2021) 6:eabg5669. doi: 10.1126/sciimmunol.abg5669

232. Dhawan M, Rabaan AA, Alwarthan S, Alhajri M, Halwani MA, Alshengeti A, et al. Regulatory T cells (Tregs) and COVID-19: unveiling the mechanisms, and therapeutic potentialities with a special focus on long COVID. *Vaccines* (2023) 11:699. doi: 10.3390/vaccines11030699

233. Wang K, Chen W, Zhang Z, Deng Y, Lian J-Q, Du P, et al. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduct Target Ther* (2020) 5:283. doi: 10.1038/s41392-020-00426-x

234. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect* (2020) 80:607–13. doi: 10.1016/j.jinf.2020.03.037

235. Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, et al. SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet* (2020) 395:1517–20. doi: 10.1016/S0140-6736(20)30920-X

236. Peteranderl C, Herold S. The impact of the interferon/TNF-related apoptosis-inducing ligand signaling axis on disease progression in respiratory viral infection and beyond. *Front Immunol* (2017) 8:313. doi: 10.3389/fimmu.2017.00313

237. André S, Picard M, Cezar R, Roux-Dalvai F, Alleaume-Butaux A, Soundaramourty C, et al. T cell apoptosis characterizes severe Covid-19 disease. *Cell Death Differ* (2022) 29:1486–99. doi: 10.1038/s41418-022-00936-x

238. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Front Immunol* (2020) 11:827. doi: 10.3389/fimmu.2020.00827

239. Carreto-Binaghi LE, Herrera MT, Guzmán-Beltrán S, Juárez E, Sarabia C, Salgado-Cantú MG, et al. Reduced IL-8 secretion by NOD-like and toll-like receptors in blood cells from COVID-19 patients. *Biomedicines* (2023) 11:1078. doi: 10.3390/biomedicines11041078

240. Jiang Y, Wei X, Guan J, Qin S, Wang Z, Lu H, et al. COVID-19 pneumonia: CD8+ T and NK cells are decreased in number but compensatory increased in cytotoxic potential. *Clin Immunol* (2020) 218:108516. doi: 10.1016/j.clim.2020.108516

241. Alahdal M, Elkord E. Exhaustion and over-activation of immune cells in COVID-19: Challenges and therapeutic opportunities. *Clin Immunol* (2022) 245:109177. doi: 10.1016/j.clim.2022.109177

242. Suthar MS, Zimmerman MG, Kauffman RC, Mantus G, Linderman SL, Hudson WH, et al. Rapid generation of neutralizing antibody responses in COVID-19 patients. *Cell Rep Med* (2020) 1:100040. doi: 10.1016/j.xcrm.2020.100040

243. Premkumar L, Segovia-Chumbez B, Jadi R, Martinez DR, Raut R, Markmann A, et al. The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Sci Immunol* (2020) 5:eabc8413. doi: 10.1126/sciimmunol.abc8413

244. Muecksch F, Wise H, Batchelor B, Squires M, Semple E, Richardson C, et al. Longitudinal serological analysis and neutralizing antibody levels in coronavirus disease 2019 convalescent patients. *J Infect Dis* (2021) 223:389–98. doi: 10.1093/infdis/jiaa659

245. Wang Z, Muecksch F, Schaefer-Babajew D, Finkin S, Viant C, Gaebler C, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* (2021) 595:426–31. doi: 10.1038/s41586-021-03696-9

246. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* (2021) 371:eabf4063. doi: 10.1126/science.abf4063

247. Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science* (2020) 370:1227–30. doi: 10.1126/science.abd7728

248. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). *Clin Infect Dis* (2020) 71:778–85. doi: 10.1093/cid/ciaa310

249. Isho B, Abe KT, Zuo M, Jamal AJ, Rathod B, Wang JH, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol* (2020) 5:eabe5511. doi: 10.1126/sciimmunol.abe5511

250. Zohar T, Alter G. Dissecting antibody-mediated protection against SARS-CoV-2. *Nat Rev Immunol* (2020) 20:392–4. doi: 10.1038/s41577-020-0359-5

251. Winkler ES, Gilchuk P, Yu J, Bailey AL, Chen RE, Chong Z, et al. Human neutralizing antibodies against SARS-CoV-2 require intact Fc effector functions for optimal therapeutic protection. *Cell* (2021) 184:1804–1820.e16. doi: 10.1016/j.cell.2021.02.026

252. Yamin R, Jones AT, Hoffmann H-H, Schäfer A, Kao KS, Francis RL, et al. Fc-engineered antibody therapeutics with improved anti-SARS-CoV-2 efficacy. *Nature* (2021) 599:465–70. doi: 10.1038/s41586-021-04017-w

253. Lee WS, Selva KJ, Davis SK, Wines BD, Reynaldi A, Esterbauer R, et al. Decay of Fc-dependent antibody functions after mild to moderate COVID-19. *Cell Rep Med* (2021) 2:100296. doi: 10.1016/j.xcrm.2021.100296

254. Anand SP, Prévost J, Nayrac M, Beaudoin-Bussières G, Benlarbi M, Gasser R, et al. Longitudinal analysis of humoral immunity against SARS-CoV-2 Spike in convalescent individuals up to 8 months post-symptom onset. *Cell Rep Med* (2021) 2:100290. doi: 10.1016/j.xcrm.2021.100290

255. Yu Y, Wang M, Zhang X, Li S, Lu Q, Zeng H, et al. Antibody-dependent cellular cytotoxicity response to SARS-CoV-2 in COVID-19 patients. *Signal Transduct Target Ther* (2021) 6:346. doi: 10.1038/s41392-021-00759-1

256. Piccoli L, Park Y-J, Tortorici MA, Czudnochowski N, Walls AC, Beltramello M, et al. Mapping neutralizing and immunodominant sites on the SARS-coV-2 spike receptor-binding domain by structure-guided high-resolution serology. *Cell* (2020) 183:1024–1042.e21. doi: 10.1016/j.cell.2020.09.037

257. Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KHD, Dingens AS, et al. Deep mutational scanning of SARS-coV-2 receptor binding domain reveals constraints on folding and ACE2 binding. *Cell* (2020) 182:1295–1310.e20. doi: 10.1016/j.cell.2020.08.012

258. McCallum M, Bassi J, De Marco A, Chen A, Walls AC, Di Iulio J, et al. SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* (2021) 373:648–54. doi: 10.1126/science.abi7994

259. McCallum M, De Marco A, Lempp FA, Tortorici MA, Pinto D, Walls AC, et al. N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *Cell* (2021) 184:2332–2347.e16. doi: 10.1016/j.cell.2021.03.028

260. Lempp FA, Soriaga LB, Montiel-Ruiz M, Benigni F, Noack J, Park Y-J, et al. Lectins enhance SARS-CoV-2 infection and influence neutralizing antibodies. *Nature* (2021) 598:342–7. doi: 10.1038/s41586-021-03925-1

261. Kuri-Cervantes L, Pampena MB, Meng W, Rosenfeld AM, Ittner CAG, Weisman AR, et al. Comprehensive mapping of immune perturbations associated with severe COVID-19. *Sci Immunol* (2020) 5:eabd7114. doi: 10.1126/sciimmunol.abd7114

262. Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* (2020) 26:1200–4. doi: 10.1038/s41591-020-0965-6

263. Burgener S, Rochat P, Dollenmaier G, Benz G, Kistler AD, Fulchini R. Progression of COVID-19 in a patient on anti-CD20 antibody treatment: case report and literature review. *Case Rep Infect Dis* (2022) 2022:8712424. doi: 10.1155/2022/8712424

264. Russell MW, Moldoveanu Z, Ogra PL, Mestecky J. Mucosal immunity in COVID-19: A neglected but critical aspect of SARS-coV-2 infection. *Front Immunol* (2020) 11:611337. doi: 10.3389/fimmu.2020.611337

265. De Biasi S, Lo Tartaro D, Meschiari M, Gibellini L, Bellinazzi C, Borella R, et al. Expansion of plasmablasts and loss of memory B cells in peripheral blood from COVID-19 patients with pneumonia. *Eur J Immunol* (2020) 50:1283–94. doi: 10.1002/eji.202048838

266. Bernardes JP, Mishra N, Tran F, Bahmer T, Best L, Blase JI, et al. Longitudinal multi-omics analyses identify responses of megakaryocytes, erythroid cells, and plasmablasts as hallmarks of severe COVID-19. *Immunity* (2020) 53:1296–1314.e9. doi: 10.1016/j.immuni.2020.11.017

267. Slight SR, Khader SA. Chemokines shape the immune responses to tuberculosis. *Cytokine Growth Factor Rev* (2013) 24:105–13. doi: 10.1016/j.cytogfr.2012.10.002

268. Olmos S, Stukes S, Ernst JD. Ectopic activation of Mycobacterium tuberculosis-specific CD4+ T cells in lungs of CCR7-/- mice. *J Immunol* (2010) 184:895–901. doi: 10.4049/jimmunol.0901230

269. Lin PL, Pawar S, Myers A, Pegu A, Fuhrman C, Reinhart TA, et al. Early events in Mycobacterium tuberculosis infection in cynomolgus macaques. *Infect Immun* (2006) 74:3790–803. doi: 10.1128/IAI.00064-06

270. Chackerian AA, Alt JM, Perera TV, Dascher CC, Behar SM. Dissemination of Mycobacterium tuberculosis is influenced by host factors and precedes the initiation of T-cell immunity. *Infect Immun* (2002) 70:4501–9. doi: 10.1128/IAI.70.8.4501-4509.2002

271. Srivastava S, Ernst JD. Cell-to-cell transfer of M. tuberculosis antigens optimizes CD4 T cell priming. *Cell Host Microbe* (2014) 15:741–52. doi: 10.1016/j.chom.2014.05.007

272. Gideon HP, Hughes TK, Tzouanas CN, Wadsworth MH, Tu AA, Gierahn TM, et al. Multimodal profiling of lung granulomas in macaques reveals cellular correlates of tuberculosis control. *Immunity* (2022) 55:827–846.e10. doi: 10.1016/j.immuni.2022.04.004

273. Donovan ML, Bielefeldt-Ohmann H, Rollo RF, McPherson SJ, Schultz TE, Mori G, et al. Distinct contributions of the innate immune receptors TLR2 and RP105 to formation and architecture of structured lung granulomas in mice infected with Mycobacterium tuberculosis. *Immunology* (2023) 169:13–26. doi: 10.1111/imm.13606

274. Guirado E, Mbawuike U, Keiser TL, Arcos J, Azad AK, Wang S-H, et al. Characterization of host and microbial determinants in individuals with latent tuberculosis infection using a human granuloma model. *mBio* (2015) 6:e02537–02514. doi: 10.1128/mBio.02537-14

275. Wayne LG, Lin KY. Glyoxylate metabolism and adaptation of Mycobacterium tuberculosis to survival under anaerobic conditions. *Infect Immun* (1982) 37:1042–9. doi: 10.1128/iai.37.3.1042-1049.1982

276. de la Barrera S, Aleman M, Musella R, Schierloh P, Pasquinelli V, Garcia V, et al. IL-10 down-regulates costimulatory molecules on Mycobacterium tuberculosis-pulsed macrophages and impairs the lytic activity of CD4 and CD8 CTL in tuberculosis patients. *Clin Exp Immunol* (2004) 138:128–38. doi: 10.1111/j.1365-2249.2004.02577.x

277. Mogues T, Goodrich ME, Ryan L, LaCourse R, North RJ. The relative importance of T cell subsets in immunity and immunopathology of airborne Mycobacterium tuberculosis infection in mice. *J Exp Med* (2001) 193:271–80. doi: 10.1084/jem.193.3.271

278. Lin PL, Rutledge T, Green AM, Bigbee M, Fuhrman C, Klein E, et al. CD4 T cell depletion exacerbates acute Mycobacterium tuberculosis while reactivation of latent infection is dependent on severity of tissue depletion in cynomolgus macaques. *AIDS Res Hum Retroviruses* (2012) 28:1693–702. doi: 10.1089/AID.2012.0028

279. Fabri M, Stenger S, Shin D-M, Yuk J-M, Liu PT, Realegeno S, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. *Sci Transl Med* (2011) 3:104ra102. doi: 10.1126/scitranslmed.3003045

280. Sallin MA, Sakai S, Kauffman KD, Young HA, Zhu J, Barber DL. Th1 differentiation drives the accumulation of intravascular, non-protective CD4 T cells during tuberculosis. *Cell Rep* (2017) 18:3091–104. doi: 10.1016/j.celrep.2017.03.007

281. Goletti D, Weissman D, Jackson RW, Graham NM, Vlahov D, Klein RS, et al. Effect of Mycobacterium tuberculosis on HIV replication. *Role Immune activation. J Immunol* (1996) 157:1271–8. doi: 10.4049/jimmunol.157.3.1271

282. Goletti D, Weissman D, Jackson RW, Collins F, Kinter A, Fauci AS. The in *vitro* induction of human immunodeficiency virus (HIV) replication in purified protein derivative-positive HIV-infected persons by recall antigen response to Mycobacterium tuberculosis is the result of a balance of the effects of endogenous interleukin-2 and proinflammatory and antiinflammatory cytokines. *J Infect Dis* (1998) 177:1332–8. doi: 10.1086/515276

283. Jasenosky LD, Scriba TJ, Hanekom WA, Goldfeld AE. T cells and adaptive immunity to Mycobacterium tuberculosis in humans. *Immunol Rev* (2015) 264:74–87. doi: 10.1111/imr.12274

284. Boom WH, Schaible UE, Achkar JM. The knowns and unknowns of latent Mycobacterium tuberculosis infection. *J Clin Invest* (2021) 131:e136222. doi: 10.1172/JCI136222

285. Cantini F, Niccoli L, Capone A, Petrone L, Goletti D. Risk of tuberculosis reactivation associated with traditional disease modifying anti-rheumatic drugs and non-anti-tumor necrosis factor biologics in patients with rheumatic disorders and suggestion for clinical practice. *Expert Opin Drug Saf* (2019) 18:415–25. doi: 10.1080/14740338.2019.1612872

286. Chen CY, Huang D, Wang RC, Shen L, Zeng G, Yao S, et al. A critical role for CD8 T cells in a nonhuman primate model of tuberculosis. *PloS Pathog* (2009) 5:e1000392. doi: 10.1371/journal.ppat.1000392

287. Gaffen SL, Jain R, Garg AV, Cua DJ. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat Rev Immunol* (2014) 14:585–600. doi: 10.1038/nri3707

288. Freches D, Korf H, Denis O, Havaux X, Huygen K, ROmano M. Mice genetically inactivated in interleukin-17A receptor are defective in long-term control of Mycobacterium tuberculosis infection. *Immunology* (2013) 140:220–31. doi: 10.1111/imm.12130

289. Cruz A, Khader SA, Torrado E, Fraga A, Pearl JE, Pedrosa J, et al. Cutting edge: IFN-gamma regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. *J Immunol* (2006) 177:1416–20. doi: 10.4049/jimmunol.177.3.1416

290. Desvignes L, Ernst JD. Interferon-gamma-responsive nonhematopoietic cells regulate the immune response to Mycobacterium tuberculosis. *Immunity* (2009) 31:974–85. doi: 10.1016/j.immuni.2009.10.007

291. Najafi-Fard S, Petruccioli E, Farroni C, Petrone L, Vanini V, Cuzzi G, et al. Evaluation of the immunomodulatory effects of interleukin-10 on peripheral blood immune cells of COVID-19 patients: Implication for COVID-19 therapy. *Front Immunol* (2022) 13:984098. doi: 10.3389/fimmu.2022.984098

292. Cicchese JM, Evans S, Hult C, Joslyn LR, Wessler T, Millar JA, et al. Dynamic balance of pro- and anti-inflammatory signals controls disease and limits pathology. *Immunol Rev* (2018) 285:147–67. doi: 10.1111/imr.12671

293. Boussiotis VA, Tsai EY, Yunis EJ, Thim S, Delgado JC, Dascher CC, et al. IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. *J Clin Invest* (2000) 105:1317–25. doi: 10.1172/JCI9918

294. Irvine EB, O'Neil A, Darrah PA, Shin S, Choudhary A, Li W, et al. Robust IgM responses following intravenous vaccination with Bacille Calmette-Guérin associate with prevention of Mycobacterium tuberculosis infection in macaques. *Nat Immunol* (2021) 22:1515–23. doi: 10.1038/s41590-021-01066-1

295. Watson A, Li H, Ma B, Weiss R, Bendayan D, Abramovitz L, et al. Human antibodies targeting a Mycobacterium transporter protein mediate protection against tuberculosis. *Nat Commun* (2021) 12:602. doi: 10.1038/s41467-021-20930-0

296. Alter G, Ottenhoff THM, Joosten SA. Antibody glycosylation in inflammation, disease and vaccination. *Semin Immunol* (2018) 39:102–10. doi: 10.1016/j.smim.2018.05.003

297. Roy Chowdhury R, Vallania F, Yang Q, Lopez Angel CJ, Darboe F, Penn-Nicholson A, et al. A multi-cohort study of the immune factors associated with M. tuberculosis infection outcomes. *Nature* (2018) 560:644–8. doi: 10.1038/s41586-018-0439-x

298. Lu LL, Chung AW, Rosebrock TR, Ghebremichael M, Yu WH, Grace PS, et al. A functional role for antibodies in tuberculosis. *Cell* (2016) 167:433–443.e14. doi: 10.1016/j.cell.2016.08.072

299. Grace PS, Dolatshahi S, Lu LL, Cain A, Palmieri F, Petrone L, et al. Antibody subclass and glycosylation shift following effective TB treatment. *Front Immunol* (2021) 12:679973. doi: 10.3389/fimmu.2021.679973

300. Lu LL, Smith MT, Yu KKQ, Luedemann C, Suscovich TJ, Grace PS, et al. IFN-γ-independent immune markers of Mycobacterium tuberculosis exposure. *Nat Med* (2019) 25:977–87. doi: 10.1038/s41591-019-0441-3

301. Melkie ST, Arias L, Farroni C, Jankovic Makek M, Goletti D, Vilaplana C. The role of antibodies in tuberculosis diagnosis, prophylaxis and therapy: a review from the ESGMYC study group. *Eur Respir Rev* (2022) 31:210218. doi: 10.1183/16000617.0218-2021

302. Sy KTL, Haw NJL, Uy J. Previous and active tuberculosis increases risk of death and prolongs recovery in patients with COVID-19. *Infect Dis (Lond)* (2020) 52:902–7. doi: 10.1080/23744235.2020.1806353

303. Dheda K, Perumal T, Moultrie H, Perumal R, Esmail A, Scott AJ, et al. The intersecting pandemics of tuberculosis and COVID-19: population-level and patient-level impact, clinical presentation, and corrective interventions. *Lancet Respir Med* (2022) 10:603–22. doi: 10.1016/S2213-2600(22)00092-3

304. Gao Y, Liu M, Chen Y, Shi S, Geng J, Tian J. Association between tuberculosis and COVID-19 severity and mortality: A rapid systematic review and meta-analysis. *J Med Virol* (2021) 93:194–6. doi: 10.1002/jmv.26311

305. TB/COVID-19 Global Study Group. Tuberculosis and COVID-19 co-infection: description of the global cohort. *Eur Respir J* (2022) 59:2102538. doi: 10.1183/13993003.02538-2021

306. Jassat W, Mudara C, Ozougwu L, Tempia S, Blumberg L, Davies M-A, et al. Difference in mortality among individuals admitted to hospital with COVID-19 during the first and second waves in South Africa: a cohort study. *Lancet Glob Health* (2021) 9:e1216–25. doi: 10.1016/S2214-109X(21)00289-8

307. Tadolini M, García-García J-M, Blanc F-X, Borisov S, Goletti D, Motta I, et al. On tuberculosis and COVID-19 co-infection. *Eur Respir J* (2020) 56:2002328. doi: 10.1183/13993003.02328-2020

308. Tadolini M, Codecasa LR, García-García J-M, Blanc F-X, Borisov S, Alffenaar J-W, et al. Active tuberculosis, sequelae and COVID-19 co-infection: first cohort of 49 cases. *Eur Respir J* (2020) 56:2001398. doi: 10.1183/13993003.01398-2020

309. Sheerin D, Abhimanyu n, Peton N, Vo W, Allison CC, Wang X, et al. Immunopathogenic overlap between COVID-19 and tuberculosis identified from transcriptomic meta-analysis and human macrophage infection. *iScience* (2022) 25:104464. doi: 10.1016/j.isci.2022.104464

310. Petrone L, Petruccioli E, Vanini V, Cuzzi G, Gualano G, Vittozzi P, et al. Coinfection of tuberculosis and COVID-19 limits the ability to in *vitro* respond to SARS-CoV-2. *Int J Infect Dis* (2021) 113 Suppl 1:S82–7. doi: 10.1016/j.ijid.2021.02.090

311. Riou C, du Bruyn E, Stek C, Daroowala R, Goliath RT, Abrahams F, et al. Relationship of SARS-CoV-2-specific CD4 response to COVID-19 severity and impact of HIV-1 and tuberculosis coinfection. *J Clin Invest* (2021) 131:e149125. doi: 10.1172/JCI149125

312. Stochino C, Villa S, Zucchi P, Parravicini P, Gori A, Raviglione MC. Clinical characteristics of COVID-19 and active tuberculosis co-infection in an Italian reference hospital. *Eur Respir J* (2020) 56:2001708. doi: 10.1183/13993003.01708-2020

313. Najafi-Fard S, Aiello A, Navarra A, Cuzzi G, Vanini V, Migliori GB, et al. Characterization of the immune impairment of tuberculosis and COVID-19 coinfected patients. *Int J Infect Dis* (2023) 130, Supplement 1, S34–S42. doi: 10.1016/j.ijid.2023.03.021

314. du Bruyn E, Stek C, Daroowala R, Said-Hartley Q, Hsiao M, Schafer G, et al. Effects of tuberculosis and/or HIV-1 infection on COVID-19 presentation and immune response in Africa. *Nat Commun* (2023) 14:188. doi: 10.1038/s41467-022-35689-1

315. Musso M, Di Gennaro F, Gualano G, Mosti S, Cerva C, Fard SN, et al. Concurrent cavitary pulmonary tuberculosis and COVID-19 pneumonia with in *vitro* immune cell anergy. *Infection* (2021) 49:1061–4. doi: 10.1007/s15010-021-01576-y

316. Sheerin D, Phan TK, Eriksson EM, Consortium CP, Coussens AK. Distinct and overlapping immunological responses to SARS-CoV-2 and Mycobacterium tuberculosis identified by single-cell RNA-seq of co-infected whole blood. (2023) 2023:. doi: 10.1101/2023.05.24.23290499

317. Hildebrand RE, Chandrasekar SS, Riel M, Touray BJB, Aschenbroich SA, Talaat AM. Superinfection with SARS-CoV-2 Has Deleterious Effects on Mycobacterium bovis BCG Immunity and Promotes Dissemination of Mycobacterium tuberculosis. *Microbiol Spectr* (2022) 10:e0307522. doi: 10.1128/spectrum.03075-22

318. Rosas Mejia O, Gloag ES, Li J, Ruane-Foster M, Claeys TA, Farkas D, et al. Mice infected with Mycobacterium tuberculosis are resistant to acute disease caused by secondary infection with SARS-CoV-2. *PloS Pathog* (2022) 18:e1010093. doi: 10.1371/journal.ppat.1010093

319. Rajamanickam A, Kumar NP, Padmapriyadarsini C, Nancy A, Selvaraj N, Karunanithi K, et al. Latent tuberculosis co-infection is associated with heightened levels of humoral, cytokine and acute phase responses in seropositive SARS-CoV-2 infection. *J Infect* (2021) 83:339–46. doi: 10.1016/j.jinf.2021.07.029

320. Rajamanickam A, Pavan Kumar N, Chandrasekaran P, Nancy A, Bhavani PK, Selvaraj N, et al. Effect of SARS-CoV-2 seropositivity on antigen - specific cytokine and chemokine responses in latent tuberculosis. *Cytokine* (2022) 150:155785. doi: 10.1016/j.cyto.2021.155785

321. Pozdnyakov A, Jin A, Bader M. Reactivation of pulmonary tuberculosis in a patient with COVID-19: case report and review of literature. *Infect Dis Clin Pract (Baltim Md)* (2021) 29:e468–70. doi: 10.1097/IPC.0000000000001032

322. Leonso A-A, Brown K, Prol R, Rawat S, Khunger A, Bromberg R. A rare case of latent tuberculosis reactivation secondary to a COVID-19 infection. *Infect Dis Rep* (2022) 14:446–52. doi: 10.3390/idr14030048

323. Garg N, Lee YI. REACTIVATION TB WITH SEVERE COVID-19. *Chest* (2020) 158:A777. doi: 10.1016/j.chest.2020.08.724

324. Khayat M, Fan H, Vali Y. COVID-19 promoting the development of active tuberculosis in a patient with latent tuberculosis infection: A case report. *Respir Med Case Rep* (2021) 32:101344. doi: 10.1016/j.rmcr.2021.101344

325. COLBY S, SHAH R. TB REACTIVATION FOLLOWING COVID-19 INFECTION. *Chest* (2022) 162:A329. doi: 10.1016/j.chest.2022.08.255

326. Tang W, Leonhardt L, Pervez A, Sarvepalli S. A case of pleural tuberculosis vs latent tuberculosis reactivation as a result of COVID-19 infection and treatment. *J Community Hosp Intern Med Perspect* (2022) 12:89–93. doi: 10.55729/2000-9666.1078

327. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Brüggen M-C, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. *Allergy* (2020) 75:1564–81. doi: 10.1111/all.14364

328. Khan N, Vidyarthi A, Amir M, Mushtaq K, Agrewala JN. T-cell exhaustion in tuberculosis: pitfalls and prospects. *Crit Rev Microbiol* (2017) 43:133–41. doi: 10.1080/1040841X.2016.1185603

329. Kumar NP, Moideen K, Banurekha VV, Nair D, Babu S. Plasma proinflammatory cytokines are markers of disease severity and bacterial burden in pulmonary tuberculosis. *Open Forum Infect Dis* (2019) 6:ofz257. doi: 10.1093/ofid/ofz257

330. Srivastava S, Jaggi N. TB positive cases go up in ongoing COVID-19 pandemic despite lower testing of TB: An observational study from a hospital from Northern India. *Indian J Tuberc* (2022) 69:157–60. doi: 10.1016/j.ijtb.2021.04.014

331. Luke E, Swafford K, Shirazi G, Venketaraman V. TB and COVID-19: an exploration of the characteristics and resulting complications of co-infection. *Front Biosci (Schol Ed)* (2022) 14:6. doi: 10.31083/j.fbs1401006

332. Pajuelo D, Gonzalez-Juarbe N, Tak U, Sun J, Orihuela CJ, Niederweis M. NAD + Depletion triggers macrophage necroptosis, a cell death pathway exploited by mycobacterium tuberculosis. *Cell Rep* (2018) 24:429–40. doi: 10.1016/j.celrep.2018.06.042

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The impact of the COVID-19 pandemic on the global tuberculosis epidemic

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Tuberculosis (TB) is a major cause of ill health worldwide. Until the coronavirus (COVID-19) pandemic, TB was the leading cause of death from a single infectious agent. COVID-19 has caused enormous health, social and economic upheavals since 2020, impairing access to essential TB services. In marked contrast to the steady global increase in TB detection between 2017 and 2019, TB notifications dropped substantially in 2020 compared with 2019 (-18%), with only a partial recovery in 2021. TB epidemiology worsened during the pandemic: the estimated 10.6 million people who fell ill with TB worldwide in 2021 is an increase of 4.5% from the previous year, reversing many years of slow decline. The annual number of TB deaths worldwide fell steadily between 2005 and 2019, reaching 1.4 million in 2019, but this trend was reversed in 2020 (1.5 million), and by 2021 global TB deaths were back to the level of 2017 (1.6 million). Intensified efforts backed by increased funding are urgently required to reverse the negative impacts of COVID-19 on TB worldwide, made more pressing by ongoing conflicts, a global energy crisis and uncertainties in food security that are likely to worsen the broader determinants of TB.

KEYWORDS

tuberculosis, tuberculosis/prevention and control, COVID-19, SARS-CoV-2, epidemiology, pandemics

Introduction

Tuberculosis (TB) is a communicable disease that is a major cause of ill health worldwide (1). It is caused by the bacillus *Mycobacterium tuberculosis*, which is spread when people who have TB disease expel bacteria into the air (e.g. by coughing). About a quarter of the global population is estimated to have been infected with TB bacilli (2), but only about 5-10% of people infected develop disease in their lifetime (3). About 90% of the people who develop TB each year are adults, with more cases among men than women. The disease typically affects the lungs but can affect other sites as well. Without treatment, the death rate from TB disease is high (about 50%). There is a strong geographical bias in the global burden of TB, and much of the TB incidence and mortality is concentrated in Asian and African countries (Figures 1A, B). The TB epidemic is strongly influenced by

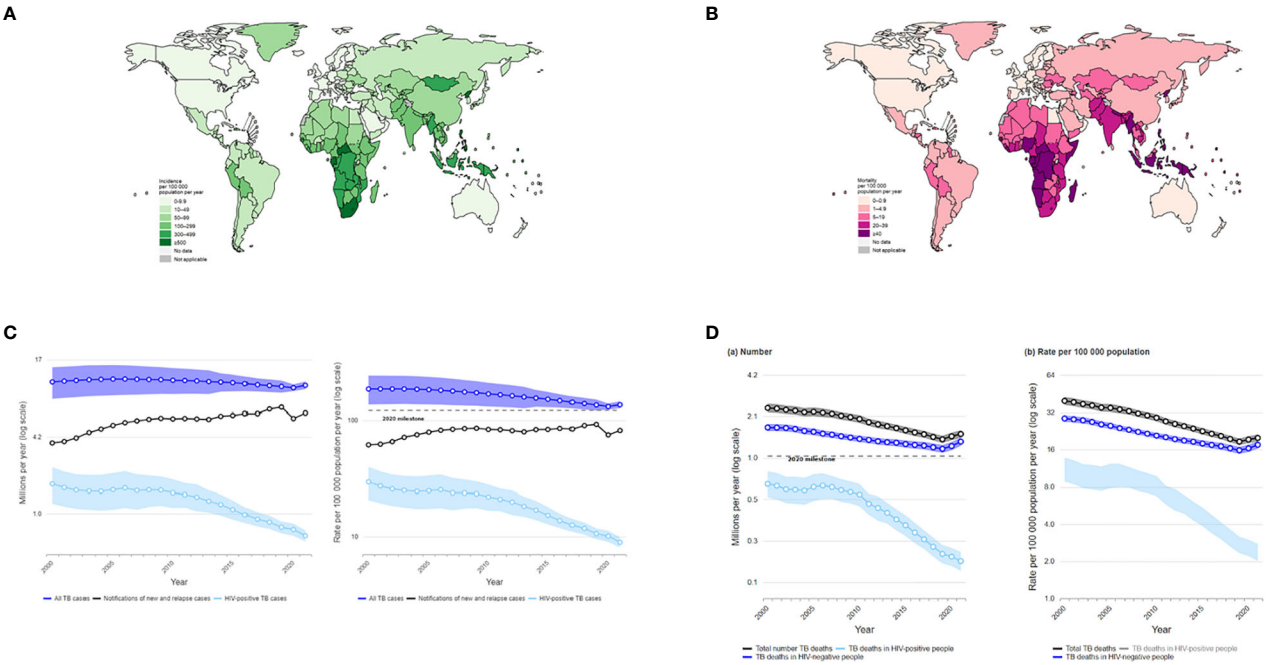

FIGURE 1
(A) Estimated TB incidence rates, 2021. **(B)** Estimated TB mortality rates in HIV-negative people, 2021. **(C)** Global trends in the estimated number of incident TB cases (left) and the incidence rate (right), 2000–2021 (shaded areas represent uncertainty intervals. The horizontal dashed line shows the 2020 milestone of the End TB Strategy). **(D)** Global trends in the estimated number of deaths caused by TB (left) and the mortality rate (right), 2000–2021 (shaded areas represent uncertainty intervals. The horizontal dashed line shows the 2020 milestone of the End TB Strategy).

social and economic development and health-related risk factors such as undernourishment, diabetes, HIV infection, alcohol use disorders and smoking (Figure 2).

Until the advent of the coronavirus (COVID-19) pandemic, TB was the leading cause of death from a single infectious agent for several years, ranking above HIV/AIDS. COVID-19 has caused enormous health, social and economic impacts since 2020. This includes impacts on the provision of and access to essential TB services, the number of people with TB diagnosed and reported through national disease surveillance systems (TB notification), and the TB epidemiology (TB burden in terms of incidence and mortality).

We present the current situation and recent trends in TB notification and epidemiology worldwide, with a focus on how COVID-19 has impacted upon the main indicators that are used to assess the global TB burden and the response of national health authorities to mitigate it.

Method

The main indicators used in this article - namely TB notification, TB incidence and TB mortality - were derived primarily from data collected yearly by the World Health Organization (WHO) from national ministries of health as part of its mandate to coordinate international work. Estimates of TB incidence and mortality are based on a well-documented approach (4). For the years 2020 and 2021, TB incidence and mortality were estimated using dynamic models for 28 countries that collectively accounted for 95% of the drop in global TB notifications during these two years. The new methods rely heavily on country and region-specific dynamic models for low and middle-income countries, in the absence of reliable and up-to-date mortality data from national vital registration systems that include standardised coding of causes of death. We compare TB notification and estimates of TB burden with targets set in WHO's End TB Strategy of 2015 and by the United Nations in 2018 (5, 6). Since 1997, WHO has published annual reports based on the data that it collects from Member States. By 2022, 202 countries and territories with more than 99% of the world's population and TB cases reported aggregated data to WHO on a series of established indicators. Since the onset of the COVID-19 pandemic, countries can also report monthly or quarterly TB notifications to WHO (7). In addition to TB notification, we also comment on the implementation of TB preventive activities during the pandemic, namely TB preventive treatment and vaccination with bacille Calmette-Guérin (BCG).

Results

TB incidence

An estimated global total of 10.6 million people (95% uncertainty interval [UI]: 9.9–11 million) fell ill with TB in 2021, equivalent to a TB notification rate of 134 cases (95% UI: 125–143) per 100 000 population. Similarly, the TB incidence rate (new TB cases per 100 000 population per year) is estimated to have

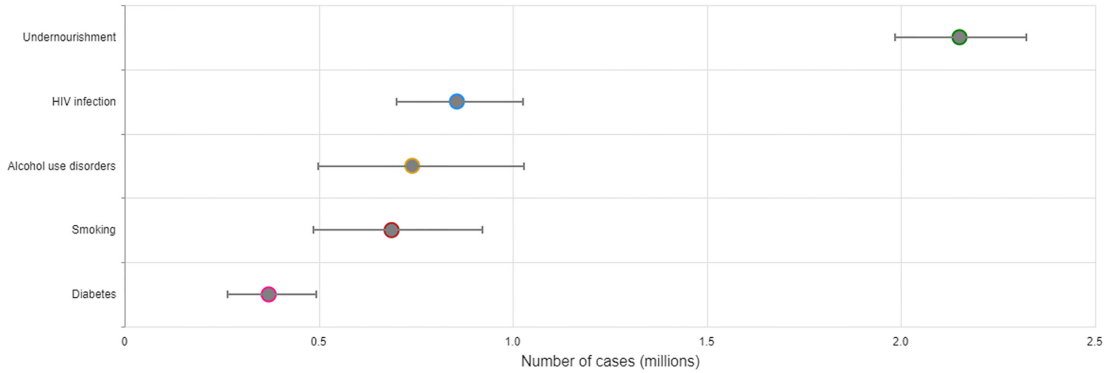

FIGURE 2
Global estimates of the number of TB cases attributable to selected risk factors, 2021. Sources of data used to produce estimates were: Imtiaz S et al. Eur Resp Jour (2017); Hayashi S et al. Trop Med Int Health (2018); Lönnroth K et al. Lancet (2010); World Bank Sustainable Development Goals Database (http://datatopics.worldbank.org/sdgs/); WHO Global Health Observatory (https://www.who.int/data/gho); and WHO Global TB Programme.

increased by 3.6% between 2020 and 2021, following declines of about 2% per year for most of the past two decades (Figure 1C). Among all TB cases in 2021, 6.7% were among people living with HIV. In 2021, eight countries accounted for more than two thirds of global TB cases: India (28%), Indonesia (9.2%), China (7.4%), the Philippines (7.0%), Pakistan (5.8%), Nigeria (4.4%), Bangladesh (3.6%) and Democratic Republic of the Congo (2.9%). The estimated 10.6 million people who fell ill with TB worldwide in 2021 is an increase of 4.5% from the previous year, reversing many years of slow decline. The cumulative fall in the TB incidence rate was 13.5% between 2015 and 2020, but the level of 2021 was only 10% below that of 2015. This was only half-way to the first End TB Strategy milestone of a 20% reduction between 2015 and 2020 and a long way from the second milestone of a 50% reduction by 2025.

TB mortality

In 2021, there were an estimated 1.4 million TB deaths among HIV-negative people (95% UI: 1.3–1.5 million) and 187 000 (95% UI, 158 000–218 000) among HIV-positive people, for a combined total of 1.6 million. The annual number of TB deaths worldwide fell steadily between 2005 and 2019, reaching 1.4 million in 2019, but this trend was reversed in 2020 (1.5 million), and by 2021 global TB deaths were back to the level of 2017 (Figure 1D). Progress previously made towards the first milestone of the End TB Strategy - reducing TB deaths by 35% between 2015 and 2020 - has been reversed and the net reduction from 2015 to 2021 was only 5.9%.

TB notification

Globally in 2021, 6.4 million people with a new episode of TB (new and relapse cases) were diagnosed and notified. Of these, 83% had pulmonary TB and almost 90% of total notifications were from Asia and Africa. In marked contrast to large increases between 2017 and 2019, there was a substantial fall (-18%) in TB notifications in 2020 compared with 2019, with a partial recovery in TB notifications in 2021 (-10% compared with 2019). However, the gap between the global TB notifications and the estimated incident TB remains similar in 2021 to 2020. Globally, the cumulative total number of people diagnosed with TB and officially reported from 2018 to 2021 is 26 million, only 66% of the 5-year target of 40 million between 2018 and 2022 that was set at the UN high-level meeting on TB in 2018.

TB preventive activities

The global number of people living with HIV and household contacts of people diagnosed with TB who were provided with TB preventive treatment increased from 1.0 million in 2015 to 3.6 million in 2019, after which there was a sizeable reduction in 2020 (to 3.2 million) followed by an almost complete return to the levels of reporting of 2019 by 2021 (to 3.5 million). Global BCG vaccination coverage decreased from 88% in 2019 to 84% in 2021, reflecting declines in most WHO regions. These trends are likely to be due to concurrent disruptions to health services caused by the COVID-19 pandemic.

Discussion

The COVID-19 pandemic has caused enormous social and economic impacts, and disrupted healthcare services worldwide. Data reported by countries point to a disproportionate impact on access to essential TB services (8). This has been characterised by pronounced drops in the number of TB cases notified on national information systems. The monitoring of TB notifications at monthly or quarterly intervals allowed a more timely assessment of the impact of the COVID-19 pandemic on TB activities in reporting countries (7).

Decreases in TB notification are likely to reflect two distinct challenges: under-reporting and missed or delayed TB diagnosis on a large scale. Countries have reported disruptions in disease surveillance activities during the pandemic (9, 10). Enhancing

health services monitoring and evaluation capacities was one of the most frequently cited needs to be addressed.

Missed and delayed TB diagnosis may have resulted from less opportunities to seek care by people who were unwell during "lockdowns" and prolonged periods of intense activity in primary healthcare clinics. This implies that more people in the community have undiagnosed and untreated TB, and for longer than before, increasing the pool of infectious individuals. Increased transmission and reduced access to proper care could explain at least in part the increments in global and regional TB incidence and mortality observed shortly after notification declined. This is made more plausible by the fact that these epidemiological trends were an abrupt reversal of a steady, albeit slow, decline in the global burden of TB for many years until 2020.

Decreasing TB notification could, however, also indicate less transmission of *Mycobacterium tuberculosis* and less infection. Restrictions in physical mobility and closure of clinics imposed by the authorities of countries during the pandemic may have offset transmission, by as much as 50% according to some modelling studies (11). This would likely happen for short periods of time such as during lockdowns. In such a situation the increased TB mortality could be explained by shortages in timely care of TB patients.

Apart from the effect of pandemic disruptions on TB healthcare services, another concern has been the risk of disease synergy between TB and COVID-19 in the same individual. There is evidence that COVID-19 patients with past and concurrent TB are more likely to have a fatal outcome in high TB burden settings (12, 13). However, it is less clear if TB patients who develop COVID-19 in the course of their illness have a substantially increased risk of dying after adjusting for other risk factors (14). There is also no clear evidence that SARS-CoV-2 infection can increase the progression from TB infection to disease, although no purpose-built studies of the impact of SARS-CoV-2 infection on TB treatment outcomes are known to have been mounted to address this question appropriately.

In addition to the effect on main TB indicators, the COVID-19 pandemic has also impacted negatively on other components of TB programmes in the last three years, such as the provision of TB preventive treatment, vaccination with bacille Calmette-Guérin (BCG) and overall spending on TB (1). Moreover, the negative impact of the disruptions on gainful employment, and key TB determinants such as nutrition and access to care for diabetes and HIV are bound to influence TB incidence and the wellbeing of people affected by TB (Figure 2). It is estimated that the COVID-19 pandemic will result in an additional 2.6 million chronically malnourished children by 2022, reversing the decreasing curve for the first time in 3 decades (15). COVID-19 has been associated with both severe COVID-19 at admission and in-hospital mortality in people living with HIV (16). Diabetes control has also been effected in both high and low-resource settings (17, 18).

By mid 2023, close to 770 million confirmed cases of COVID-19 and 7 million deaths had been reported globally since the start of the pandemic (19). In May 2023 WHO declared COVID-19 as an established and ongoing health issue which no longer constitutes a public health emergency of international concern and advised on the transitioning to long-term management of COVID-19 (20). The latest WHO survey on essential health service performance at the end of 2022 registered the first major signs of recovery since the start of the COVID-19 pandemic (10). Recovery from the economic adversities created by the pandemic is likely to take longer in emerging economies and economically disadvantaged groups (21). Intensified efforts backed by increased funding are urgently required to mitigate and reverse the negative impacts of the COVID-19 pandemic on TB. The need for action has become even more pressing in the context of ongoing conflicts, a global energy crisis and associated risks to food security (22), which are likely to worsen some of the broader determinants of TB. The dearth of evidence on disease synergy between COVID-19 and TB is likely to have forfeited opportunities to improve the clinical management of people with both conditions and public health decision making for those at risk. This underlines the need to equip pandemic preparedness plans with research methods for rapid action on major diseases like TB as the world switches gears from emergency phase to contingency strategies for future pandemics.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. World Health Organization. *Global tuberculosis report 2022*. Geneva: World Health Organization (2022). Available at: https://www.who.int/tb/publications/global_report/en/.

2. Houben RMGJ, Dodd PJ. The global burden of latent tuberculosis infection: A re-estimation using Mathematical modelling. *PloS Med* (2016) 13:e1002152. doi: 10.1371/journal.pmed.1002152

3. Nelson LJ, Wells CD. Global epidemiology of childhood tuberculosis. *Int J Tuberc Lung Dis* (2004) 8:636–47.

4. *Methods used by WHO to estimate the global burden of TB disease*. Geneva: World Health Organization (2022). Available at: https://www.who.int/publications/m/item/methods-used-by-who-to-estimate-the-global-burden-of-tb-disease-2022.

5. Uplekar M, Weil D, Lönnroth K, Jaramillo E, Lienhardt C, Dias HM, et al. WHO's new end TB strategy. *Lancet* (2015) 385:1799–801. doi: 10.1016/S0140-6736(15)60570-0

6. United Nations General Assembly. *Resolution A/RES/73.3. Political declaration of the high-level meeting of the General Assembly on the fight against tuberculosis* (2018). Available at: https://digitallibrary.un.org/record/1645268/files/A_73_L-4-EN.pdf?ln=en.

7. *Provisional tuberculosis (TB) notifications* . Available at: https://worldhealthorg.shinyapps.io/tb_pronto/ (Accessed 21 February 2023).

8. Pai M, Kasaeva T, Swaminathan S. Covid-19's devastating effect on tuberculosis care — A path to recovery. *N Engl J Med* (2022) 386:1490–3. doi: 10.1056/NEJMp2118145

9. *Second round of the national pulse survey on continuity of essential health services during the COVID-19 pandemic: January-March 2021*. (Geneva, Switzerland: World Health Organization) (2021). Available at: https://www.who.int/publications-detail-redirect/WHO-2019-nCoV-EHS-continuity-survey-2021.1.

10. *Fourth round of the global pulse survey on continuity of essential health services during the COVID-19 pandemic: November 2022 - January 2023. Interim report. 1 May 2023*. (Geneva, Switzerland: World Health Organization) (2023). Available at: https://www.who.int/publications/i/item/WHO-2019-nCoV-EHS_continuity-survey-2023.1.

11. Cilloni L, Fu H, Vesga JF, Dowdy D, Pretorius C, Ahmedov S, et al. The potential impact of the COVID-19 pandemic on the tuberculosis epidemic a modelling analysis. *EClinicalMedicine* (2020) 28:100603. doi: 10.1016/j.eclinm.2020.100603

12. Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases, South Africa, Boulle A, Davies MA, Hussey H, Ismail M, Morden E, et al. Risk factors for coronavirus disease 2019 (COVID-19) death in a population cohort study from the Western Cape Province, South Africa. *Clin Infect Dis* (2021) 73:e2005–15. doi: 10.1093/cid/ciaa1198

13. Song W, Zhao J, Zhang Q, Liu S, Zhu X, An Q, et al. COVID-19 and tuberculosis coinfection: an overview of case reports/case series and meta-analysis. *Front Med* (2021) 8:657006. doi: 10.3389/fmed.2021.657006

14. Vadlamudi N, Basham CA, Johnston JC, Ahmad Khan F, Battista Migliori G, Centis R, et al. The association of SARS-CoV-2 infection and tuberculosis disease with unfavorable treatment outcomes: A systematic review. *PLOS Glob Public Health* (2023) 3(7):e0002163. doi: 10.2139/ssrn.4292852

15. *Malnutrition in Children* . Available at: https://data.unicef.org/topic/nutrition/malnutrition/ (Accessed 23 May 2023).

16. Bertagnolio S, Thwin SS, Silva R, Nagarajan S, Jassat W, Fowler R, et al. Clinical features of, and risk factors for, severe or fatal COVID-19 among people living with HIV admitted to hospital: analysis of data from the WHO Global Clinical Platform of COVID-19. *Lancet HIV* (2022) 9:e486–95. doi: 10.1016/S2352-3018(22)00097-2

17. Ojo O, Wang X-H, Ojo OO, Orjih E, Pavithran N, Adegboye ARA, et al. The effects of COVID-19 lockdown on glycaemic control and lipid profile in patients with type 2 diabetes: A systematic review and meta-analysis. *Int J Environ Res Public Health* (2022) 19:1095. doi: 10.3390/ijerph19031095

18. Carr MJ, Wright AK, Leelarathna L, Thabit H, Milne N, Kanumilli N, et al. Impact of COVID-19 restrictions on diabetes health checks and prescribing for people with type 2 diabetes: a UK-wide cohort study involving 618 161 people in primary care. *BMJ Qual Saf* (2022) 31:503–14. doi: 10.1136/bmjqs-2021-013613

19. *WHO Coronavirus (COVID-19) Dashboard* . Available at: https://covid19.who.int (Accessed 23 May 2023).

20. Statement on the fifteenth meeting of the IHR. *Emergency Committee on the COVID-19 pandemic* (2005). Available at: https://www.who.int/news/item/05-05-2023-statement-on-the-fifteenth-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-coronavirus-disease-(covid-19)-pandemic (Accessed 23 May 2023).

21. *World development report 2022: finance for an equitable recovery*. Washington, DC: World Bank (2022). Available at: https://openknowledge.worldbank.org/bitstream/handle/10986/36883/9781464817304.pdf.

22. Carwile ME, Hochberg NS, Sinha P. Undernutrition is feeding the tuberculosis pandemic: A perspective. *J Clin Tuberculosis Other Mycobacterial Dis* (2022) 27:100311. doi: 10.1016/j.jctube.2022.100311

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Co-infection of mice with SARS-CoV-2 and *Mycobacterium tuberculosis* limits early viral replication but does not affect mycobacterial loads

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Viral co-infections have been implicated in worsening tuberculosis (TB) and during the COVID-19 pandemic, the global rate of TB-related deaths has increased for the first time in over a decade. We and others have previously shown that a resolved prior or concurrent influenza A virus infection in *Mycobacterium tuberculosis* (*Mtb*)-infected mice resulted in increased pulmonary bacterial burden, partly through type I interferon (IFN-I)-dependent mechanisms. Here we investigated whether SARS-CoV-2 (SCV2) co-infection could also negatively affect bacterial control of *Mtb*. Importantly, we found that K18-hACE2 transgenic mice infected with SCV2 one month before, or months after aerosol *Mtb* exposure did not display exacerbated *Mtb* infection-associated pathology, weight loss, nor did they have increased pulmonary bacterial loads. However, pre-existing *Mtb* infection at the time of exposure to the ancestral SCV2 strain in infected K18-hACE2 transgenic mice or the beta variant (B.1.351) in WT C57Bl/6 mice significantly limited early SCV2 replication in the lung. *Mtb*-driven protection against SCV2 increased with higher bacterial doses and did not require IFN-I, TLR2 or TLR9 signaling. These data suggest that SCV2 co-infection does not exacerbate *Mtb* infection in mice, but rather the inflammatory response generated by *Mtb* infection in the lungs at the time of SCV2 exposure restricts viral replication.

KEYWORDS

lung, mycobacterium tuberculosis, SARS-CoV-2, tuberculosis, COVID-19, Type-I interferon, co-infection

Introduction

Pulmonary viral infections have been shown to both increase the likelihood and exacerbate the severity of secondary bacterial infections in the lung (1–7). The underlying immunological mechanisms are diverse and range from lung epithelial barrier breakdown and augmented adhesion of pathogens to the subversion of both adaptive and innate immunity from protective anti-bacterial pathways towards detrimental anti-viral inflammatory pathways like type-I interferon (IFN-I) (5, 8). Viral co-infections also play a role in the exacerbation of *Mycobacterium tuberculosis* (*Mtb*) infection (9), one of the leading causes of infectious disease-related mortality worldwide (10). For example, co-infection with cytomegalovirus (CMV) has been associated with an enhanced risk of tuberculosis (TB) disease (11–13). Furthermore, there are marked associations between influenza A virus (IAV) co-infection at the time of TB diagnosis and elevated *Mtb* burden (14), as well as increased risk of mortality in TB patients co-infected with both *Mtb* and IAV (15). *Mtb*-infected mice that were either simultaneously or subsequently infected with murine pneumonia virus (PVM) or IAV have been shown to have exacerbated lung tissue pathology (16). Our previous work demonstrated that simultaneous or prior IAV co-infection elevates pulmonary *Mtb* bacterial burden and reduces host survival after *Mtb* infection (17, 18). When IAV infection coincided with initial priming of *Mtb*-specific T cell responses, loss of bacterial control was dependent on elevated IFN-I and interleukin-10 (IL-10) signaling ultimately resulting in a reduced *Mtb*-specific $CD4^+$ T cell response (17, 18).

Since the beginning of the COVID-19 pandemic, caused by SARS-CoV-2 (SCV2), TB diagnosis and case reporting reduced globally by 18% despite no change in the actual incidence of TB infection (10, 19). Importantly, a 7.5% increase in global TB deaths was observed, marking the first year-on-year increase in the global TB death toll since 2005 (10). A clear understanding of whether co-infection with SCV2 and *Mtb* has immunological consequences on the outcome of TB or COVID-19 is confounded by non-biological factors of the COVID-19 pandemic, including reduced BCG vaccination rates, disrupted TB outreach services and amplified global poverty (20). In addition, there have been reduced rates of early TB diagnosis during the COVID-19 pandemic attributed to reduced availability of staff and equipment for clinics and diagnostic labs (20–23) and reduced patient presentation due to fear of COVID-19 infection or increased social stigma around respiratory symptoms (24). TB treatment regimens, which already faced significant challenges before the pandemic because of the intensive and prolonged course of antibiotics required, have also been negatively impacted in TB-endemic countries during the pandemic (23, 25–27). Alongside negative TB outcomes, clinical reports have shown that *Mtb* and SCV2 co-infection results in a greater likelihood of severe COVID-19 disease (by an odds ratio of 2.21), COVID-19-related death (by an odds ratio of 2.77) (28) and overall elevated risk of negative clinical outcomes in co-infected individuals (29). Mechanistic studies into the possibility of immunological interactions critically influencing the outcome of *Mtb* and SCV2 co-infections are needed to develop effective strategies to reduce the mortality rate of both diseases (30, 31).

To directly ask whether co-infection with *Mtb* and SCV2 has a biological impact on the outcome of either TB or COVID-19, we sequentially infected mice with SCV2 followed by *Mtb* or co-infected *Mtb*-infected mice with SCV2. We show here that regardless of the order of infection co-infection with SCV2, unlike co-infection with IAV, does not alter the outcome of *Mtb* infection in mice. Moreover, we show that early pulmonary SCV2 replication is suppressed in chronically *Mtb*-infected mice through a mechanism that is dependent on mycobacterial dose but does not require signaling through type-I interferon (IFN-I) or toll-like receptor 2 (TLR2) or TLR9.

Results

Infection of mice with SCV2 one month before *Mtb* exposure does not alter pulmonary *Mtb* burden or pathology

We have previously shown that in mice, prior IAV infection leads to elevated pulmonary bacterial burden 16 weeks following subsequent *Mtb* infection (17). To determine whether prior infection with SCV2 similarly impacts the outcome of *Mtb* infection we used human Angiotensin Converting Enzyme 2 transgenic (K18-hACE2 Tg) mice, which are susceptible to infection with the ancestral strain of SCV2. K18-hACE2 Tg mice were infected with a sub-lethal dose of the hCoV-19/USA-WA1/2020 (USA-WA1/2020) isolate of SCV2 and 28 days later infected with *Mtb* (Figure 1A). *Mtb* disease was allowed to develop, and lungs and spleens were collected at 4 weeks (Figures 1B–D) or 20 weeks (Figures 1E–G) post *Mtb* infection. SCV2 infection resulted in transient weight loss 5 – 8 days post infection (Figure 1A). Lung pathology or bacterial distribution was determined by hematoxylin and eosin (H&E), and acid fast (AF) staining of lung sections 4 weeks post-*Mtb* infection, however no difference was detected when comparing mice previously infected with SCV2 to those animals that received *Mtb* alone (Figure 1B). Importantly, pulmonary (Figure 1C) or splenic (Figure 1D) bacterial loads were unchanged in mice previously infected with SCV2 compared to *Mtb*-only mice. To test whether prior SCV2 infection may affect the control of *Mtb* at a later timepoint, we assessed lung pathology and bacterial burden at 20 weeks post-*Mtb* infection. Again, H&E and AF staining did not reveal changes in lung pathology or bacterial localization between mice with prior SCV2 infection compared to mice that were only *Mtb* infected (Figure 1E). Similarly, previous SCV2 infection did not alter pulmonary (Figure 1F) or splenic (Figure 1G) bacterial loads at this later 20-week timepoint. Taken together, and in contrast to findings with prior IAV infection (17), our data here suggest that prior infection with SCV2 does not lead to increased *Mtb*-driven disease or impairment of *Mtb* bacterial replication in mice.

Co-infection with SCV2 does not affect *Mtb* burden or lung pathology

We and others have reported that concurrent or sequential infection with IAV and *Mtb* resulted in loss of bacterial control

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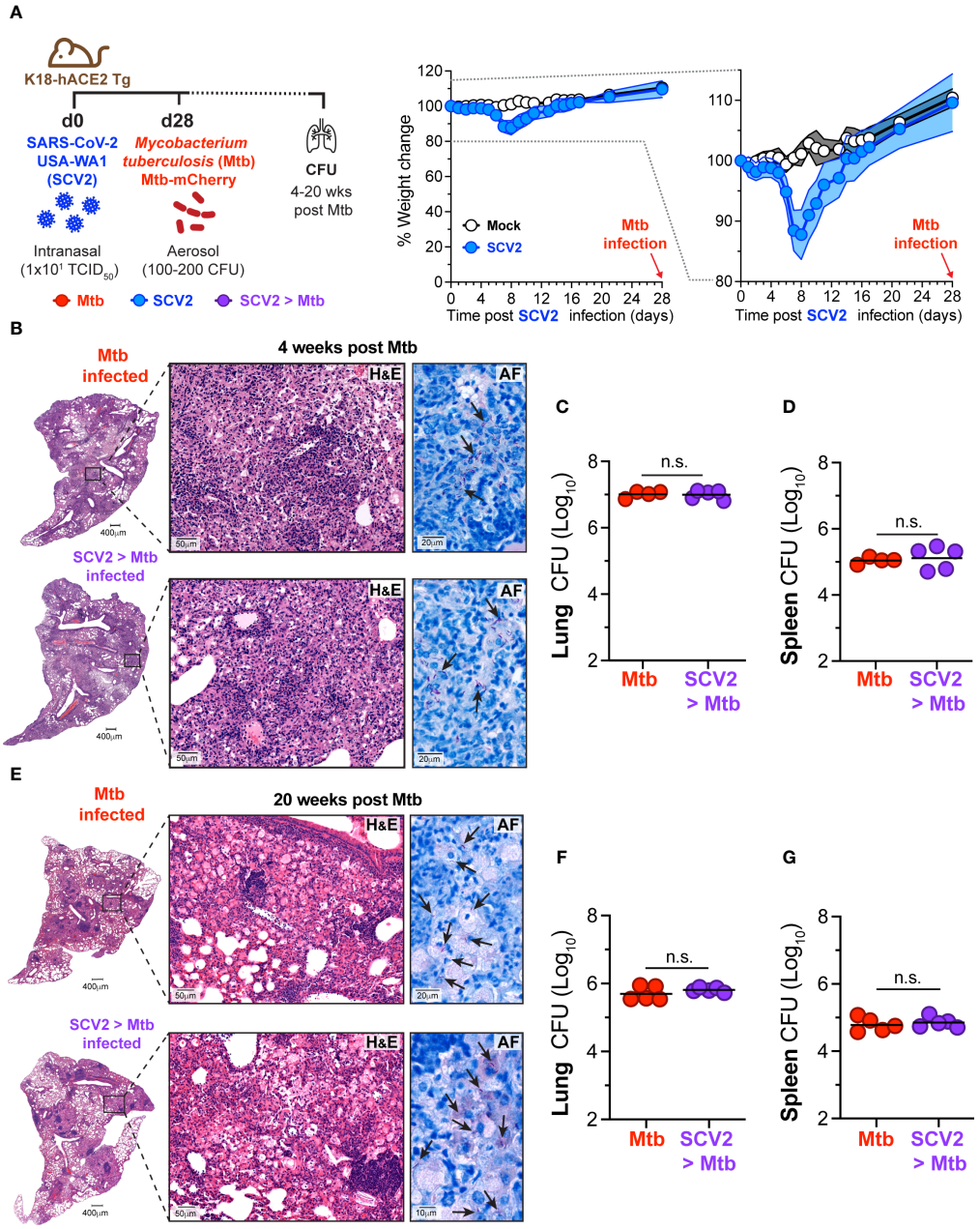

FIGURE 1
SCV2 infection one month before Mtb infection does not exacerbate Mtb disease. **(A)** Left: Schematic of experimental set-up where K18-hACE2 Tg mice were infected intranasally with 10 $TCID_{50}$ SCV2 (USA-WA1/2020) or mock supernatant 28 days before aerosol infection with 100 – 200 CFU *Mtb* and mice were euthanized either 4 or 20 weeks after *Mtb* infection. Middle: Weight loss of K18-hACE2 Tg mice after SCV2 infection and before *Mtb* infection Right: Selected range of weight change curve to highlight differences in weight loss between SCV2 and Mock infected groups (n= 4-5 per group from one experiment representative of two independent experiments, mean ± SD as traveling error bars). **(B)** Representative hematoxylin and eosin (H&E) and acid-fast AF staining of lung tissue from mice at 4 weeks post *Mtb* infection, with or without prior SCV2 infection (arrows indicate examples of *Mtb* bacteria, scale bars indicate magnification). **(C, D)** *Mtb* CFU in **(C)** lungs and **(D)** spleens of mice at 4 weeks post *Mtb* infection. **(E)** Representative H&E and AF staining of lung tissue 20 weeks post *Mtb* infection with and without prior SCV2 infection (arrows indicate examples of *Mtb* bacteria, scale bars indicate magnification). **(F, G)** *Mtb* CFU in **(F)** lungs and **(G)** spleens of mice at 20 weeks post *Mtb* infection (n= 4-5 per group from one independent experiment per timepoint, geometric mean, two-tailed Mann Whitney test). n.s. = not significant.

(17, 18). To ask whether SCV2 co-infection could equally compromise *Mtb* replication, K18-hACE2 Tg mice were first infected with *Mtb*. At a later stage of infection (day 170 post-*Mtb*) *Mtb*-infected mice and age-matched controls were then infected with a sub-lethal dose of USA-WA1/2020 SCV2 and monitored for 28 days after which bacterial burdens and lung pathology were assessed (Figure 2A). Importantly, SCV2 co-infection did not impact the bacterial burden of *Mtb* in bronchoalveolar lavage (BAL), lungs, or spleens (Figure 2B). Additionally, no differences were seen in lung pathology or bacterial localization as determined by H&E and AF staining (Figure 2C) or scoring of affected lung areas (Figure 2D).

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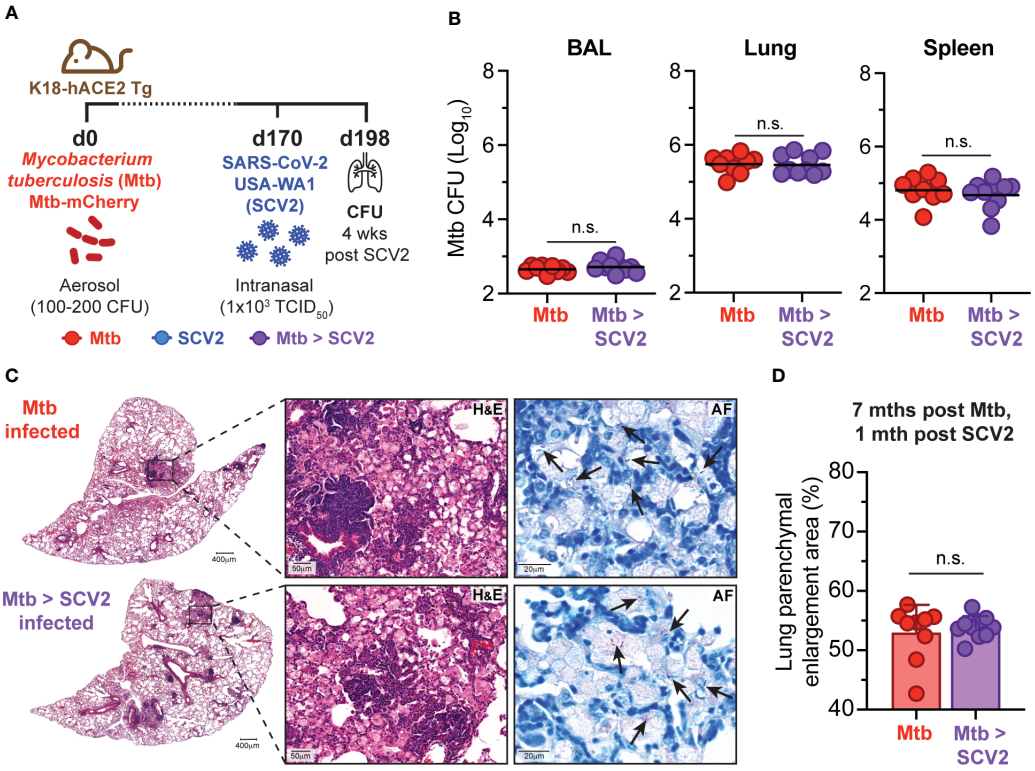

FIGURE 2
SCV2 co-infection does not exacerbate *Mtb* disease. **(A)** Schematic of experimental set-up where K18-hACE2 Tg mice were aerosol infected with 100 – 200 CFU *Mtb* (H37Rv-mCherry) 170 days before being intranasally infected with $1x10^3$ $TCID_{50}$ SCV2 (USA-WA1/2020) or mock supernatant, mice were euthanized 1 month after SCV2 infection. **(B)** *Mtb* CFU in BALs, lungs and spleens (n= 9-10 per group, data combined from two independent experiments, geometric mean). **(C)** Representative H&E and AF staining of lung tissue from mice as described in **(A)** (arrows indicate examples of *Mtb* bacteria, scale bars indicate magnification) **(D)** Quantification of percentage of parenchymal enlargement from H&E shown in **C**) (n= 9-10 per group from two independent experiments, mean ± S.D., two-tailed Mann Whitney test). n.s. = not significant.

Next, we asked whether co-infection with SCV2 at 16 weeks post *Mtb* infection could negatively impact the existing *Mtb*-specific $CD4^+$ or $CD8^+$ T cell responses. When we quantified *Mtb* ($ESAT6_{4-17}$)-specific $CD4^+$ T cells *via* MHC-II tetramer straining one month following co-infection with SCV2, the frequency of antigen-specific $CD4^+$ T cells was unchanged between mice infected solely with *Mtb* or those co-infected with SCV2 (Figure 3A). There were also no differences in the proportion of lung parenchyma-residing $ESAT6_{4-17}$-specific $CD4^+$ T cells, as assessed by lack of intravenous CD45 staining ($i.v.^{neg}$) (32), nor in the expression of Ki-67 or levels of the transcription factor T-bet on those cells (Figure 3B). Likewise, when we examined *Mtb*-specific $CD8^+$ T cell responses the overall abundance (Figure 3C) and proportion of parenchymal or KLRG1-expressing cells within *Mtb* $TB10.4_{4-11}$ and *Mtb* $32c_{93-102}$ MHC-I tetramer positive $CD8^+$ T cells were similar between co-infected lungs and lung from mice infected with only *Mtb* (Figure 3D). Together these data suggest that the pre-existing pulmonary *Mtb*-specific $CD4^+$ and $CD8^+$ T cell responses are not negatively impacted over the course of 4 weeks following SCV2 co-infection.

We next measured SCV2 antigen-specific T cell responses 4 weeks after SCV2 infection in mice with and without an underlying *Mtb* infection using SCV2-specific tetramers (33). To measure SCV2 specific $CD4^+$ T cells we utilized an $ORF3A_{266-280}$ MHC-II $I\text{-}A^b$ tetramer and 4 weeks after sub-lethal infection detected approximately 1-2% of effector CD4 T cells that stained positive for the reagent (blue symbols) by flow cytometry, compared to less than 1% in *Mtb* co-infected mice (purple symbols) and 0.5% non-specific staining background in SCV2 unexposed animals (red symbols) (Figure 4A). Thus the overall frequency of $ORF3_{266-280}$ specific $CD4^+$ T cells was significantly reduced in the lungs of co-infected mice compared to their SCV2-only counterparts. Conversely, proportionately more $ORF3_{266-280}$ specific cells were residing in the lung parenchyma ($i.v.^{neg}$) and expressed a small but significant increase in T-bet expression (Figure 4B). Importantly, both the SCV2 $S_{539-546}$-specific and SCV2 $N_{219-227}$-specific $CD8^+$ T cell responses were significantly reduced in mice with an underlying *Mtb* infection compared to SCV2 alone (Figure 4C). Furthermore, while the overall proportion of $i.v.^{neg}$, KLRG1-expressing $S_{539-546}$-specific $CD8^+$ T cells was the same regardless of *Mtb* infection status, the frequency of tissue-resident memory (T_{RM}, $CD69^+$) T cells, was significantly reduced within that fraction in co-infected lungs (Figure 4D). Thus, ongoing *Mtb* infection resulted in a significant reduction in the magnitude of the pulmonary SCV2 $S_{539-546}$ specific T_{RM} response.

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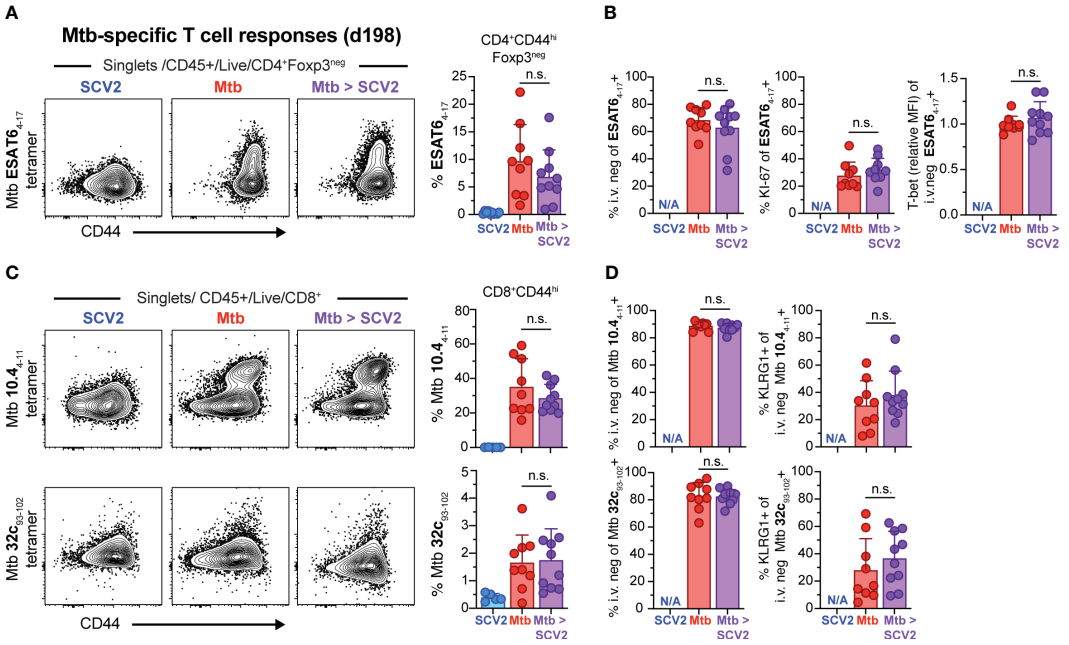

FIGURE 3
SCV2 co-infection does not negatively affect *Mtb*-specific CD4$^+$ or CD8$^+$ T cells. Example FACS plots and summary data from the lungs of mice described in Figure 2A. **(A)** Example FACS plots of ESAT6$_{4-17}$ MHC-II tetramer staining of CD4$^+$ Foxp3$^-$ cells and proportion of ESAT6$_{4-17}$-specific cells within activated CD44^{hi}CD4$^+$Foxp3$^-$ T cells **(B)** Quantification of ESAT6$_{4-17}$ tetramer-positive cells that are recruited into the lung parenchyma (CD45 i.v.^{neg}), positive for Ki-67 and relative expression intensity (geometric mean fluorescent intensity, MFI) of T-bet in lung resident ESAT6$_{4-17}$ tetramer-positive CD4$^+$ T cells. **(C)** Example FACS plots of *Mtb* TB10.4$_{4-11}$ (top) and *Mtb* 32c$_{93-102}$ (bottom) MHC-I tetramer staining of CD8$^+$ T cells and proportion of *Mtb* TB10.4$_{4-11}$ or *Mtb* 32c$_{93-102}$ -specific CD8$^+$ T cells gated on activated CD44^{hi}CD8$^+$ T cells **(D)** Quantification of *Mtb* TB10.4$_{4-11}$ (top) and *Mtb* 32c$_{93-102}$ (bottom) tetramer-positive cells recruited into the lung parenchyma (CD45 i.v.^{neg}) and their expression of KLRG1 (N/A= not applicable, n= 9-10 per group, data combined from 2 independent experiments, mean ± S.D., two-tailed Mann Whitney test). n.s. = not significant.

Underlying *Mtb* infection reduces initial SCV2 viral burden independent of IFN-I

Considering that antigen burden can directly impact T cell expansion and memory development (34), we asked whether the decrease in SCV2-specific CD8$^+$ T$_{RM}$ frequency 4 weeks after SCV2 infection in *Mtb*-infected mice was caused by a change in the initial SCV2 viral burden. We suspected that viral loads were reduced in co-infected mice as susceptible K18-hACE2 Tg mice lost 10% of their pre-SCV2 infection body weight 5 – 8 days after SCV2 infection but did not lose any weight if they were also infected with *Mtb* (Figure 5A). To determine whether SCV2 viral titers were reduced in the lungs of co-infected mice, we infected K18-hACE2 Tg mice with USA-WA1/2020 either with or without underlying *Mtb* infection and collected lungs at 3 days post SCV2 infection, which is early enough to determine viral loads. Indeed, ongoing *Mtb* infection reduced SCV2 lung viral titers by 1-2 logs at 3 days post-infection as measured by both TCID$_{50}$ assay on Vero E6 cells (Figure 5B) and quantitative PCR (qPCR) to measure the number of copies of the SCV2 E gene in both its actively replicating (sub-genomic, sgRNA) and typical (genomic, gRNA) conformations (35) (Figure 5C). Recognizing that the K18-hACE2 model has changes in viral tropism due to the artificial nature of the hACE2 transgene expression (36), we utilized a SCV2 variant of concern (VOC, beta variant, B.1.351), which carries an asparagine to tyrosine substitution at amino acid 501 of the spike protein, allowing binding to murine ACE2 and establishment of transient SCV2 infection in wild type (WT) C57Bl/6 mice (37, 38). Strikingly, we observed a significant reduction in B.1.351 SCV2 viral titers in the lungs of *Mtb*-infected C57Bl/6 mice as early as 1 day post SCV2 infection, and the magnitude of viral restriction correlated with increasing *Mtb*-infectious dose, with no replicating virus detectable in the lungs of mice that previously received a high dose of *Mtb* (1000 - 2000 CFU) (Figures 5D, E). Taken together, these results suggest that an underlying pulmonary *Mtb* infection restricts early viral replication, leading to a reduction in overall viral antigens and a decrease in the magnitude of the T cell and T$_{RM}$ response.

Because our findings showed restriction of viral replication as early as one day after SCV2 infection in a *Mtb* dose-dependent manner, we speculated that *Mtb*-driven innate inflammation, alongside induction of antiviral interferons, may mediate the observed protective effects. *Mtb* carries several pathogen-associated molecular patterns (PAMPs) that activate pattern recognition receptors (PRRs), including TLR2 and TLR9 (39). TLR activation leads to production of several inflammatory cytokines, including IFN-I. Due to the potent antiviral nature of IFN-I (8), we next examined whether *Mtb* sensing *via* TLR2 or TLR9 or a *Mtb*-driven IFN-I response were required for SCV2 restriction in *Mtb*-infected mouse lungs. We infected mice deficient in the IFNα receptor 1 (IFNAR1, *Ifnar1*$^{-/-}$), TLR2 (*Tlr2*$^{-/-}$), or TLR9 (*Tlr9*$^{-/-}$) with *Mtb* and 1-2 months later with B.1.351 SCV2 (Figure 6A). Of note, without underlying *Mtb* infection (blue

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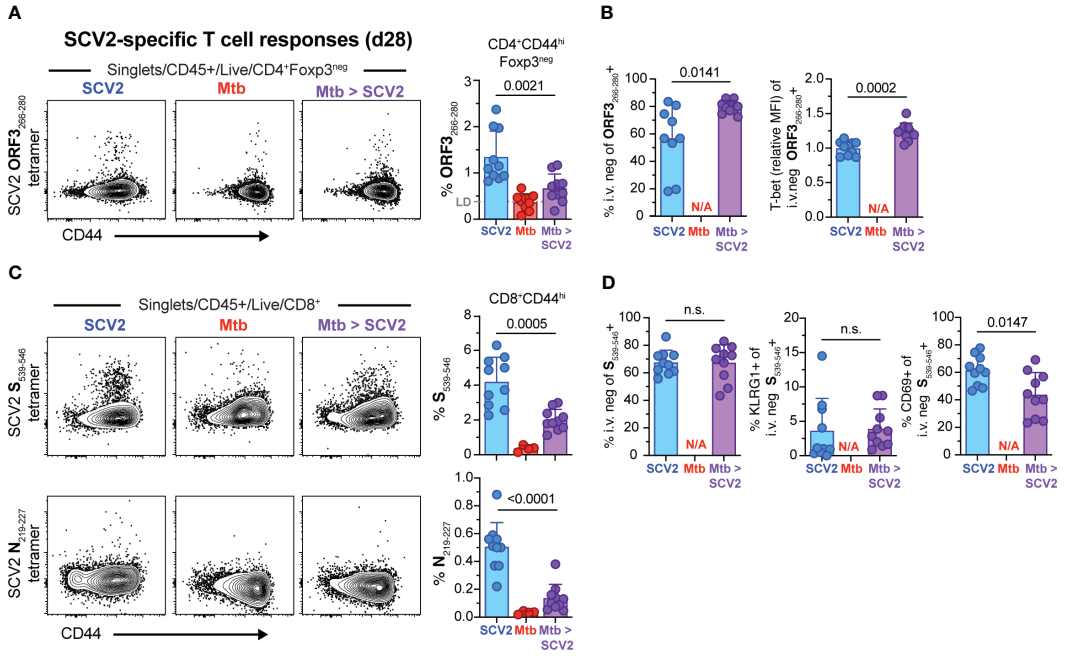

FIGURE 4
SCV2 co-infection of *Mtb* infected mice results in decreased SCV2-specific $CD4^+$ and $CD8^+$ T cells in the lungs 4 weeks later. Example FACS plots and summary data from the lungs of mice described in Figure 2A. **(A)** Example FACS plots of SCV2 $ORF3_{266-280}$ MHC-II tetramer staining of $CD4^+$ $Foxp3^-$ cells and proportion of $ORF3_{266-28}$-specific cells within activated $CD44^{hi}CD4^+Foxp3^-$ T cells 4 weeks after SCV2 infection of naïve (blue) or 7-month *Mtb*-infected mice (purple). LD = Limit of Detection is indicated based on non-specific tetramer binding in *Mtb* only infected groups (red) **(B)** Quantification of $ORF3_{266-280}$ tetramer-positive cells residing in lung parenchyma ($CD45$ $i.v.^{neg}$) and relative expression intensity (geometric mean fluorescent intensity, MFI) of T-bet in lung resident $ORF3_{266-280}$ tetramer-positive $CD4^+$ T cells. **(C)** Example FACS plots of SCV2 $S_{539-546}$ (top) and SCV2 $N_{219-227}$ (bottom) MHC-I tetramer staining of $CD8^+$ T cells and proportion of $S_{539-546}$- or $N_{219-227}$- specific $CD8^+$ T cells gated on activated $CD44^{hi}CD8^+$ T cells **(D)** Quantification of SCV2 $S_{539-546}$ tetramer-positive cells recruited into the lung parenchyma ($CD45$ $i.v.^{neg}$) and their expression of KLRG1 and CD69. (N/A= not applicable, n= 9-10 per group, data combined from 2 independent experiments, mean ± S.D., two-tailed Mann Whitney test). n.s. = not significant.

symbols), *Ifnar1*$^{-/-}$ mice displayed a 1.0 – 1.5 log significant increase in viral titers at three days after B.1.351 SCV2 infection compared to WT mice as measured by both $TCID_{50}$ (Figure 6A) and qPCR (Figure 6B). SCV2-infected *Tlr2*$^{-/-}$ mice had a significant increase in viral titers when measured by $TCID_{50}$ (Figure 6A) but not when assessed by qPCR (Figure 6B). *Tlr9*$^{-/-}$ mice showed no differences in lung viral titers (Figures 6A, B). Importantly and irrespective of these baseline increases in viral titers, we consistently observed a 1.5 – 2.0 log reduction in SCV2 viral loads in lungs of mice with an underlying *Mtb* infection, regardless of their expression of TLR2, TLR9, or IFNAR1 (Figures 6A, B). These results indicate that *Mtb*-induced restriction of SCV2 is not dependent solely on TLR2, TLR9 or IFN-I signaling and likely is a consequence of multiple innate inflammatory immune alterations during *Mtb* infection compared to the lungs of immunologically naïve mice.

Discussion

To investigate the immunological consequences of *Mtb* and SCV2 interactions we have utilized various murine co-infection models. We have shown that *Mtb*-infected mice that have recovered from a prior SCV2 infection showed no significant changes in *Mtb* bacterial burden or lung pathology. In addition, SCV2 co-infection of chronically *Mtb*-infected mice did not negatively impact bacterial control, lung pathology, or existing *Mtb*-specific T-cell responses. Importantly, using these models we have also demonstrated that early SCV2 replication is dampened in the lungs of *Mtb*-infected K18-hACE2 Tg and C57Bl/6 mice compared to mice without an underlying *Mtb* infection. This protective effect was *Mtb* dose-dependent, prevented SCV2-induced weight loss, and was associated with lower SCV2-specific memory T cell responses compared to mice infected only with SCV2. Our observations agree with previous data published by Rosas-Mejia and colleagues who first showed that concurrent co-infection with *Mtb* and SCV2 in both K18-hACE2 Tg and WT C57Bl/6 mice reduced SCV2 viral titers but did not affect *Mtb* bacterial loads (31). The Rosas-Meija study also showed that co-infection of mice with *Mtb* and SCV2 altered cytokine production and the abundance of immune cell subsets as determined by single cell RNA sequencing (scRNASeq) at 4 – 7 days post SCV2 infection compared to mice infected with either pathogen individually (31). Compared to lungs from SCV2 only mice, co-infected lungs had elevated IFNγ protein, increased Tumor Necrosis Factor (*Tnf*) transcript, reduced IFN-induced protein with tetratricopeptide repeats 2 (*Ifit2)* and *Ifit3* transcripts, and scRNASeq indicated an increased proportion of B cells and a reduced frequency of $CD8^+$ T cells. Our data adds that these perturbations do not affect the *Mtb*-specific T cell response, and instead may contribute to the reduction in SCV2-specific T cell responses that we have

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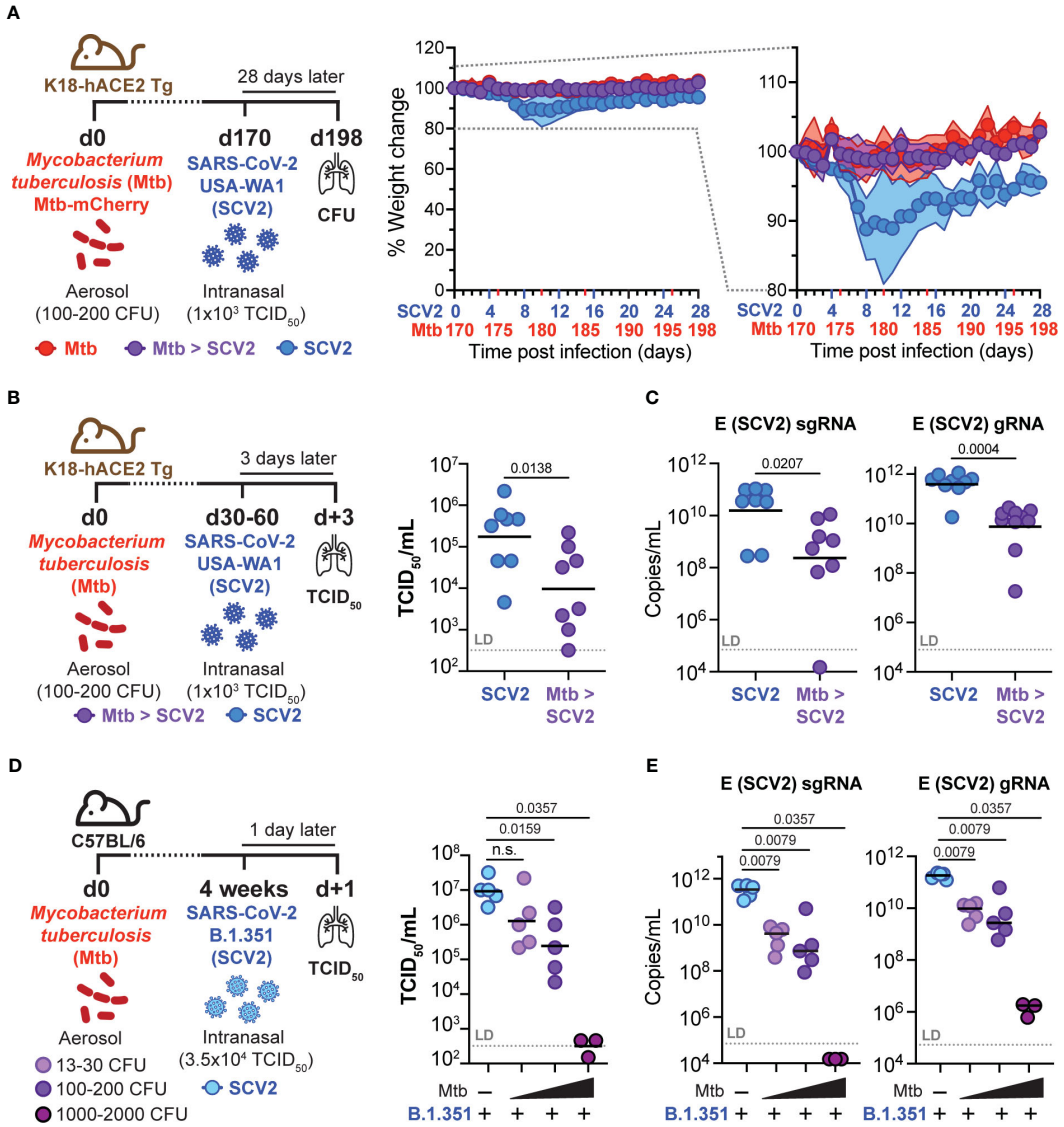

FIGURE 5
Pre-existing *Mtb* infection lowers early SCV2 viral burden in an *Mtb* dose-dependent manner. **(A)** Left: Schematic of experimental set-up where K18-hACE2 Tg mice were infected with *Mtb* 170 days before being intranasally infected with $1x10^3$ $TCID_{50}$ SCV2 (USA-WA1/2020) or mock supernatant, mice were euthanized 28 days after SCV2 infection. Middle: Weight loss of SCV2 infected K18-hACE2 Tg mice with (purple) or without (blue) underlying *Mtb* infection Right: Selected range of weight change curve to highlight differences in weight loss between SCV2 only and co-infected groups (n= 9-10 per group pooled from 2 independent experiments, mean ± SD as traveling error bars). **(B)** Left: Schematic of experimental set-up where K18-hACE2 Tg mice were infected with *Mtb* by aerosol exposure 1-2 months before infection with $1x10^3$ $TCID_{50}$ SCV2 (USA-WA1/2020), mice were euthanized 3 days after SCV2 infection. Right: SCV2 viral load in lungs as measured by $TCID_{50}$ and **(C)** qPCR for the sub-genomic (sg) or genomic (g) SCV2 E gene (n= 8 per group, data combined from two independent experiments, geometric mean, two-tailed Mann Whitney test, LD= limit of detection). **(D)** Left: Schematic of experimental set-up where C57Bl/6 WT mice were infected with various doses of *Mtb* (H37Rv-mCherry) by aerosol exposure 4 weeks before being intranasally infected with $3.5x10^4$ $TCID_{50}$ SCV2 (B.1.351), mice were euthanized 1 day later. Right: Viral loads in lung as measured by $TCID_{50}$ on Vero E6 cells and **(E)** qPCR for the SCV2 E gene sgRNA and gRNA (right) (n= 3 – 5 per group from one experiment representative of two independent experiments, geometric mean, statistical significance calculated by two-tailed Mann Whitney test, LD= limit of detection). n.s. = not significant.

observed at one month post SCV2 co-infection. In addition, Hildebrand et al. reported a significant decrease in SCV2 viral titers at four days post SCV2 infection of *Mtb*-infected mice (30), but interestingly, they saw a significant increase in splenic *Mtb* loads while pulmonary *Mtb* burdens were not significantly changed (16). The discrepancy in bacterial replication seen by Hildebrand et al. may be due to their use of the Erdmann strain of *Mtb* compared to the H37Rv laboratory strain used both herein and in the Rosas Mejia study, suggesting the outcome of *Mtb* and SCV2 co-infection may be modulated by differences in the strain of *Mtb*. In turn, it is also likely that the strain and variant of SCV2 itself can influence disease during an underlying *Mtb* infection. Future studies directly comparing diverse strains of *Mtb* and SCV2 in mouse coinfection models would advance our understanding of how virulence factors expressed by each pathogen contribute to the overall outcome of both diseases.

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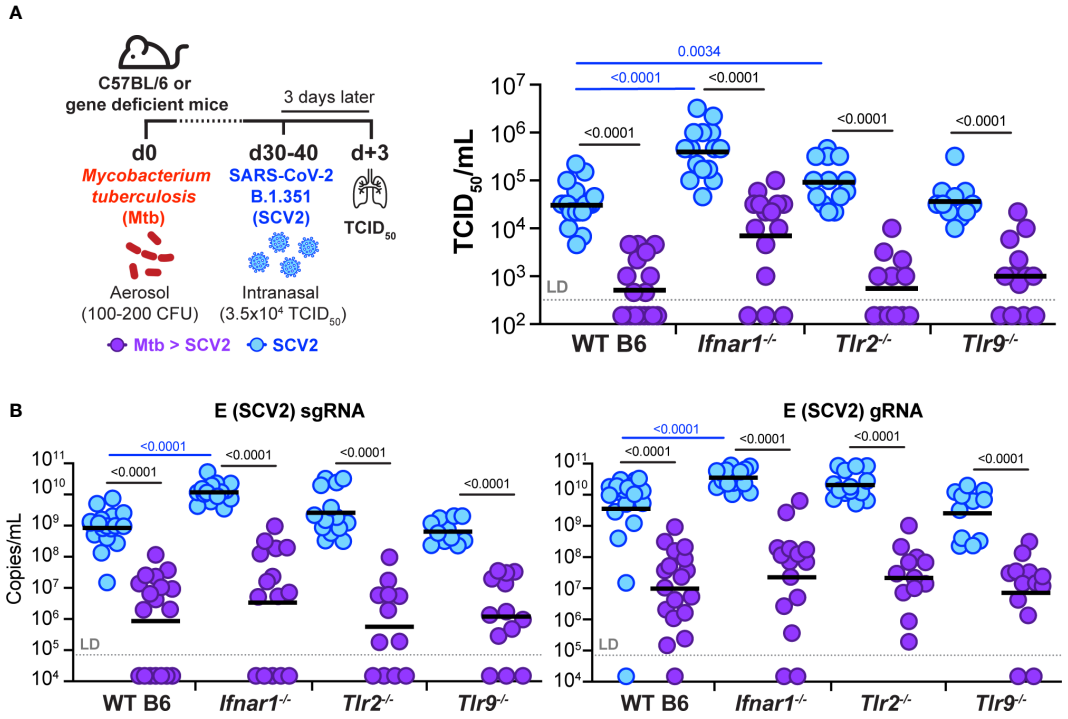

FIGURE 6
Underlying *Mtb* infection reduces SCV2 viral burden independent of IFNAR1, TLR2, or TLR9. **(A)** Left: Schematic of experimental set-up where C57Bl/6 WT, *Ifnar1*$^{-/-}$, *Tlr2*$^{-/-}$ or *Tlr9*$^{-/-}$ mice were infected with *Mtb* 30-40 days prior to being intranasally infected with 3.5x10^4 $TCID_{50}$ SCV2 (B.1.351), mice were euthanized 3 days after SCV2 infection. Right: SCV2 viral load in lungs as measured by $TCID_{50}$ on Vero E6 cells. **(B)** SCV2 viral loads in lungs as measured by qPCR for the SCV2 E sgRNA (left) or gRNA (right) (n= 11-19 per group, data combined from four independent experiments, geometric mean, two-tailed Mann Whitney test (only significant p values shown), LD= limit of detection; significant differences are indicated by blue comparisons between SCV2 only groups (blue), significant differences are indicated by black comparisons between SCV2 (blue) and coinfected groups (purple).

The inability of SCV2 to increase mycobacterial load and lung pathology in mice subsequently infected with *Mtb* contrasts with our previous experiments with sequential IAV and *Mtb* infections (17). SCV2 infection likely engages immune pathways differently in both quantity and/or quality compared to IAV, such as IFN-I production. Others have shown through *in vitro* infection of a human airway epithelial cell line susceptible to both viruses that IAV was a more potent inducer of IFN activity (as measured by STAT1 phosphorylation) than USA-WA1/2020 SCV2 (40). Additionally, COVID-19 patients hospitalized with pneumonia have comparatively low and delayed production of IFN-I when contrasted with severe IAV pneumonia patients (41). As IFN-I has a detrimental impact on *Mtb*-driven disease outcomes in mice and humans (17, 42–48), this raises the possibility that SCV2 may not be able to exacerbate *Mtb* infection due to the induction of a weaker IFN response compared to IAV infection. Future studies must systematically address commonalities and differences between the long-term impacts of IAV or SCV2 infection on the lung microenvironment and subsequent respiratory immune responses to secondary infections. Our data also do not exclude the possibility that *Mtb* burden may be altered at timepoints different from those tested here or whether SCV2 co-infection, similar to IAV co-infection (17, 18), can alter the mortality of *Mtb*-infected animals.

Using flow cytometric analyses, we investigated the impact of SCV2 infection 16 weeks post *Mtb* infection on existing *Mtb*-specific T cell responses, however no reduction was detected compared to mice infected with *Mtb* alone. Other studies where viruses were administered within the first two weeks after *Mtb*-infection (17, 18, 49), a critical time during which initial T cell priming to *Mtb* antigens occurs (50), have reported dampened *Mtb*-specific T cell responses and increased susceptibility to *Mtb*. We intentionally did not explore SCV2 co-infection within the first two weeks after *Mtb* infection because we wanted to mimic the most common clinical scenarios (i.e., individuals who had recovered from a previous SCV2 infection or individuals with a latent, underlying *Mtb* infection). While isolated *Mtb* components such as those present in Complete Freund's Adjuvant can serve as adjuvants to amplify adaptive responses to specific peptide antigens (51, 52), any potential adjuvant effect caused by infection with live *Mtb* bacteria here, was unable to boost antigen-specific T cell responses to SCV2. In contrast, we showed that ongoing *Mtb* infection reduced the frequency of SCV2-specific CD8$^+$ T_{RM}, which was likely due to strongly enhanced early innate viral control in *Mtb* infected mice. We cannot, however, completely rule out that the underlying *Mtb* infection additionally influenced the priming, expansion, contraction, and migration of SCV2-specific T cells into the lungs of co-infected mice; Therefore, further studies are needed to address how simultaneous infection with SCV2 and *Mtb* impacts the priming of adaptive immune responses and disease outcomes for each pathogen.

The *Mtb* infection-mediated suppression of SCV2 replication in our studies was apparent as early as one day post SCV2 exposure. As such, we propose that underlying *Mtb* infection enhances early anti-viral innate immunity in the lung. In contrast to the detrimental role IFN-I plays in *Mtb* infection, IFN-I is a critical family of cytokines promoting innate anti-viral immunity (8). However, our data suggest that IFN-I signaling did not mediate the increased viral control in *Mtb*-infected lungs, as we showed that *Ifnar1*$^{-/-}$ mice had a similarly reduced SCV2 burden as WT mice when previously infected with *Mtb*. Nevertheless, we also showed that IFNAR1-deficient mice had a higher viral load at 3 days post-infection independently of *Mtb* infection status, in line with previously published *in vivo* data that support IFN-I-dependent control of SCV2 replication in mice (53–57) and hamsters (58, 59). Genes induced by IFN-I signaling (Interferon Stimulate Genes, ISGs) overlap significantly, but not completely with genes induced by IFNγ (IFN-II) and IFNλ (IFN-III) (60, 61), suggesting that production of any family of IFNs may have similar effects during SCV2 infection. Several studies utilizing mice deficient in IFNγ signaling have demonstrated a direct role for IFN-II in restriction of SCV2 (62–64). Moreover, IFNγ plays a central role in SCV2 restriction following intravenous infection of mice with *Mycobacterium bovis* bacille Calmette-Guérin (BCG), an attenuated relative of *Mtb* (62, 65). Studies of IFNλ in SCV2-infected mice indicate that the IFN-III response can also restrict viral replication in the mouse lung (66). However, IFNλ signaling-deficient hamsters did not exhibit a similar defect in viral control (67). Thus, while *Mtb*-driven control of early viral replication occurred independently of IFN-I in our studies, it is possible that IFN-III and/or IFN-II, the latter of which is highly induced after mycobacterial infections in mice and is responsible for reduced SCV2 burden following infection with BCG (65), may contribute to the anti-viral state in *Mtb*-infected lungs.

Finally, because early viral restriction was *Mtb*-infection dose-dependent, we explored whether suppression of viral replication in *Mtb*-infected mice was mediated through mycobacterial sensing by TLR2 or TLR9. In addition to recognition of mycobacterial ligands, a role for TLR2 in recognizing the envelope (E) protein of SCV2 leading to innate viral control has been previously reported (68), and we show here that TLR2-deficient mice indeed displayed higher $TCID_{50}$ viral titers in their lungs three days after infection with B.1.351 SCV2 in the absence of *Mtb* infection. Nevertheless, neither TLR2 nor TLR9 signals were individually responsible for suppressing SCV2 replication in the lungs of *Mtb* co-infected C57Bl/6 mice. Our data do not exclude the possibility that multiple TLRs may act in concert to induce a SCV2-suppressive immune environment during *Mtb* infection, and further functional studies are needed to uncover the complex immunological mechanisms responsible for increased innate anti-viral immunity. These mechanisms may include but are not limited to down-regulation of viral entry receptors, enhanced antiviral activation of lung epithelial cells, trained innate immunity or modulation of the innate immune cell milieu in the lung (64, 65, 69–71).

Together, our data suggest that, in the K18-hACE2 Tg mouse model, infection with the ancestral strain of SCV2 does not exacerbate ongoing or subsequent *Mtb* infection at the timepoints tested. Further studies utilizing different strains of mice, *Mtb* strains and SCV2 variants that explore the entire course of infection would further strengthen these observations. While our data do not rule out the possibility of immunological influences in the exacerbation of TB or COVID-19 in co-infected humans they do point to the impact of sociological and healthcare disruptions as more significant factors underlying the reported increase in TB mortality rates during the COVID-19 pandemic.

Methods

Mice

K18-hACE2 Tg hemizygous transgenic mice (B6.Cg-Tg(K18-ACE2)2Prlmn/J; JAX stock #034860) (36), were purchased from Jackson Laboratories (Bar Harbor, ME). C57Bl/6 mice or C57Bl/6 mice expressing a *Foxp3*-GFP reporter (C57BL/6-Foxp3^{tm1Kuch}) (72) were used as wild type C57Bl/6 controls. *Foxp3*-GFP mice and *Ifnar1* KO mice (B6-[KO]IFNa/bR1) (73) were obtained through a supply contract between NIAID and Taconic Farms. *Tlr2* KO mice (74) and *Tlr9* KO mice (75) were originally generated by the laboratory of Dr. Shizuo Akira (Osaka University, Japan) and were kind gifts of Dr. Alan Sher (NIH/NIAID) and Dr. Giorgio Trinchieri (NIH/NCI) respectively. All mouse strains were confirmed to be on a C57Bl/6 background by genetic background analysis submitted through Transnetyx and performed by Neogen using the MiniMUGA platform. Both male and female mice were used and were 8-16 weeks old at the onset of experiments and mice within experiments were age and sex matched. All animals were bred and maintained in an AAALAC-accredited ABSL2 or ABSL3 facility at the NIH and experiments were performed in compliance with an animal study proposal approved by the NIAID Animal Care and Use Committee.

Mtb infection of mice

Aerosol infections of mice with H37Rv-mCherry (50-200 CFU, or as indicated in figure legends) were carried out in a Glas-Col whole-body inhalation exposure system as previously described in detail (76). Briefly, to quantify *Mtb* CFU, lung or spleen homogenates, BALF or inocula were serial-diluted in PBS + 0.1% Tween-80 and plated on Middlebrook 7H11 agar (Sigma Aldrich) supplemented with oleic acid-albumin-dextrose-catalase (OADC) for 3 weeks at 37°C before colonies were counted.

SARS-CoV-2 infection of mice

SARS-CoV-2 hCoV-19/USA-WA1/2020 (Pango lineage A, GISAID reference: EPI_ISL_404895.2) (USA-WA1/2020) and SARS-CoV-2/human/ZAF/KRISP-K005325/2020 beta variant of concern (Pango lineage B.1.351, GISAID reference: EPI_ISL_678615) (B.1.351) were obtained from BEI resources (NIAID, NIH). Viral stocks were generated by infection of Vero

cells (CCL-81, American Type Culture Collection) without (USA-WA1/2020) or with (B.1.351) stable expression of TMPRSS2 (77) at a multiplicity of infection of 0.01 for 48hrs. Cell culture media was harvested and centrifuged at 3500 x g, pooled, aliquoted, and stored at -80°C until use. Virus stocks were sequenced using the Illumina platform; USA-WA1/2020 was consistent with the reference sequence MN985325.1 except for H655Y in S, S6L in E, T7I in M, and S194T in N; B.1.351 was consistent with reference sequence MZ376663.1. Mice were anesthetized with isoflurane and infected intranasally with 35µL inoculum containing $1.0x10^1$ - $1.0x10^3$ $TCID_{50}$ USA-WA1/2020 or $3.5x10^4$ $TCID_{50}$ B.1.351. Inoculum was quantified by $TCID_{50}$ assay in Vero E6 cells (CRL-1586; American Type Culture Collection).

Viral quantification by $TCID_{50}$ assay

$2.5x10^4$ Vero E6 cells were seeded in 100µL DMEM + 10% FCS per well of 96-well tissue culture cluster plates, incubated at 37°C + 5% CO_2 for 16-24 hrs and washed twice with 100µL DMEM + 2% FCS before the assay was conducted. After harvesting lungs from mice, the inferior lobe, post-caval lobe and left lung were homogenized in 600µL PBS using 2.7mm glass beads on a Precellys tissue homogenizer (Bertin Instruments) before dilution with PBS to a final volume of 1.7mL. Viral titers were determined by performing 10-fold serial dilutions of homogenates in DMEM + 2% FCS in quadruplicate, then plating 100µL serial-diluted homogenate and 100µL DMEM + 2% FCS on washed Vero E6 cells and incubating at 37°C + 5% CO_2 for 96 hours. $TCID_{50}$ was measured by removing supernatants and staining wells with crystal violet before scoring for cytopathic effect and calculation using the Reed–Muench method.

RNA extraction and quantitative PCR of viral genomes

For RNA extraction, the superior lobe from each mouse was placed in RNAlater (Invitrogen) and stored at −80°C. RNAlater-stabilized lung lobes were thawed at RT for 20 min, then homogenized in RLT Plus buffer with β-mercaptoethanol (QIAGEN). Total RNA was then isolated from the RLT-homogenized tissue using the RNeasy Plus Mini Kit (QIAGEN), including on-column DNase treatment using the RNase-Free DNase set (QIAGEN) following the manufacturer's instructions and eluted in 60µL RNAse-free water. SCV2 genome copy quantitation was performed in duplicate from 2.5uL of eluted RNA per reaction using the Taqpath 1-step RT-qPCR Master Mix (Thermo) as described by the manufacturer. The SCV2 E gene in both typical (genomic, gRNA) and actively replicating (sub-genomic, sgRNA) conformations (35) was detected using primers at 500nM as follows: E (genomic) Forward (5'- ACAGGTACGTTAATAGTT AATAGCGT-3'), E (sub-genomic) Forward (5'- CGATCTCTTG TAGATCTGTTCTC-3'), E (genomic and sub-genomic) Reverse (5'- ATATTGCAGCAGTACGCACACA -3') and the probe for both E genomic and sub-genomic reactions was used at 125nM (5′- (FAM)-ACACTAGCCATCCTTACTGCGCTTCG-(3IABkFQ) -3′). Cycling conditions: Initial: 25°C for 2 min, 50°C for 15 min, and 95°C for 2 min, Cycling: 95°C for 3 sec, 60°C for 30 sec, for 40 cycles. Copy number was calculated based on standard curves generated for each RT-qPCR run, with SCV2 RNA standard of known quantity and eleven 5-fold dilutions run in duplicate (78).

Cell isolation for flow cytometry

Lungs from infected mice were dissociated using a GentleMACS dissociator (Miltenyi Biotec) in digestion buffer comprised of 0.33mg/mL Liberase TL (Roche), 7U/mL benzonase (Sino Biological), 10µM cytochalasin D (Sigma-Aldrich) and 200µg/mL hyaluronidase (Sigma-Aldrich) followed by 30 - 45 minutes at 37°C. Digested lung was fully dispersed by passage through a 100µm pore size cell strainer and an aliquot was removed for bacterial CFU measurements when needed. Isolated cells were stained with MHC-II tetramers for 40min at 37°C in complete RPMI with 1mM aminoguanidine (Sigma-Aldrich), 100nM dasatinib (Cayman Chemical), 3µg/mL brefeldin A (ThermoFisher) and 2µM monensin (ThermoFisher). Cells were then washed and stained with MHC-I tetramers, surface antibodies and Molecular Probes LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (ThermoFisher) for 20 min at 4°C before permeabilization and fixation using eBioscience™ Foxp3 Transcription Factor Staining Buffer Set (ThermoFisher) at 4°C overnight. Intracellular staining was performed in eBioscience™ Permeabilization Buffer (ThermoFisher) for 40 minutes at 4°C. Samples were acquired on a FACSymphony (BD Biosciences). FACS data were analyzed using FlowJo10 (Treestar). Antibodies were purchased from BioLegend, BD and ThermoFisher as follows: anti-CD45 (30-F11), anti-CD4 (GK1.5), anti-FoxP3 (FJK-16s), anti-CD8a (53-6.7), anti-CD44 (IM7), anti-Ki-67 (B56), anti-T-bet (4B10), anti-KLRG1 (2F1), anti-CD69 (H1.2F3). Tetramer reagents were obtained from the NIH Tetramer Core Facility as follows: *Mtb* $ESAT6_{4-17}$ MHC-II I-A^b tetramer, *Mtb* $TB10.4_{4-11}$ MHC-I H-$2K^b$ tetramer, *Mtb* $32c_{93-102}$ MHC-I H2-D^b tetramer, SCV2 $ORF3A_{266-280}$ MHC-II I-A^b tetramer, SCV2 $S_{532-546}$ MHC-I H-$2K^b$ tetramer, SCV2 $N_{219-227}$ MHC-I H-$2D^b$ tetramer.

Histopathology

The middle right lung lobe from each mouse was fixed in 4% paraformaldehyde, transferred to 70% ethanol and paraffin-embedded before sectioning and mounting on glass slides for staining with hematoxylin and eosin (H&E) or the Kinyoun method for visualization of acid-fast (AF) mycobacteria. Stained slides were imaged by light microscopy on an Aperio Versa microscope (Leica Microsystems). Images were processed using QuPath v0.3.2 (Bankhead et al., 2017) and ImageJ v1.53t (NIH) for quantification and visualization as previously described (79).

Statistical analyses

Statistical analyses were performed using GraphPad Prism v9.0 for Mac OS X (GraphPad Software). Each figure legend lists all the statistical details of experiments, including the statistical tests used. Data are expressed as mean ± SD. Significant differences are indicated by the p value in each figure.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by National Institute of Allergy and Infectious Diseases IACUC. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

Conceptualization: PB and KM-B. Investigation: PB, EA, EC, AB, FT-J, CJ, KH and KM-B. Resources: CN, DB, RJ and KM-B. Data Analysis and Curation: PB, EA and KM-B. Data Visualization: PB, EA and KM-B. Writing – Original Draft: PB and KM-B, Writing – Review & Editing: all co-authors. Supervision: DB and KM-B. Funding Acquisition: RJ, DB, and KM-B. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Brundage JF. Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. *Lancet Infect Dis* (2006) 6:303–12. doi: 10.1016/S1473-3099(06)70466-2

2. Morris DE, Cleary DW, Clarke SC. Secondary bacterial infections associated with influenza pandemics. *Front Microbiol* (2017) 8:1041. doi: 10.3389/fmicb.2017.01041

3. Bakaletz LO. Viral potentiation of bacterial superinfection of the respiratory tract. *Trends Microbiol* (1995) 3:110–4. doi: 10.1016/S0966-842X(00)88892-7

4. Bakaletz LO. Viral–bacterial co-infections in the respiratory tract. *Curr Opin Microbiol* (2017) 35:30–5. doi: 10.1016/j.mib.2016.11.003

5. Obar JJ, Shepardson KM. Coinfections in the lung: How viral infection creates a favorable environment for bacterial and fungal infections. *PloS Pathog* (2023) 19: e1011334. doi: 10.1371/journal.ppat.1011334

6. Jamieson AM, Pasman L, Yu S, Gamradt P, Homer RJ, Decker T, et al. Role of tissue protection in lethal respiratory viral-bacterial coinfection. *Science* (2013) 340:1230–4. doi: 10.1126/science.1233632

7. Jamieson AM, Yu S, Annicelli CH, Medzhitov R. Influenza virus-induced glucocorticoids compromise innate host defense against a secondary bacterial infection. *Cell Host Microbe* (2010) 7:103–14. doi: 10.1016/j.chom.2010.01.010

8. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. *Nat Rev Immunol* (2015) 15:87–103. doi: 10.1038/nri3787

9. Ong CWM, Migliori GB, Raviglione M, MacGregor-Skinner G, Sotgiu G, Alffenaar J-W, et al. Epidemic and pandemic viral infections: impact on tuberculosis and the lung: A consensus by the World Association for Infectious Diseases and Immunological Disorders (WAidid), Global Tuberculosis Network (GTN), and members of the European Society of Clinical Microbiology and Infectious Diseases Study Group for Mycobacterial Infections (ESGMYC). *Eur Respir J* (2020) 56:2001727. doi: 10.1183/13993003.01727-2020

10. World Health Organization. *Global Tuberculosis Report 2022* (2022). Geneva: World Health Organization. Available at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022 (Accessed November 1, 2022).

11. Müller J, Tanner R, Matsumiya M, Snowden MA, Landry B, Satti I, et al. Cytomegalovirus infection is a risk factor for tuberculosis disease in infants. *JCI Insight* (2019) 4:e130090. doi: 10.1172/jci.insight.130090

12. Stockdale L, Nash S, Farmer R, Raynes J, Mallikaarjun S, Newton R, et al. Cytomegalovirus antibody responses associated with increased risk of tuberculosis disease in Ugandan adults. *J Infect Dis* (2020) 221:1127–34. doi: 10.1093/infdis/jiz581

13. van der Heijden YF, Zhang B, Chougnet CA, Huaman MA. Cytomegalovirus infection is associated with increased prevalence of latent tuberculosis infection. *Open Forum Infect Dis* (2021) 8:ofab539. doi: 10.1093/ofid/ofab539

14. Mendy J, Jarju S, Heslop R, Bojang AL, Kampmann B, Sutherland JS. Changes in Mycobacterium tuberculosis-Specific Immunity With Influenza co-infection at Time of TB Diagnosis. *Front Immunol* (2019) 9:3093. doi: 10.3389/fimmu.2018.03093

15. Walaza S, Tempia S, Dawood H, Variava E, Moyes J, Cohen AL, et al. Influenza virus infection is associated with increased risk of death amongst patients hospitalized with confirmed pulmonary tuberculosis in South Africa, 2010–2011. *BMC Infect Dis* (2015) 15:26. doi: 10.1186/s12879-015-0746-x

16. Volkert M, Pierce C, Horsfall FLJr, Dubos RJ. The enhancing effect of concurrent infection with pneumotropic viruses on pulmonary tuberculosis in mice. *J Exp Med* (1947) 86:203–14. doi: 10.1084/jem.86.3.203

17. Redford PS, Mayer-Barber KD, McNab FW, Stavropoulos E, Wack A, Sher A, et al. Influenza A virus impairs control of mycobacterium tuberculosis coinfection through a type I interferon receptor–dependent pathway. *J Infect Dis* (2014) 209:270–4. doi: 10.1093/infdis/jit424

18. Ring S, Eggers L, Behrends J, Wutkowski A, Schwudke D, Kröger A, et al. Blocking IL-10 receptor signaling ameliorates Mycobacterium tuberculosis infection during influenza-induced exacerbation. *JCI Insight* (2019) 4:e126533. doi: 10.1172/jci.insight.126533

19. Malik AA, Safdar N, Chandir S, Khan U, Khowaja S, Riaz N, et al. Tuberculosis control and care in the era of COVID-19. *Health Policy Plan* (2020) 35:1130–2. doi: 10.1093/heapol/czaa109

20. Zimmer AJ, Klinton JS, Oga-Omenka C, Heitkamp P, Nyirenda CN, Furin J, et al. Tuberculosis in times of COVID-19. *J Epidemiol Community Health* (2022) 76:310–6. doi: 10.1136/jech-2021-217529

21. Odume B, Falokun V, Chukwuogo O, Ogbudebe C, Useni S, Nwokoye N, et al. Impact of COVID-19 on TB active case finding in Nigeria. *Public Health Action* (2020) 10:157–62. doi: 10.5588/pha.20.0037

22. Klinton JS, Heitkamp P, Rashid A, Faleye BO, Win Htat H, Hussain H, et al. One year of COVID-19 and its impact on private provider engagement for TB: A rapid assessment of intermediary NGOs in seven high TB burden countries. *J Clin Tuberc Mycobact Dis* (2021) 25:100277. doi: 10.1016/j.jctube.2021.100277

23. McQuaid CF, Vassall A, Cohen T, Fiekert KCOVID/TB Modelling Working Group, , White RG. The impact of COVID-19 on TB: a review of the data. *Int J Tuberc Lung Dis* (2021) 25:436–46. doi: 10.5588/ijtld.21.0148

24. Action global health advocacy partnership, global coalition of TB activists, global TB caucus, et al. *The Impact of COVID-19 on the TB Epidemic: A Community Perspective*. Available at: https://express.adobe.com/page/xJ7pygvhrIAqW/ (Accessed April 3, 2023).

25. Zimmer AJ, Heitkamp P, Malar J, Dantas C, O'Brien K, Pandita A, et al. Facility-based directly observed therapy (DOT) for tuberculosis during COVID-19: A community perspective. *J Clin Tuberc Mycobact Dis* (2021) 24:100248. doi: 10.1016/j.jctube.2021.100248

26. Benade M, Long L, Meyer-Rath G, Miot J, Evans D, Tucker J-M, et al. Reduction in initiations of drug-sensitive tuberculosis treatment in South Africa during the COVID-19 pandemic: Analysis of retrospective, facility-level data. *PloS Glob Public Health* (2022) 2:e0000559. doi: 10.1371/journal.pgph.0000559

27. Coutinho I, Alves LC, Werneck GL, Trajman A. The impact of the COVID-19 pandemic in tuberculosis preventive treatment in Brazil: a retrospective cohort study using secondary data. *Lancet Reg Health – Am* (2023) 19:100444. doi: 10.1016/j.lana.2023.100444

28. Song W, Zhao J, Zhang Q, Liu S, Zhu X, An Q, et al. COVID-19 and tuberculosis coinfection: an overview of case reports/case series and meta-analysis. *Front Med* (2021) 8:657006. doi: 10.3389/fmed.2021.657006

29. Chiok KR, Dhar N, Banerjee A. Mycobacterium tuberculosis and SARS-CoV-2 co-infections: The knowns and unknowns. *iScience* (2023) 26:106629. doi: 10.1016/j.isci.2023.106629

30. Hildebrand RE, Chandrasekar SS, Riel M, Touray BJB, Aschenbroich SA, Talaat AM. Superinfection with SARS-CoV-2 Has Deleterious Effects on Mycobacterium bovis BCG Immunity and Promotes Dissemination of Mycobacterium tuberculosis. *Microbiol Spectr* (2022) 0:e03075–22. doi: 10.1128/spectrum.03075-22

31. Rosas-Mejia O, Gloag ES, Li J, Ruane-Foster M, Claeys TA, Farkas D, et al. Mice infected with Mycobacterium tuberculosis are resistant to acute disease caused by secondary infection with SARS-CoV-2. *PloS Pathog* (2022) 18:e1010093. doi: 10.1371/journal.ppat.1010093

32. Anderson KG, Mayer-Barber K, Sung H, Beura L, James BR, Taylor JJ, et al. Intravascular staining for discrimination of vascular and tissue leukocytes. *Nat Protoc* (2014) 9:209–22. doi: 10.1038/nprot.2014.005

33. Joag V, Wijeyesinghe S, Stolley JM, Quarnstrom CF, Dileepan T, Soerens AG, et al. Cutting edge: mouse SARS-coV-2 epitope reveals infection and vaccine-elicited CD8 T cell responses. *J Immunol* (2021) 206:931–5. doi: 10.4049/jimmunol.2001400

34. Kaech SM, Cui W. Transcriptional control of effector and memory CD8+ T cell differentiation. *Nat Rev Immunol* (2012) 12:749–61. doi: 10.1038/nri3307

35. Long S. SARS-coV-2 subgenomic RNAs: characterization, utility, and perspectives. *Viruses* (2021) 13:1923. doi: 10.3390/v13101923

36. McCray PB, Pewe L, Wohlford-Lenane C, Hickey M, Manzel L, Shi L, et al. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. *J Virol* (2007) 81:813–21. doi: 10.1128/JVI.02012-06

37. Niu Z, Zhang Z, Gao X, Du P, Lu J, Yan B, et al. N501Y mutation imparts cross-species transmission of SARS-CoV-2 to mice by enhancing receptor binding. *Signal Transduct Target Ther* (2021) 6:1–3. doi: 10.1038/s41392-021-00704-2

38. Kant R, Kareinen L, Smura T, Freitag TL, Jha SK, Alitalo K, et al. Common laboratory mice are susceptible to infection with the SARS-coV-2 beta variant. *Viruses* (2021) 13:2263. doi: 10.3390/v13112263

39. Faridgohar M, Nikoueinejad H. New findings of Toll-like receptors involved in Mycobacterium tuberculosis infection. *Pathog Glob Health* (2017) 111:256–64. doi: 10.1080/20477724.2017.1351080

40. Lokugamage KG, Hage A, de Vries M, Valero-Jimenez AM, Schindewolf C, Dittmann M, et al. Type I interferon susceptibility distinguishes SARS-coV-2 from SARS-coV. *J Virol* (2020) 94:e01410–20. doi: 10.1128/JVI.01410-20

41. Galani I-E, Rovina N, Lampropoulou V, Triantafyllia V, Manioudaki M, Pavlos E, et al. Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. *Nat Immunol* (2021) 22:32–40. doi: 10.1038/s41590-020-00840-x

42. Manca C, Tsenova L, Bergtold A, Freeman S, Tovey M, Musser JM, et al. Virulence of a Mycobacterium tuberculosis clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN-α/β. *Proc Natl Acad Sci* (2001) 98:5752–7. doi: 10.1073/pnas.091096998

43. Stanley SA, Johndrow JE, Manzanillo P, Cox JS. The type I IFN response to infection with mycobacterium tuberculosis requires ESX-1-mediated secretion and contributes to pathogenesis. *J Immunol* (2007) 178:3143–52. doi: 10.4049/jimmunol.178.5.3143

44. Berry MPR, Graham CM, McNab FW, Xu Z, Bloch SAA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* (2010) 466:973–7. doi: 10.1038/nature09247

45. Antonelli LRV, Gigliotti Rothfuchs A, Gonçalves R, Roffê E, Cheever AW, Bafica A, et al. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. *J Clin Invest* (2010) 120:1674–82. doi: 10.1172/JCI40817

46. Mayer-Barber KD, Andrade BB, Barber DL, Hieny S, Feng CG, Caspar P, et al. Innate and Adaptive Interferons Suppress IL-1α and IL-1β Production by Distinct Pulmonary Myeloid Subsets during Mycobacterium tuberculosis Infection. *Immunity* (2011) 35:1023–34. doi: 10.1016/j.immuni.2011.12.002

47. Ji DX, Yamashiro LH, Chen KJ, Mukaida N, Kramnik I, Darwin KH, et al. Type I interferon-driven susceptibility to Mycobacterium tuberculosis is mediated by IL-1Ra. *Nat Microbiol* (2019) 4:2128–35. doi: 10.1038/s41564-019-0578-3

48. Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, Murray PJ, et al. tuberculosis W/beijing strains upregulate type I IFNs and increase expression of negative regulators of the jak-stat pathway. *J Interferon Cytokine Res* (2005) 25:694–701. doi: 10.1089/jir.2005.25.694

49. Xu W, Snell LM, Guo M, Boukhaled G, Macleod BL, Li M, et al. Early innate and adaptive immune perturbations determine long-term severity of chronic virus and Mycobacterium tuberculosis coinfection. *Immunity* (2021) 54:526–541.e7. doi: 10.1016/j.immuni.2021.01.003

50. Urdahl KB, Shafiani S, Ernst JD. Initiation and regulation of T-cell responses in tuberculosis. *Mucosal Immunol* (2011) 4:288–93. doi: 10.1038/mi.2011.10

51. Lewis PA, Loomis D. Allergic irritability. *J Exp Med* (1924) 40:503–15. doi: 10.1084/jem.40.4.503

52. Billiau A, Matthys P. Modes of action of Freund's adjuvants in experimental models of autoimmune diseases. *J Leukoc Biol* (2001) 70:849–60. doi: 10.1189/jlb.70.6.849

53. Israelow B, Song E, Mao T, Lu P, Meir A, Liu F, et al. Mouse model of SARS-CoV-2 reveals inflammatory role of type I interferon signaling. *J Exp Med* (2020) 217:e20201241. doi: 10.1084/jem.20201241

54. Sun J, Zhuang Z, Zheng J, Li K, Wong RL-Y, Liu D, et al. Generation of a broadly useful model for COVID-19 pathogenesis, vaccination, and treatment. *Cell* (2020) 182:734–743.e5. doi: 10.1016/j.cell.2020.06.010

55. Ogger PP, Garcia Martín M, Michalaki C, Zhou J, Brown JC, Du Y, et al. Type I interferon receptor signalling deficiency results in dysregulated innate immune responses to SARS-CoV-2 in mice. *Eur J Immunol* (2022) 52:1768–75. doi: 10.1002/eji.202249913

56. Uddin MB, Liang Y, Shao S, Palani S, McKelvey M, Weaver SC, et al. Type I IFN signaling protects mice from lethal SARS-coV-2 neuroinvasion. *ImmunoHorizons* (2022) 6:716–21. doi: 10.4049/immunohorizons.2200065

57. Leist SR, Dinnon KH, Schäfer A, Tse LV, Okuda K, Hou YJ, et al. A mouse-adapted SARS-coV-2 induces acute lung injury and mortality in standard laboratory mice. *Cell* (2020) 183:1070–1085.e12. doi: 10.1016/j.cell.2020.09.050

58. Bessière P, Wasniewski M, Picard-Meyer E, Servat A, Figueroa T, Foret-Lucas C, et al. Intranasal type I interferon treatment is beneficial only when administered before clinical signs onset in the SARS-CoV-2 hamster model. *PloS Pathog* (2021) 17:e1009427. doi: 10.1371/journal.ppat.1009427

59. Hoagland DA, Møller R, Uhl SA, Oishi K, Frere J, Golynker I, et al. Leveraging the antiviral type I interferon system as a first line of defense against SARS-CoV-2 pathogenicity. *Immunity* (2021) 54:557–570.e5. doi: 10.1016/j.immuni.2021.01.017

60. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol* (2005) 5:375–86. doi: 10.1038/nri1604

61. Schoggins JW. Interferon-stimulated genes: what do they all do? *Annu Rev Virol* (2019) 6:567–84. doi: 10.1146/annurev-virology-092818-015756

62. Hilligan KL, Namasivayam S, Clancy CS, Baker PJ, Old SI, Peluf V, et al. Pre-existing interferon gamma conditions the lung to mediate early control of SARS-CoV-2. *bioRxiv* (2023) 2023:07.15.549135. doi: 10.1101/2023.07.15.549135

63. Karki R, Sharma BR, Tuladhar S, Williams EP, Zalduondo L, Samir P, et al. Synergism of TNF-α and IFN-γ Triggers inflammatory cell death, tissue damage, and mortality in SARS-coV-2 infection and cytokine shock syndromes. *Cell* (2021) 184:149–168.e17. doi: 10.1016/j.cell.2020.11.025

64. Gawish R, Starkl P, Pimenov L, Hladik A, Lakovits K, Oberndorfer F, et al. ACE2 is the critical in *vivo* receptor for SARS-CoV-2 in a novel COVID-19 mouse model with TNF- and IFNγ-driven immunopathology. *eLife* (2022) 11:e74623. doi: 10.7554/eLife.74623

65. Hilligan KL, Namasivayam S, Clancy CS, O'Mard D, Oland SD, Robertson SJ, et al. Intravenous administration of BCG protects mice against lethal SARS-CoV-2 challenge. *J Exp Med* (2021) 219:e20211862. doi: 10.1084/jem.20211862

66. Chong Z, Karl CE, Halfmann PJ, Kawaoka Y, Winkler ES, Keeler SP, et al. Nasally delivered interferon-λ protects mice against infection by SARS-CoV-2 variants including Omicron. *Cell Rep* (2022) 39:110799. doi: 10.1016/j.celrep.2022.110799

67. Boudewijns R, Thibaut HJ, Kaptein SJF, Li R, Vergote V, Seldeslachts L, et al. STAT2 signaling restricts viral dissemination but drives severe pneumonia in SARS-CoV-2 infected hamsters. *Nat Commun* (2020) 11:5838. doi: 10.1038/s41467-020-19684-y

68. Zheng M, Karki R, Williams EP, Yang D, Fitzpatrick E, Vogel P, et al. TLR2 senses the SARS-CoV-2 envelope protein to produce inflammatory cytokines. *Nat Immunol* (2021) 22:829–38. doi: 10.1038/s41590-021-00937-x

69. Tamir H, Melamed S, Erez N, Politi B, Yahalom-Ronen Y, Achdout H, et al. Induction of innate immune response by TLR3 agonist protects mice against SARS-coV-2 infection. *Viruses* (2022) 14:189. doi: 10.3390/v14020189

70. Martin-Sancho L, Lewinski MK, Pache L, Stoneham CA, Yin X, Becker ME, et al. Functional landscape of SARS-CoV-2 cellular restriction. *Mol Cell* (2021) 81:2656–2668.e8. doi: 10.1016/j.molcel.2021.04.008

71. Zhang B-Z, Shuai H, Gong H-R, Hu J-C, Yan B, Yuen TT-T, et al. Bacillus Calmette-Guérin–induced trained immunity protects against SARS-CoV-2 challenge in K18-hACE2 mice. *JCI Insight* (2022) 7:e157393. doi: 10.1172/jci.insight.157393

72. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* (2006) 441:235–8. doi: 10.1038/nature04753

73. Müller U, Steinhoff U, Reis LFL, Hemmi S, Pavlovic J, Zinkernagel RM, et al. Functional role of type I and type II interferons in antiviral defense. *Science* (1994) 264:1918–21. doi: 10.1126/science.8009221

74. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* (1999) 11:443–51. doi: 10.1016/S1074-7613(00)80119-3

75. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, et al. A Toll-like receptor recognizes bacterial DNA. *Nature* (2000) 408:740–5. doi: 10.1038/35047123

76. Bohrer AC, Tocheny C, Assmann M, Ganusov VV, Mayer–Barber KD. Cutting edge: IL-1R1 mediates host resistance to mycobacterium tuberculosis by trans-protection of infected cells. *J Immunol* (2018) 201:1645–50. doi: 10.4049/jimmunol.1800438

77. Liu X, Luongo C, Matsuoka Y, Park H-S, Santos C, Yang L, et al. A single intranasal dose of a live-attenuated parainfluenza virus-vectored SARS-CoV-2 vaccine is protective in hamsters. *Proc Natl Acad Sci* (2021) 118:e2109744118. doi: 10.1073/pnas.2109744118

78. Nelson CE, Namasivayam S, Foreman TW, Kauffman KD, Sakai S, Dorosky DE, et al. Mild SARS-CoV-2 infection in rhesus macaques is associated with viral control prior to antigen-specific T cell responses in tissues. *Sci Immunol* (2022) 7:eabo0535 doi: 10.1126/sciimmunol.abo0535

79. Amaral EP, Ribeiro SCM, Lanes VR, Almeida FM, de Andrade MRM, CCB B, et al. Pulmonary infection with hypervirulent mycobacteria reveals a crucial role for the P2X7 receptor in aggressive forms of tuberculosis. *PloS Pathog* (2014) 10:e1004188. doi: 10.1371/journal.ppat.1004188

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BCG mediated protection of the lung against experimental SARS-CoV-2 infection

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The observation of reduced COVID-19 incidence and severity in populations receiving neonatal intradermal BCG vaccination vaccine raised the question of whether BCG can induce non-specific protection against the SARS-CoV-2 (SCV2) virus. Subsequent epidemiologic studies and clinical trials have largely failed to support this hypothesis. Furthermore, in small animal model studies all investigators have failed to observe resistance to viral challenge in response to BCG immunization by the conventional and clinically acceptable intradermal or subcutaneous routes. Nevertheless, BCG administered by the intravenous (IV) route has been shown to strongly protect both hamsters and mice against SCV2 infection and disease. In this Perspective, we review the current data on the effects of BCG vaccination on resistance to COVID-19 as well as summarize recent work in rodent models on the mechanisms by which IV administered BCG promotes resistance to the virus and discuss the translational implications of these findings.

KEYWORDS

COVID-19, Bacille Calmette-Guérin (BCG), lung, interferon gamma (IFNγ), trained immunity

Introduction

The innate compartment of the immune system differs from the adaptive in its ability to provide non-specific defense against a wide variety of threats encountered by the body and its stimulation is an important strategy for enhancing host resistance to pathogens. Avirulent microbes and their products are themselves important triggers of innate immune function and recently have been described to do so with long term effects (1). BCG (Bacille Calmette Guérin) is a well-studied microbial stimulus for its effects on innate immunity. This attenuated *Mycobacterium bovis* strain is widely used to vaccinate against extrapulmonary tuberculosis (TB) in infants and children and was discovered in the mid-20th century to also promote non-specific resistance against tumors, a finding that led to its current employment as a treatment for some forms of bladder cancer (2). More recently BCG vaccination has been associated with lowering all-cause mortality in infants (3), reducing viremia after a yellow fever vaccine challenge in adults (4), and decreasing risk of respiratory infections in the elderly (5). Multiple mechanisms have been proposed to

explain these effects, the most prominent of which involve the induction of "trained immunity" in which myelopoietic-derived innate effector cells become epigenetically modified so that they remain in a long-term primed state (up to 1 year in humans) (6–8).

BCG is typically administered to vaccinees by intradermal (ID) or subcutaneous (SC) injection although other routes (e.g. oral) have been employed in the past (9, 10). Although not clinically approved, the intravenous (IV) route of BCG administration has recently been employed in two important studies related to TB vaccination. In the first study, Kaufmann and colleagues showed that IV BCG preferentially induces trained immunity in mice because of its ability to access and infect long lived myelopoietic stem cells in the bone marrow (7). In the second report, Darrah and colleagues showed that IV in contrast to SC administered BCG induces sterile immunity against *M. tuberculosis* (Mtb) challenge in a rhesus monkey model (11), a dramatic finding that the authors attributed to the direct targeting of the lung and the induction of a strong local memory T cell response when the vaccine is given by this route (12). Recent studies indicate that in macaques such resistance can persist after the clearance of culturable BCG bacilli (13). Nevertheless, the contribution of BCG stimulated innate immune mechanisms to this striking protection is at present unclear.

Clinical evidence for or against the association of BCG vaccination with host resistance to COVID-19

Given its previously demonstrated ability to stimulate non-specific host resistance to certain other viral infections, BCG immunization was suggested in the early months of the COVID-19 pandemic as a possible prophylactic measure for the prevention of SCV2 infection and disease (14, 15). This concept was initially supported by a number of ecological/epidemiologic studies suggesting an association of prior BCG vaccination with a lower incidence of COVID-19 disease (16, 17) despite the relatively short period (up to 1 year) that "trained" responses have been reported to persist *in vivo* (8). This early work was followed up with a large number of more extensive investigations (summarized in Table 1) that in general have failed to confirm the protective effects of BCG vaccination on the incidence and severity of SCV2 infection (18–21, 26–29), including a recently published international multi-cohort randomized trial (BRACE) involving ID administration of BCG to adult health care workers (22). One study conducted with a small cohort of older adults in Greece did note some protection against the incidence of COVID-19 symptoms; however, the existence of SCV2 infection in these individuals was not confirmed by PCR or antibody testing (24). A significant reduction in the incidence and symptom severity of COVID-19 was also observed in a different study involving the follow-up of adult diabetes patients given 3 ID doses of intramural BCG over a 2-3 year period before the onset of the pandemic (25). The explanation for the unusual efficacy observed in the latter study is unclear but may relate to the multiple dosage, the use of a highly virulent BCG isolate (Tokyo strain) (30, 31), the spacing between BCG vaccination and SCV2 exposure, or possibly the diabetic state of the participants. Overall, there is currently no compelling evidence that a single-dose intradermal BCG inoculation provides protection against SCV2 infection and disease; however, there may be certain conditions that favor the protective outcomes observed with multiple BCG doses (25). Future studies examining prolonged or repeated mycobacterial exposures, either due to population level exposure to environmental mycobacteria and/or BCG re-vaccination strategies, may provide further insights into any potential protective effects (32).

Evidence in animal models for BCG induced protection against SARS-CoV-2

The hypothesis that prior BCG vaccination might offer protection against COVID-19 prompted a series of studies in different animal models to examine the effects of prior BCG administration on resistance to SCV2 challenge (Table 2). This work has generated a consensus that when inoculated by the conventional ID (or subcutaneous) route to mice (33, 35–37) or hamsters (35, 38) or by aerosol to monkeys (39), BCG fails to trigger significant protection against intranasal or intra-bronchial infection with the virus. Nevertheless, a number of independent studies have shown that when administered by the IV route to mice or hamsters, BCG can confer high levels of resistance to both SCV2 infection and disease (Table 2) (33, 34, 38, 40, 41). In the initial description of this effect, K18 transgenic mice which express the human ACE2 receptor (K18-hACE2) for the virus were IV inoculated with BCG (Pasteur strain) before intranasal SCV2 infection with a lethal dose of the WA/2020 strain (33). At 42 days following BCG administration, the virus challenged mice showed a striking protection from SCV2 induced weight loss and mortality along with pronounced reductions in pulmonary viral loads at 5 days post infection. This protection was still evident 112 days following BCG inoculation but at lower levels (33). To confirm that the COVID-19 resistance induced by IV BCG is not peculiar to hACE2 transgenic mice, the experiments were repeated using a second model in which wild type C57BL/6 mice were challenged with the more virulent B.1.1.7 SCV2 variant. In this situation unvaccinated mice support viral replication for 3-4 days before clearing the infection with minimal accompanying disease. Again, IV BCG induced striking protection against SCV2 with the majority of the BCG exposed mice showing no detectable virus in their lungs at 3 days following B.1.1.7 challenge (33). Consistent with the other studies cited above, no significant resistance against SCV2 was observed in mice inoculated with the same dose of BCG by the SC route in either of the two murine models. The ability of IV BCG to protect K18-hACE2 mice from early SCV2 infection was confirmed in a second study using the Tokyo strain of BCG and intranasal viral challenge with either an original "wild-type" strain or more virulent kappa or delta variants (40). In additional work, IV administered BCG (Tice strain) was shown to reduce viral loads and bronchopneumonia in Syrian hamsters challenged intranasally with the Wuhan-1 strain

TABLE 1 Summary of human trials investigating BCG efficacy against COVID-19.

Trial design	Participant characteristics	BCG strain	SCV2 outcomes (versus control arm)			Reference
			Incidence	Severity	Other parameters	
RCT, ~1000 individuals/ arm	Adult, 60y+	Danish 1331	NC	NA	Higher SCV2 antibody titers in BCG vaccinated participants	(18)
RCT, ~1000 individuals/ arm	Adult, 60y+	VPM1002	NC	NA	NC in self-reported duration of illness with respiratory tract infection, but trend towards lower duration in BCG vaccinated individuals within the cohort who did not received COVID-19-specific vaccines.	(19)
RCT, ~750 individuals/ arm	Adult, health care workers	Danish 1331	NC	NC		(20, 21)
RCT, ~1700 individuals/ arm	Adult, health care workers	Danish 1331	NC	NC	Lower cytokine responses in whole blood samples exposed to irradiated SCV2 in BCG vaccinated individuals (n=25)	(22, 23)
RCT, ~150 individuals/ arm	Adult, 50y+	Moscow	Reduced*	NA		(24)
RCT, 48 in placebo arm, 96 in BCG arm	Adult, type-1 diabetes patients	Tokyo 172, 3 doses	Reduced	Reduced		(25)
RCT, ~3000 individuals/ arm	Adult, 60y+ with >1 co-morbidities	Danish 1331	NC	NC	NC in incidence of other respiratory infections	(26)
RCT, ~130 individuals/ arm	Adult, health care workers	Moscow or Moreau	NC	NA		(27)
RCT, ~70 individuals/ arm	Adult, health care workers	Moscow	NC	NA		(28)
RCT, ~250 individuals/ arm	Adults	Not specified	NC	Reduced		(29)

NC, no change; NA, not assessed.
*COVID-19 incidence was defined as "possible/probable/definitive" in this study.
These citations are based on a literature search in May 2023.

SCV2 (38). In contrast to the above findings, Kaufmann et al. reported that K18-hACE2 mice or hamsters given IV (or SC) BCG (Tice strain) showed no significant protection against intranasal (or in the case of mice either intranasal or intratracheal) challenge with a SCV2-B lineage variant. Nevertheless, the same BCG exposed mice displayed resistance to intranasally administered Influenza A virus (35). Ongoing follow up studies suggest that the negative results with SCV2 obtained in the latter study may relate to the BCG strain (42), its preparation and/or the dose employed for vaccination (Kaufmann and Hilligan, unpublished).

The consistent failure of SC or ID inoculated BCG to provide protection against SCV2 infection suggests that the resistance conferred by IV BCG may relate to the long-term presence mycobacteria in the lungs and accompanying granulomatous inflammation occurring in animals inoculated by that route (33, 40). Consistent with this hypothesis, K18-hACE2 or non-transgenic mice infected by aerosol with virulent *Mycobacterium tuberculosis* and developing pulmonary TB, display high levels of resistance to SCV2 comparable to that reported in IV BCG exposed animals (37, 43, 44). Nevertheless, as noted above, in rhesus macaques BCG given by the aerosol route failed to induce protection against SCV2 challenge (39). Since pulmonary bacterial infection and local tissue responses were not evaluated in that study, it is difficult to ascertain whether this discrepancy with the rodent studies reflects the different host species employed or the local levels of BCG and/or immune responses occurring at that site. Indeed, a comparison between IV and aerosol inoculation of rhesus macaques by Darrah et al, showed that only IV BCG resulted in the formation of "microgranuloma" structures in the lung as well as increased numbers of CD4+ T cells and CD11c+ antigen-presenting cells (11).

TABLE 2 Summary of animal studies assessing efficacy of BCG against SCV2 infection and disease.

Animal model	Route of BCG administration	BCG strain	SCV2 outcomes (versus control group)		Reference
			Disease phenotype	Viral titers	
Mouse, K18-hACE2	SC	Pasteur	NC (survival and weight loss)	NC	(33)
Mouse, K18-hACE2	IV	Pasteur	Improved (survival and weight loss)	Reduced	(33)
Mouse, wildtype B6	SC	Pasteur	n/a	NC	(33)
Mouse, wildtype B6	IV	Pasteur	n/a	Reduced	(33, 34)
Mouse, K18-hACE2	SC	Tice	NC (survival and weight loss)	NC	(35)
Mouse, K18-hACE2	IV	Tice	NC (survival and weight loss)	NC	(35)
Hamster, Syrian Golden	SC	Tice	NC (weight loss)	NC	(35)
Hamster, Syrian Golden	IV	Tice	NC (weight loss)	NC	(35)
Hamster, Roborovski	SC	Tice	NC (survival and weight loss)	NC	(35)
Hamster, Roborovski	IV	Tice	NC (survival and weight loss)	NC	(35)
Mouse, K18-hACE2	SC	Pasteur	NC (weight loss)	NC	(36)
Mouse, K18-hACE2	SC	Pasteur	NC (survival and weight loss)	NC	(37)
Hamster, Syrian Golden	IV	Tice	Improved (bronchopneumonia score)	Reduced	(38)
Rhesus macaque	aerosol	Danish 1331	NC (pathology score)	NC	(39)
Mouse, K18-hACE2	SC	Tokyo 172	NA	NC	(40)
Mouse, K18-hACE2	IV	Tokyo 172	Modestly improved (weight loss)	Reduced	(40)
Mouse, wildtype B6	IV	Tice	Improved (weight loss)*	Reduced*	(41)

NC, no change; NA, not assessed; n/a, not applicable.
*protective effect only apparent from 21 days after IV BCG inoculation.

Mechanisms underlying BCG induced resistance to SCV2 infection and disease

It was originally proposed that ID (or SC) administered BCG might offer protection against COVID-19 because of its previously documented ability to enhance clinical resistance to other viral infections, effects that were attributed to the induction of trained immunity (14, 15). Since in nearly all studies humans vaccinated with BCG by this route fail to display significant resistance to COVID-19, it would appear that any response induced by a single-dose BCG inoculation is not sufficient to restrict SCV2. Nevertheless, it is still possible that boosting of the response by intradermal re-vaccination could induce more effective immunity and this could be the basis of the protection against COVID-19 observed by Faustman and colleagues in diabetes patients given multiple BCG inoculations (25).

Since with the latter exception BCG induced protection against SCV2 has not been documented in humans or non-human primates, nearly all the current information on anti-viral mechanisms derives from the studies on murine and hamster rodents involving IV administered bacteria. That route of inoculation has been previously shown in mice to preferentially stimulate myelopoiesis and the generation of monocyte/macrophages with a trained phenotype (7). Consistent with these earlier findings, Zhang and colleagues reported that IV BCG vaccinated mice challenged with SCV2 display enhanced bone marrow myelopoiesis, augmented pulmonary monocyte/macrophage infiltration and upregulated innate immune and metabolic gene signatures previously described as associated with training (40). Although not specifically addressing the issue of trained immunity, both the NIH murine model study of Hilligan et al. and hamster study of Singh and colleagues described enhanced pulmonary macrophage numbers in IV BCG inoculated animals that likely arise from bone marrow monocytes (33, 38). Given the long-term persistence of both mycobacteria and granulomatous inflammation in the lungs of IV BCG vaccinated mice (33, 40), it is unlikely that resistance to SCV2 challenge would require the type of trained myeloid cells previously described as arising in hosts exposed to a prior single intradermal bacterial inoculation.

In each of the three studies documenting protection against SCV2 induced by IV BCG, vaccination was shown to simultaneously reduce pulmonary viral load and virus induced bronchopneumonia, in some cases as early as 2 days post challenge. Consistent with the latter observation, in both mouse studies BCG inoculation resulted in lowered production of SCV2 induced IL-6 and MCP1 (CCL2) (33, 40). Although this decrease could reflect an effect of reduced viral load in the vaccinated animals, the results of a multivariate analysis performed in the NIH murine study revealed an inhibitory effect of prior IV BCG administration on the induction of these pathology associated cytokines independent of viral titer (33). These data align with results from the BRACE clinical trial that showed that while BCG vaccination did not protect against COVID-19 (22), BCG did limit

SCV2-induced pro-inflammatory cytokine responses *ex vivo*, suggesting that BCG inoculation can modulate virus triggered immune responses independent of its protective effect (23).

In both mice and hamsters, IV BCG administration led to pronounced elevations in pulmonary T cells, while only a minor response was seen in mice given SC BCG. In mice, IV BCG enhanced lymphocytes were characterized as CD8+, FoxP3− CD4+, and FoxP3+ CD4+ T cells, as well as MAIT cells and their levels did not significantly increase following viral challenge (33, 38). Indeed, if anything, prior IV BCG administration appeared to suppress the CD8+ T cell expansion triggered by SCV2 infection. Somewhat in contrast, in the hamster model, prior IV BCG inoculation resulted in an expansion of cells with Th1, Th17, Treg, CTLs or Tmem transcriptional markers after viral challenge as well as the emergence of a new plasma cell population not present prior to SCV2 exposure and expressing genes associated with immunoglobulin production suggestive of accelerated antibody production. In the same hamster study, IV BCG vaccination also appeared to dampen the expression of T cell exhaustion markers triggered by SCV2 infection (38). Together these observations show that IV BCG triggers the recruitment of adaptive immune cells into the lung tissue that in addition to supplying a potential source of protective antibodies may be important in providing cytokines and other signals that shape the innate immune landscape. Another interesting possibility is that the response to the bacteria has hindered the ability of the host to respond to another inflammatory stimuli.

Type I IFNs are important for control of viral pathogens but in SCV2 and other virus infections these cytokines can also promote pathology (45, 46). Interestingly in the NIH mouse model study prior IV BCG inoculation appeared to suppress rather than augment the SCV2 triggered Type I IFN response consistent with the suppression of COVID-19-like pathology observed in these animals. BCG infection is classically associated with strong IFNγ production from CD4+ T, CD8+ T and NK cells and the cytokine was found to be heavily induced in the lungs of both mice and hamsters months after IV BCG inoculation (33, 38, 40). Importantly, this local Type II IFN response was minimal in mice vaccinated by the SC route consistent with the dearth of both BCG and its associated granulomatous tissue inflammation in lungs of these animals in contrast to IV inoculated mice. Recent functional studies in the murine models suggest that this IFNγ response deriving primarily from CD4+ T cells and acting on non-hematopoietic cells in the lung is required for the reduction in both SCV2 virus and its associated pathology and that the recombinant cytokine itself can trigger these effects (34, 41). Whether IV BCG induced protection against SCV2 is mediated entirely through this mechanism or also involves the myeloid, T or B lymphocytes changes reported to be associated with resistance in the studies discussed above is at present unclear. A summary of the different effector mechanisms currently proposed to explain the protection against SCV2 induced by IV BCG is presented in Figure 1.

Discussion and translational implications

The findings reviewed above establish a proof of principle in animal models that single dose BCG can stimulate protection against SCV2 but only when given IV, a mode of administration that is currently not clinically acceptable. The data do not rule out the possibility that through repeated boosting (25) or the use of a specially engineered bacterial strain (36) protection against COVID-19 could be generated through conventional ID or SC vaccination although it is likely that such resistance would involve a different mechanism. There is currently considerable interest in the possible use of IV administered BCG for vaccination against *M. tuberculosis* because of its ability to confer sterile immunity against this important pathogen in rhesus monkeys (11, 13). This has stimulated efforts to develop attenuated BCG mutants (e.g. auxotrophs) that would be safe for human intravenous use and such strains could be tested as candidates for protection against COVID-19 (47).

Regardless, the demonstration that bacterial stimulation of the lung can induce high levels of resistance against SCV2 could lead to the discovery of novel mechanisms of anti-viral protection with potential clinical applicability. For example, the recent evidence that BCG induced IFNγ can protect mice from SCV2 challenge (34, 41) raises the question of whether the cytokine could be used intranasally to protect subjects at high risk of infection possibly with less risk of toxicity than Type I IFN. It is also becoming clear that

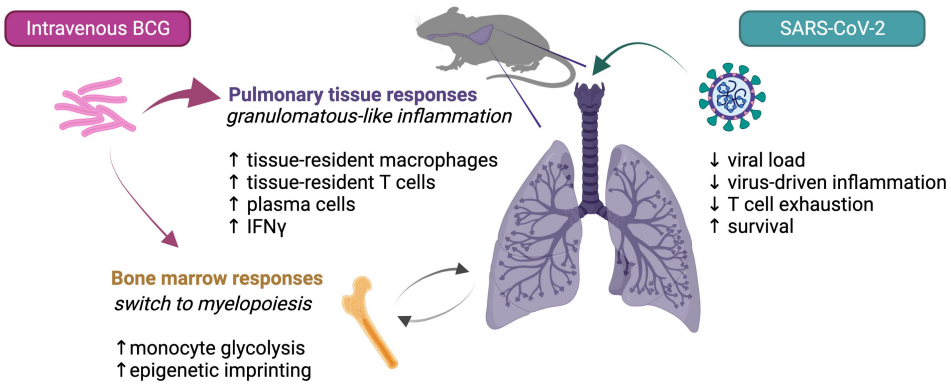

FIGURE 1
Possible mechanisms contributing to IV BCG conferred protection against SCV2 in mice.

IV BCG is not a unique non-specific stimulus for host protection against experimental SCV2. In addition to prior *M. tuberculosis* infection (37, 43, 44), recent findings indicate that intranasally administered PRR ligands can also trigger host resistance in the same murine models (48–50) as can prior infection with a lung-transiting helminth (51). While seemingly distinct stimuli, it is possible that they all act by triggering the production of anti-viral effectors by pulmonary myeloid or epithelial cells.

As noted in the studies reviewed here, IV BCG infection can trigger long term changes in the cellular composition and adaptive immune responsiveness of lung tissue. While trained immunity may contribute [recently reviewed by Netea et al. (52)], other factors such as bacterial induced tissue remodeling and continuous immune stimulation by the bacteria surviving within granuloma-like structures in the lung are in this situation likely to play a more important role in promoting the long-lived property of the protection triggered by IV BCG at that tissue site (Figure 1).

Despite its limitations as a vaccine, studies on BCG continue to provide important insights on the interplay of innate and adaptive immunity in the host response to pathogens and in this case hopefully add to our understanding of how the lung can be stimulated to control both SCV2 and COVID-19 associated pathology.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Dominguez-Andres J, Dos Santos JC, Bekkering S, Mulder WJM, van der Meer JWM, Riksen NP, et al. Trained immunity: adaptation within innate immune mechanisms. *Physiol Rev* (2023) 103(1):313–46. doi: 10.1152/physrev.00031.2021

2. Pettenati C, Ingersoll MA. Mechanisms of BCG immunotherapy and its outlook for bladder cancer. *Nat Rev Urol* (2018) 15(10):615–25. doi: 10.1038/s41585-018-0055-4

3. Biering-Sorensen S, Aaby P, Lund N, Monteiro I, Jensen KJ, Eriksen HB, et al. Early BCG-Denmark and neonatal mortality among infants weighing <2500 g: A randomized controlled trial. *Clin Infect Dis* (2017) 65(7):1183–90. doi: 10.1093/cid/cix525

4. Arts RJW, Moorlag S, Novakovic B, Li Y, Wang SY, Oosting M, et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. *Cell Host Microbe* (2018) 23(1):89–100.e5. doi: 10.1016/j.chom.2017.12.010

5. Giamarellos-Bourboulis EJ, Tsilika M, Moorlag S, Antonakos N, Kotsaki A, Dominguez-Andres J, et al. Activate: randomized clinical trial of BCG vaccination against infection in the elderly. *Cell* (2020) 183(2):315–23.e9. doi: 10.1016/j.cell.2020.08.051

6. Cirovic B, de Bree LCJ, Groh L, Blok BA, Chan J, van der Velden W, et al. BCG vaccination in humans elicits trained immunity *via* the hematopoietic progenitor compartment. *Cell Host Microbe* (2020) 28(2):322–34.e5. doi: 10.1016/j.chom.2020.05.014

7. Kaufmann E, Sanz J, Dunn JL, Khan N, Mendonca LE, Pacis A, et al. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. *Cell* (2018) 172(1-2):176–90.e19. doi: 10.1016/j.cell.2017.12.031

8. Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LA, Jacobs C, et al. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. *J Innate Immun* (2014) 6(2):152–8. doi: 10.1159/000355628

9. Cho T, KhatChadourian C, Nguyen H, Dara Y, Jung S, Venketaraman V. A review of the BCG vaccine and other approaches toward tuberculosis eradication. *Hum Vaccin Immunother* (2021) 17(8):2454–70. doi: 10.1080/21645515.2021.1885280

10. Calmette A, Guerin C BW-H. Essai d'immunisation contre l'infection tuberculeuse. *Bull Acad Med* (1924) 91:787–96.

11. Darrah PA, Zeppa JJ, Maiello P, Hackney JA, Wadsworth MH 2nd, Hughes TK, et al. Prevention of tuberculosis in macaques after intravenous BCG immunization. *Nature* (2020) 577(7788):95–102. doi: 10.1038/s41586-019-1817-8

12. Darrah PA, Zeppa JJ, Wang C, Irvine EB, Bucsan AN, Rodgers MA, et al. Airway T cells are a correlate of i.v. Bacille Calmette-Guerin-mediated protection against tuberculosis in rhesus macaques. *Cell Host Microbe* (2023) 31(6):962–77 e8. doi: 10.1016/j.chom.2023.05.006

13. Larson E, Ellis-Connell A, Rodgers M, Gubernat A, Gleim J, Moriarty R, et al. Vaccination with intravenous BCG protects macaques with pre-existing SIV infection from tuberculosis. *Res Sq* (2023). doi: 10.21203/rs.3.rs-2802306/v1

14. O'Neill LAJ, Netea MG. BCG-induced trained immunity: can it offer protection against COVID-19? *Nat Rev Immunol* (2020) 20(6):335–7. doi: 10.1038/s41577-020-0337-y

15. Curtis N, Sparrow A, Ghebreyesus TA, Netea MG. Considering BCG vaccination to reduce the impact of COVID-19. *Lancet* (2020) 395(10236):1545–6. doi: 10.1016/S0140-6736(20)31025-4

16. Escobar LE, Molina-Cruz A, Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). *Proc Natl Acad Sci USA* (2020) 117(30):17720–6. doi: 10.1073/pnas.2008410117

17. Rivas MN, Ebinger JE, Wu M, Sun N, Braun J, Sobhani K, et al. BCG vaccination history associates with decreased SARS-CoV-2 seroprevalence across a diverse cohort of health care workers. *J Clin Invest* (2021) 131(2):e147157. doi: 10.1172/JCI145157

18. Moorlag S, Taks E, Ten Doesschate T, van der Vaart TW, Janssen AB, Muller L, et al. Efficacy of BCG vaccination against respiratory tract infections in older adults during the coronavirus disease 2019 pandemic. *Clin Infect Dis* (2022) 75(1):e938–e46. doi: 10.1093/cid/ciac182

19. Blossey AM, Bruckner S, May M, Parzmair GP, Sharma H, Shaligram U, et al. VPM1002 as prophylaxis against severe respiratory tract infections including coronavirus disease 2019 in the elderly: A phase 3 randomized, double-blind, placebo-controlled, multicenter clinical study. *Clin Infect Dis* (2023) 76(7):1304–10. doi: 10.1093/cid/ciac881

20. Ten Doesschate T, van der Vaart TW, Debisarun PA, Taks E, Moorlag S, Paternotte N, et al. Bacillus Calmette-Guerin vaccine to reduce healthcare worker absenteeism in COVID-19 pandemic, a randomized controlled trial. *Clin Microbiol Infect* (2022) 28(9):1278–85. doi: 10.1016/j.cmi.2022.04.009

21. Claus J, Ten Doesschate T, Gumbs C, van Werkhoven CH, van der Vaart TW, Janssen AB, et al. BCG vaccination of health care workers does not reduce SARS-coV-2 infections nor infection severity or duration: a randomized placebo-controlled trial. *mBio* (2023) 14(2):e0035623. doi: 10.1128/mbio.00356-23

22. Pittet LF, Messina NL, Orsini F, Moore CL, Abruzzo V, Barry S, et al. Randomized trial of BCG vaccine to protect against covid-19 in health care workers. *N Engl J Med* (2023) 388(17):1582–96. doi: 10.1056/NEJMoa2212616

23. Messina NL, Germano S, McElroy R, Rudraraju R, Bonnici R, Pittet LF, et al. Off-target effects of bacillus Calmette-Guerin vaccination on immune responses to SARS-CoV-2: implications for protection against severe COVID-19. *Clin Transl Immunol* (2022) 11(4):e1387. doi: 10.1002/cti2.1387

24. Tsilika M, Taks E, Dolianitis K, Kotsaki A, Leventogiannis K, Damoulari C, et al. ACTIVATE-2: A double-blind randomized trial of BCG vaccination against COVID-19 in individuals at risk. *Front Immunol* (2022) 13:873067. doi: 10.3389/fimmu.2022.873067

25. Faustman DL, Lee A, Hostetter ER, Aristarkhova A, Ng NC, Shpilsky GF, et al. Multiple BCG vaccinations for the prevention of COVID-19 and other infectious diseases in type 1 diabetes. *Cell Rep Med* (2022) 3(9):100728. doi: 10.1016/j.xcrm.2022.100728

26. Koekenbier EL, Fohse K, van de Maat JS, Oosterheert JJ, van Nieuwkoop C, Hoogerwerf JJ, et al. Bacillus Calmette-Guerin vaccine for prevention of COVID-19 and other respiratory tract infections in older adults with comorbidities: a randomized controlled trial. *Clin Microbiol Infect* (2023) 29:781-8. doi: 10.1016/j.cmi.2023.01.019

27. Santos AP, Werneck GL, Dalvi APR, Dos Santos CC, Tierno P, Condelo HS, et al. The effect of BCG vaccination on infection and antibody levels against SARS-CoV-2-The results of ProBCG: a multicenter randomized clinical trial in Brazil. *Int J Infect Dis* (2023) 130:8–16. doi: 10.1016/j.ijid.2023.02.014

28. Dos Anjos LRB, da Costa AC, Cardoso A, Guimaraes RA, Rodrigues RL, Ribeiro KM, et al. Efficacy and safety of BCG revaccination with M. bovis BCG moscow to prevent COVID-19 infection in health care workers: A randomized phase II clinical trial. *Front Immunol* (2022) 13:841868. doi: 10.3389/fimmu.2022.841868

29. Sinha S, Ajayababu A, Thukral H, Gupta S, Guha SK, Basu A, et al. Efficacy of bacillus calmette-guerin (BCG) vaccination in reducing the incidence and severity of COVID-19 in high-risk population (BRIC): a phase III, multi-centre, quadruple-blind randomised control trial. *Infect Dis Ther* (2022) 11(6):2205–17. doi: 10.1007/s40121-022-00703-y

30. Angelidou A, Conti MG, Diray-Arce J, Benn CS, Shann F, Netea MG, et al. Licensed Bacille Calmette-Guerin (BCG) formulations differ markedly in bacterial viability, RNA content and innate immune activation. *Vaccine* (2020) 38(9):2229–40. doi: 10.1016/j.vaccine.2019.11.060

31. Ritz N, Dutta B, Donath S, Casalaz D, Connell TG, Tebruegge M, et al. The influence of bacille Calmette-Guerin vaccine strain on the immune response against tuberculosis: a randomized trial. *Am J Respir Crit Care Med* (2012) 185(2):213–22. doi: 10.1164/rccm.201104-0714OC

32. Singh S, Maurya RP, Singh RK. "Trained immunity" from Mycobacterium spp. exposure or BCG vaccination and COVID-19 outcomes. *PloS Pathog* (2020) 16(10): e1008969. doi: 10.1371/journal.ppat.1008969

33. Hilligan KL, Namasivayam S, Clancy CS, O'Mard D, Oland SD, Robertson SJ, et al. Intravenous administration of BCG protects mice against lethal SARS-CoV-2 challenge. *J Exp Med* (2022) 219(2):e20211862. doi: 10.1084/jem.20211862

34. Hilligan KL, Namasivayam S, Clancy CS, Baker PJ, Old SI, Peluf V, et al. Pre-existing interferon gamma conditions the lung to mediate early control of SARS-CoV-2. *bioRxiv* (2023) 2023:07.15.549135. doi: 10.1101/2023.07.15.549135

35. Kaufmann E, Khan N, Tran KA, Ulndreaj A, Pernet E, Fontes G, et al. BCG vaccination provides protection against IAV but not SARS-CoV-2. *Cell Rep* (2022) 38 (10):110502. doi: 10.1016/j.celrep.2022.110502

36. Counoupas C, Johansen MD, Stella AO, Nguyen DH, Ferguson AL, Aggarwal A, et al. A single dose, BCG-adjuvanted COVID-19 vaccine provides sterilising immunity against SARS-CoV-2 infection. *NPJ Vaccines* (2021) 6(1):143. doi: 10.1038/s41541-021-00406-4

37. Hildebrand RE, Chandrasekar SS, Riel M, Touray BJB, Aschenbroich SA, Talaat AM. Superinfection with SARS-CoV-2 Has Deleterious Effects on Mycobacterium bovis BCG Immunity and Promotes Dissemination of Mycobacterium tuberculosis. *Microbiol Spectr* (2022) 10(5):e0307522. doi: 10.1128/spectrum.03075-22

38. Singh AK, Wang R, Lombardo KA, Praharaj M, Bullen CK, Um P, et al. Dynamic single-cell RNA sequencing reveals BCG vaccination curtails SARS-CoV-2 induced disease severity and lung inflammation. *bioRxiv* (2022). doi: 10.1101/2022.03.15.484018

39. White AD, Sibley L, Sarfas C, Morrison AL, Bewley K, Churchward C, et al. Influence of aerosol delivered BCG vaccination on immunological and disease parameters following SARS-coV-2 challenge in rhesus macaques. *Front Immunol* (2021) 12:801799. doi: 10.3389/fimmu.2021.801799

40. Zhang BZ, Shuai H, Gong HR, Hu JC, Yan B, Yuen TT, et al. Bacillus Calmette-Guerin-induced trained immunity protects against SARS-CoV-2 challenge in K18-hACE2 mice. *JCI Insight* (2022) 7(11):e157393. doi: 10.1172/jci.insight.157393

41. Lee A, Floyd K, Wu S-Y, Fang Z, Tan TK, Li C, et al. Integrated Organ Immunity: Antigen-specific CD4-T cell-derived IFN-γ induced by BCG imprints prolonged lung innate resistance against respiratory viruses. *bioRxiv* (2023) 2023:07.31.551354. doi: 10.1101/2023.07.31.551354

42. Zhang L, Ru HW, Chen FZ, Jin CY, Sun RF, Fan XY, et al. Variable virulence and efficacy of BCG vaccine strains in mice and correlation with genome polymorphisms. *Mol Ther* (2016) 24(2):398–405. doi: 10.1038/mt.2015.216

43. Rosas Mejia O, Gloag ES, Li J, Ruane-Foster M, Claeys TA, Farkas D, et al. Mice infected with Mycobacterium tuberculosis are resistant to acute disease caused by secondary infection with SARS-CoV-2. *PloS Pathog* (2022) 18(3):e1010093. doi: 10.1371/journal.ppat.1010093

44. Baker PJ, Amaral EP, Castro E, Bohrer AC, Torres-Juarez F, Jordan CM, et al. Co-infection of mice with SARS-CoV-2 and Mycobacterium tuberculosis limits early viral replication but does not affect mycobacterial loads. *Front Immunol* (2023) 14. doi: 10.3389/fimmu.2023.1240419

45. Major J, Crotta S, Llorian M, McCabe TM, Gad HH, Priestnall SL, et al. Type I and III interferons disrupt lung epithelial repair during recovery from viral infection. *Science* (2020) 369(6504):712–7. doi: 10.1126/science.abc2061

46. Broggi A, Ghosh S, Sposito B, Spreafico R, Balzarini F, Lo Cascio A, et al. Type III interferons disrupt the lung epithelial barrier upon viral recognition. *Science* (2020) 369 (6504):706–12. doi: 10.1126/science.abc3545

47. Dellagostin OA, Borsuk S, Oliveira TL, Seixas FK. Auxotrophic mycobacterium bovis BCG: updates and perspectives. *Vaccines (Basel)* (2022) 10(5):802. doi: 10.3390/vaccines10050802

48. Humphries F, Shmuel-Galia L, Jiang Z, Wilson R, Landis P, Ng SL, et al. A diamidobenzimidazole STING agonist protects against SARS-CoV-2 infection. *Sci Immunol* (2021) 6(59):eabi9002. doi: 10.1126/sciimmunol.abi9002

49. Li M, Ferretti M, Ying B, Descamps H, Lee E, Dittmar M, et al. Pharmacological activation of STING blocks SARS-CoV-2 infection. *Sci Immunol* (2021) 6(59):eabi9007. doi: 10.1126/sciimmunol.abi9007

50. Tamir H, Melamed S, Erez N, Politi B, Yahalom-Ronen Y, Achdout H, et al. Induction of innate immune response by TLR3 agonist protects mice against SARS-coV-2 infection. *Viruses* (2022) 14(2):189. doi: 10.3390/v14020189

51. Oyesola OO, Hilligan KL, Namasivayam S, Howard N, Clancy CS, Zhao M, et al. Exposure to lung-migrating helminth protects against murine SARS-CoV-2 infection through macrophage-dependent T cell activation. *Sci Immunol* (2023) 8(86):eadf8161. doi: 10.1126/sciimmunol.adf8161

52. Netea MG, Ziogas A, Benn CS, Giamarellos-Bourboulis EJ, Joosten LAB, Arditi M, et al. The role of trained immunity in COVID-19: Lessons for the next pandemic. *Cell Host Microbe* (2023) 31(6):890–901. doi: 10.1016/j.chom.2023.05.004

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Immune interaction between SARS-CoV-2 and *Mycobacterium tuberculosis*

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SARS-CoV-2 and *Mycobacterium tuberculosis* (*Mtb*) are major infectious causes of death, with meta-analyses and population-based studies finding increased mortality in co-infected patients simultaneously diagnosed with COVID-19 and tuberculosis (TB). There is a need to understand the immune interaction between SARS-CoV-2 and *Mtb* which impacts poor outcomes for those co-infected. We performed a PubMed and preprint search using keywords [SARS-CoV-2] AND [tuberculosis] AND [Immune response], including publications after January 2020, excluding reviews or opinions. Abstracts were evaluated by authors for inclusion of data specifically investigating the innate and/or acquired immune responses to SARS-CoV-2 and *Mtb* in humans and animal models, immunopathological responses in co-infection and both trials and investigations of potential protection against SARS-CoV-2 by *Bacille Calmette Guérin* (BCG). Of the 248 articles identified, 39 were included. Incidence of co-infection is discussed, considering in areas with a high burden of TB, where reported co-infection is likely underestimated. We evaluated evidence of the clinical association between COVID-19 and TB, discuss differences and similarities in immune responses in humans and in murine studies, and the implications of co-infection. SARS-CoV-2 and *Mtb* have both been shown to modulate immune responses, particularly of monocytes, macrophages, neutrophils, and T cells. Co-infection may result in impaired immunity to SARS-CoV-2, with an exacerbated inflammatory response, while T cell responses to *Mtb* may be modulated by SARS-CoV-2. Furthermore, there has been no proven potential COVID-19 clinical benefit of BCG despite numerous large-scale clinical trials.

KEYWORDS

COVID-19, latent TB, LTBI, *Bacille Calmette Guérin*, co-infection, immune response, transcriptomics, T cells

Introduction

Tuberculosis (TB) and coronavirus disease 2019 (COVID-19) are leading causes of infectious death worldwide (1). As of 14 June 2023, there have been 6,943,390 COVID-19 deaths reported to World Health Organisation (WHO) (2). During the same three-year period, approximately 4.5 million people are estimated to have died of TB. The causative agent of COVID-19, SARS-CoV-2 has undergone various mutations since the start of its pandemic, with several major variants of concern arising and resulting in distinct waves of new infections globally. Since the emergence of the Omicron B.1.1.529 variant, with its attributes of increased transmissibility and reduced risk of mortality, coincident with increasing global vaccine coverage, SARS-CoV-2's contribution to hospital admissions and overall mortality has been in decline worldwide (3). Notwithstanding COVID-19 remains a highly significant cause of death, TB has again become the leading single infectious cause of death in 2023.

Several recent accounts have shown a detrimental effect of SARS-CoV-2 on TB prevention and care, associating with an increase in reported deaths from TB, a significant decrease in the diagnosis and treatment of TB cases, and diversion of resources allocated for essential TB services and research (4–6). This has now led to a global call to re-establish essential TB services in the wake of widespread disruptions caused by the COVID-19 pandemic.

There are clinical similarities between COVID-19 and TB. Both present predominantly with respiratory signs and symptoms, yet both can also have significant extrapulmonary manifestations (7). Disease severity is greatly influenced by host factors and co-morbidities such as diabetes mellitus, male sex, and HIV-1 co-infection. The purpose of our review was to evaluate clinical evidence of interaction between SARS-CoV-2 and *Mycobacterium tuberculosis* (*Mtb*) to determine if co-infection worsens the presentation and outcome of either disease. In addition, we focussed on evidence of potentially adverse immune interaction between the infections that may contribute to worse outcomes for those co-infected.

Method

We performed a PubMed search using keywords [SARS-CoV-2] AND [tuberculosis] AND [Immune response], including publications after January 2020, and MedRxiv search to include preprints. This search delivered 248 publications, which was reduced to 107 abstracts, based on presentation of original clinical, epidemiological, or experimental data, excluding most reviews and viewpoints. Authors evaluated abstracts to include data specifically investigating co-infection prevalence, the impact, acquired and innate immune responses with SARS-CoV-2 and *Mtb* in humans and animals, immunopathological responses in co-infection and/or trials and investigations of potential BCG protection against SARS-CoV-2. Of the 248 articles identified, 107 abstracts were evaluated and 39 were included (See Supplementary Text 1 for list of articles used in this review).

Results and discussion

Impact of TB on COVID-19 outcomes and vice versa

The COVID-19 pandemic caused global disruptions to health services, with well documented negative impacts on *Mtb* infected patients and TB-related services, not limited to reduced reporting of active TB cases, difficulty in adequate access to healthcare and health services being overwhelmed by acute COVID-19 cases (1, 4, 5). Co-infection is reported globally with several studies pointing towards increased risk of mortality for co-infected individuals, however studies from high- and low-income countries appear to reflect a marked difference in outcomes (8–12).

Early observational studies of SARS-CoV-2 and *Mtb* co-infected patients did not suggest TB was a major contributor to increased risk of death in COVID-19 patients, but rather suggested that SARS-CoV-2 infection contributed to a worsening of TB prognosis and/or TB-related death (13, 14). These studies originated from high-income countries with small sample sizes.

Motta et al. (14) reviewed eight cases of co-infected patients in high income countries that died and found SARS-CoV-2 co-infection worsened the prognosis of TB patients and contributed to mortality, with most patients who died acquiring nosocomial SARS-CoV-2 infection. Conversely, an early observational study from China found that patients with asymptomatic latent TB infection (LTBI) or symptomatic active TB were not only potentially more susceptible to SARS-CoV-2 infection, but COVID-19 disease may also progress more rapidly and be more severe in these individuals (15). Although this study was small with only 13 SARS-CoV-2/*Mtb* co-infected cases, these findings were later supported by large studies from Africa performed in settings of high prevalence of HIV/TB co-infection. These studies surmised that current and previous TB associated with increased COVID-19-related death and were an independent risk factor for mortality (8, 12, 16).

A recent meta-analysis examined the impact of TB on COVID-19 severity and found that overall, COVID-19 patients with TB tended to have an increased risk for more severe disease compared to those without TB (OR = 1.56, 95% CI: 1.13–2.16) (17). As most of the included studies were from Asia, especially from China, the potential generalisability of the findings could be determined through further meta-analyses.

Occurrence of co-infection

Dual presentation was extensively reported early in the COVID-19 pandemic, with TB and COVID-19 co-diagnosis rates ranging between one to four percent (12) although this may be an under ascertainment. A recent evaluation of confirmed co-infected cases reported the prevalence of TB in confirmed COVID-19 patients was 1.1% higher than most reported prevalence in Africa and Asia (18).

Underreporting of SARS-CoV-2 infection, specifically from countries in Africa and other low-income countries, is highly plausible. A study from Zambia identified significant underreporting of COVID-19-related deaths by post-mortem testing of patients (9). They found that most cases died whilst living in the community, where testing facilities were sparse, as opposed to in-hospital deaths. There was evidence of insufficient testing even in hospitals and, despite patient symptoms suggestive of typical COVID-19 disease, SARS-CoV-2 infection was not confirmed (9). Challenges with COVID-19 diagnostic testing and data are not unique to Zambia and have contributed to underreporting in several other African countries. Bradshaw et al. analysed the reported excess deaths data in South Africa during the COVID-19 pandemic and found a near 3-fold increase in excess death from natural causes within timelines corresponding to the peaks of SARS-CoV-2 infection rates, suggesting there was considerable underreporting of SARS-CoV-2 associated deaths (19).

With significant underreporting of SARS-CoV-2 infection in countries with a high TB burden, and decreased reporting of active TB cases in 2020 and 2021 (1), co-infection may also have been far more common than reported. A recent observational study examined the clinical presentation of COVID-19 in an African setting, describing the impact TB and/or HIV-1 infection had on patients admitted with COVID-19 (16). This study included 104 adults, of which 14% had active TB and found clinical features suggestive of either COVID-19 or TB. Chest X-rays in patients with confirmed co-infection were more likely to be classified as non-COVID-19 like, irrespective of HIV status, with a small number having radiological features predominantly suggestive of TB. Although the risk of death due to SARS-CoV-2 infection could not be specifically evaluated, 30/104 (29%) enrolled COVID-19 patients died and 6/15 (40%) of those were co-diagnosed with TB (16).

This study highlighted an important clinical lesson, emphasising that co-infection should be investigated in patients with typical TB presentation in settings with high prevalence of TB (16). This sentiment is echoed by numerous studies reporting similar presentation of signs and symptoms consistent with co-infection across various settings (10, 20) [Summarised in Table 1].

Similarities and differences in the immune response to *Mtb* and SARS-CoV-2

Both SARS-CoV-2 and *Mtb* are inhaled as a consequence of infectious aerosols and droplets produced by an infected person. In the case of *Mtb*, a spectrum of host immunological responses, both innate and acquired, with or without T cell priming either clear the mycobacteria or result in an established *Mtb* infection. Risk and incidence of infection and disease progression vary greatly depending on population demographics, co-morbidities and environmental factors (21). To establish infection, *Mtb* must overcome the robust physical barriers of the airway, to reach the lung where alveolar macrophages, neutrophils and dendritic cells are infected, activated and subsequently recruit innate and adaptive lymphocyte populations to aid bacterial containment (22).

TABLE 1 Clinical studies of TB and COVID-19 co-diagnosis.

	Du Bruyn et al. (16)	Tadolini et al. (20)	Stochino et al. (10)	Yu Chen et al. (15)
Country income	Low	High	High	Middle
Co-infected cohort	15 (Active TB)	49 (Active TB)	20 (Active TB)	13 (IGRA +)
Signs and symptoms	Either suggestive of COVID-19 or TB	- Fever 32/48 - Dry cough 27/48 - Dyspnoea 17/48	- Fever 12/20 - Cough 9/20 - Dyspnoea 3/20 - None 3/20	More rapid development of symptoms in co-infection
Chest radiographic features	- 6/14 Classic COVID-19 - 5/14 non-COVID-19-like - 3/14 Indeterminate	- Typical COVID-19 in 21/49 - TB-related lesions in 23/49	Majority showed no radiological signs of COVID-19 (16/20)	TB calcification in 3/13
Lymphopenia	Exacerbated	N/A	13/20	N/A
Inflammatory markers	Highest WCC in co-infected patients compared to COVID-19 alone. Lowest lymphocyte counts in patients with TB, HIV and COVID-19.	N/A	19/20 D-dimer >250 (5/20 >2000) 11/20 raised ferritin	N/A
Time from TB diagnosis to SARS-CoV-2 detection	Majority (9/15) were simultaneous. (Within 5 days)	Variable, SARS-CoV-2 preceded TB in 14/49 cases	Median time: 30 days	TB diagnosed retrospectively in confirmed COVID-19 patients
Conclusion	1. TB should be suspected in all COVID-19 patients at hospital admission. 2. TB may negatively impact the immune response to SARS-CoV-2, specifically in relation to antibody and T-cell responses	COVID-19 impact on TB pathogenesis not established.	Modest impact of COVID-19 on active TB	*Mtb* infection might increase susceptibility to SARS-CoV-2, with increased risk of severity.

Interferon-γ (IFN-γ) activation of alveolar macrophages is the central component of the immune response to *Mtb* infection. Activation of autophagy results in phagosome maturation and an increase in its acidification which leads to *Mtb* killing and is a fundamental process *Mtb* inhibits to maintain its infectious niche (23). Natural killer (NK) cells play a role by recognising and lysing *Mtb* infected macrophages, increasing IFN-γ production and further secreting cytokines to enhance recruitment of CD8+ T cells and NK T cells. This contributes to the characteristic granuloma formation, consisting of macrophages, neutrophils, Langhans epithelioid giant cells and those formed by fusion of macrophages, surrounded by lymphocytes and a fibrotic cuff (21). Alveolar macrophages use MHC class II molecules to present antigens to CD4+ T cells that are on the outer border of the granuloma, increasing cytokine secretion - notably IFN-γ and tumour necrosis factor (TNF). This will further activate the innate immune response and assist with T cell differentiation and other lymphocyte responses (21, 23). Granuloma morphology and fate are crucial determinants of infection outcome.

SARS-CoV-2 causes an acute infection, with most patients developing symptoms within five to six days after exposure. It predominantly affects the respiratory system; however other organ systems can also be involved. Clinical presentation varies from asymptomatic to severe disease, with symptoms generally being non-specific and includes coughing, fever, headache, and myalgia. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) receptors to enter target cells. ACE2 can be found in multiple cells, more specifically in lung epithelium, enterocytes, renal and myocardial cells, and oral mucosal epithelium (24).

Whilst ACE2 was first identified as the cell surface receptor for SARS-CoV-2 infection, L-SIGN and DC-SIGN C-type lectins receptors present on various phagocytes and Glucose-regulated protein 78 (GRP78) which translocate to the membrane can also recognise SARS-CoV-2. Binding to receptors is facilitated by proteolytic activation of SARS-CoV-2 S protein by furin-like proteases, transmembrane protease, serine 2 (TMPRSS2) and cathepsin L, whilst viral endocytosis is mediated by clatherin (25–30). Once intracellular, immune cells trigger signalling cascades either by direct endosomal TLR recognition of viral single-stranded (ss)RNA in cells such as plasmacytoid dendritic cells or cytosolic sensing of double-stranded (ds)RNA during viral replication (31). The signalling cascade that results from this recognition triggers transcription factor activation and the production of type I and III IFN and other pro-inflammatory cytokines and chemokines. However, the virus is adept at subverting host IFN responses, leading to lower levels of these cytokines, particularly during severe COVID-19 (32). Type I IFN pathway is important for antiviral responses, and it also plays a key role in TB. Our search, however, did not reveal studies that had investigated this in depth and this important interplay should form the basis for future research.

Alveolar macrophages play a critical role in responding to SARS-CoV-2 in the lungs, but single-cell and spatial transcriptomic studies of BALF and post-mortem lung samples identified depletion of this cell type in the lungs of severe COVID-19 patients as a contributing factor to immunopathology (33). Single cell RNA sequencing (scRNA-seq) has also revealed that profound dysregulation of myeloid cells, specifically increased circulation of various neutrophil subsets, including immature low density neutrophils, immature monocytes or progenitor cells, and myeloid-derived suppressor cells as hallmarks of severe COVID-19, through their contribution to creating an inflammatory cytokines storm (34–37). NK cells exert antiviral activity by clearing infected cells in response to signalling events triggered by SARS-CoV-2 recognition (38).

Clinical markers of COVID-19 deterioration and acute respiratory distress syndrome (ARDS) include elevated lactate dehydrogenase (LDH), C-reactive protein (CRP), interleukin-6 (IL-6), D-dimer, white cell count (WCC), high-sensitivity troponin I, platelet count and renal markers (39). Significant lymphopenia and neutrophilia, creating an elevated neutrophil: lymphocyte ratio is found in critically ill patients (40, 41); a marker not normally associated with viral infection but also associated with severe TB (42). Specific plasma markers: IL-1β, IL-1RA, IL-7, IL-8, IL-9, IL-10, basic FGF, G-CSF, GM-CSF, IFN-γ, CXCL10, CCL2, CCL3, CCL4, PDGF, TNF, and VEGF, show an increased presence in both ICU and non-ICU patients when compared with healthy individuals (43). ICU-admitted patients can also show increased concentrations of G-CSF, CXCL10, CCL2, CCL3, and TNF, hallmarks of the "cytokine storm" associated with COVID-19 disease severity (43).

Having noted an unusual spike in indeterminate *Mtb* IFN-γ release assay (IGRA) results in their facility, Ward et al. subsequently investigated confirmed SARS-CoV-2-positive hospitalised patients and IFN-γ production. Indeterminate QuantiFERON-TB Gold Plus results in COVID-19 patients, indicative of T cell anergy (positive control PHA-induced IFN-γ production below threshold) seemed to have decreased survival, with higher serum IL-6 and IL-10 levels, however these differences were not statistically significant (44). They also established that this decrease in IFN-γ was not related to lymphopenia or immunosuppressive therapy.

Impact of *Mtb* and SARS-CoV-2 co-infection on reciprocal immune memory and innate immune responses

Using a rapid, simplified whole blood-based multiparameter assay to quantify and phenotype SARS-CoV-2-specific T cells, Riou et al. examined SARS-CoV-2 antigen-specific CD4+ T cell responses in relation to disease severity in 95 hospitalised COVID-19 patients in South Africa, 38 of whom were HIV and/or *Mtb* co-infected (45). They found the attributes of SARS-CoV-2-specific CD4+ T cells, and not necessarily the magnitude, were associated with disease severity, characterised by reduced proliferation capacity, and enhanced HLA-DR expression, poor polyfunctional potential and increased proportions of TNF-single positive cells. On the contrary, in non-COVID-19 comparator patients, most SARS-CoV-2-reactive CD4+ T cells were distributed among triple functional cells (IL2+IFN-γ+TNF+) and cells co-producing IFN-γ and TNF.

In the same study, CD4+ T cell depletion resulting from HIV infection, related to suboptimal T cell and humoral immune SARS-CoV-2 responses. In their HIV/TB co-infected COVID-19 cohort consisting of eight patients, only three patients had an antibody response to SARS-CoV-2, and only two had a detectable CD4+ T cell response. Total CD4+ T cell frequency was much higher in SARS-CoV-2 responders compared to non-responders. Furthermore, in the HIV+ cohort, the frequency of total CD4+ T cells was associated with the magnitude of SARS-CoV-2-specific CD4+ T cells. These data suggest that lymphopenia impairs the SARS-CoV-2-specific immune response (45).

When considering the impact of COVID-19 on *Mtb*-specific responses, it was shown that patients with COVID-19 had a significant 5-fold reduction in the frequency of *Mtb*-specific CD4+ T cells compared with healthy pre-pandemic LTBI controls, and 2-fold reduction in COVID-19/HIV+ patients compared to HIV+ pre-pandemic controls. As an intact T cell response is essential to control *Mtb* infection, a decline in *Mtb*-specific CD4+ T cells could therefore affect the ability of the host to control either existing latent or new *Mtb* infection (45). *Mtb*-specific CD4+ T cell activation, previously shown to distinguish active and subclinical TB from those with latent infection, was also found to have a trend towards higher activation in COVID-19/TB patients compared to TB patients without COVID-19, whilst there was no elevation in *Mtb*-specific CD4+ T cells in COVID-19 patients not co-presenting with TB. Together, this suggests that whilst acute COVID-19 does not immediately reactivate LTBI to subclinical/active disease, it contributes to greater *Mtb*-specific T cell activation which may exacerbate existing subclinical/active disease.

Looking further into the interaction with LTBI, Rajamanickam et al. (46) examined seropositive, asymptomatic SARS-CoV-2-infected individuals in India and compared immune responses in IGRA-positive (LTBI) and -negative individuals. They showed IGRA-positive individuals had higher levels of humoral, cytokine and acute phase responses compared to IGRA-negative individuals, and thus concluded that LTBI could significantly affect systemic inflammation, as well as cytokine responses and enhanced neutralising antibody capacity in SARS-CoV-2-infected individuals (46). The same investigators also evaluated the effect of SARS-CoV-2 seropositivity on antigen-specific cytokine and chemokine responses in LTBI using QuantiFERON Gold In-tube assay plasma (47). They showed that SARS-CoV-2 seropositive individuals with LTBI had increased cytokine concentrations in both unstimulated and *Mtb* antigen-stimulated tubes, when compared to those who were SARS-CoV-2 seronegative. These differences were not observed in IGRA-negative individuals who were SARS-CoV-2 seropositive. The authors conclude that both baseline and *Mtb* antigen-induced cytokine responses are augmented by SARS-CoV-2 sensitisation, suggesting prior SARS-CoV-2 infection augments the immune response to *Mtb* in LTBI (47).

A highly cited study by Petrone et al. (48) concluded that active TB disease can negatively affect a patient's ability to generate a SARS-CoV-2-specific immune response, by looking specifically at T cell IFN-γ production in their cohort of co-infected participants. Whole-blood from TB/COVID-19 patients showed the lowest IFN-γ secretion in response to SARS-CoV-2 peptide stimulation compared with COVID-19 patients and to LTBI/COVID-19 patients. They showed that COVID-19 patients with either latent or active TB, still had the ability to respond to *Mtb*-specific antigens. However only 20% of active TB patients with COVID-19 had a positive response, compared to 64% of COVID-19 patients with LTBI, indicating that active TB depresses the COVID-19-specific host immune response (48), supporting the finding by Riou et al. in COVID-19 with TB/HIV.

A study by Najafi-Fard et al. (49) looked at 119 study participants and compared the plasma immune profile of the 14 TB/COVID-19 co-infected cohort, to the COVID-19 only patients, TB only patients, or 20 healthy controls using a 27-plex multiplex assay. They found that levels of circulating TNF had the strongest association with TB/COVID-19 co-infection compared with COVID-19. They also found that co-infected patients showed a reduced SARS-CoV-2-specific response for several pro-inflammatory cytokines and/or chemokines, anti-inflammatory cytokines, and growth factors and that co-infection negatively affected the *Mtb*-specific response (49).

Overall, these results (summarised in Table 2), indicate that T cell responses to SARS-CoV-2 and *Mtb* are both dysregulated by each co-infecting pathogen, resulting in decreased defensive capabilities against both *Mtb* and HIV-1 in COVID-19 patients, potentially contributing to more unfavourable outcomes and higher mortality in some cases.

Sheerin et al. (50) assessed transcriptional overlap between host immune responses to TB and COVID-19 by profiling scRNA-seq immune cell and severity signatures on bulk RNA-seq data from TB patients across the spectrum of disease, generating "disease risk scores" based on the enrichment of each signature. This analysis indicated that the highest disease risk scores in TB patients were associated with monocyte and neutrophil signatures from severe COVID-19 patients. By summarising gene expression changes at the immunological pathway level for TB, COVID-19 and influenza (as a control for other forms of respiratory infection), it was also shown that IFN-γ and TNF signalling was similarly enriched in COVID-19 and TB patients, but not influenza. Finally, they validated the detrimental interaction between COVID-19 and TB on innate immune cells by comparing the impact of co-culturing human monocyte-derived macrophages (MDM) in the inflammatory milieu from *Mtb* infected MDM on MDM susceptibility to SARS-CoV-2 infection and inflammatory response. They found co-cultured MDM were more susceptible to SARS-CoV-2 infection and more pro-inflammatory, with increased IFN-α, IFN-γ, TNF, IL-1β and TMPRSS2 expression.

This analysis of blood transcriptional responses from patients and asymptomatic infected persons was followed up by a more thorough exploration of direct co-infection of blood using scRNA-seq; Sheerin et al. (51) infected whole blood from healthy COVID-19 vaccinated donors *ex vivo* with *Mtb*, SARS-CoV-2, or both pathogens simultaneously and quantified single cell transcriptome

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TABLE 2 Immunological response interactions to *Mtb* and SARS-CoV-2 in co-infected persons.

Study (reference)	*Mtb* infection effect on SARS-CoV-2 specific immune responses			SARS-CoV-2 effect on *Mtb*-specific immune responses		Other findings
Riou et al. (45)	Patients co-infected with HIV and active TB showed less capacity to form SARS-CoV-2 antibodies – however this was not associated with increased mortality in their cohort.	Active TB co-infection changed the functional abilities of SARS-CoV-2–specific CD4+ T cells and caused a reduction of their polyfunctional abilities.	HIV or TB co-infection had minimal impact on the memory and activation profile of SARS-CoV-2 specific CD4+ T cells.	Patients with confirmed SARS-CoV-2 had a reduction in *Mtb*-specific CD4+ T cell responses.	Less severe disease showed improved capacity of SARS-CoV-2–specific CD4+ T cells to co-express IFN-γ, TNF, and IL-2.	Patients with pre-existing lymphopenia showed an impaired immune response to SARS-CoV-2.
Petrone et al. (48)	TB-COVID-19 patients showed the lowest quantitative IFN-γ response to CD4-S* compared to COVID-19 patients and LTBI** - COVID-19 patients.	A positive CD4-S response was found in 55.6% COVID-19-patients and 63.6% LTBI -COVID-19-patients as opposed to only 20% of active TB-COVID-19-patients.	Active TB depresses the COVID specific response: 20% TB-COVID-19-patients had a positive response, vs 63.6% LTBI-COVID-19-patients.	The IFN-γ response to *Mtb*-antigens was higher in active TB and latent TB co-infected COVID-19 patients, when compared to COVID-19 only patients.	COVID-19-patients, either with latent or active TB, retain the ability to respond to *Mtb*-specific antigens.	Cortisone treatment did not seem to have an impact on the ability to respond to SARS-CoV-2 antigens.
Rajamanickam et al. (46)	LTBI and SARS-CoV-2 co-infection was associated with higher levels of SARS-CoV-2 specific IgM, IgG and IgA antibodies.	Co-infected patients had enhanced neutralisation activity compared to SARS-CoV-2 positive patients with LTBI		Elevated plasma IFN-γ, IL-2, TNF, IL-1α, IL-1β, IL-6, IL-12, IL-15, IL-17, IL-3, GM-CSF, IL-10, IL-25, IL-33, CCL3 and CXCL10 in co-infected patients	Significantly higher levels of C-reactive protein, alpha-2 macroglobulin, VEGF and TGF-α	
Rajamanickam et al. (47)				LTBI +/IgG + *** had increased baseline levels of pro-inflammatory cytokines & chemokines, and altered levels of anti-inflammatory cytokines	LTBI +/IgG + had elevated TB- antigen stimulated levels of pro-inflammatory cytokines and chemokines, and altered levels of anti-inflammatory cytokines	No marked differences in mitogen stimulated levels of pro- and anti- inflammatory cytokines or chemokines
Najafi-Fard et al. (49)	Decreased SARS-CoV-2 specific immune responses in co-infected patients compared to COVID-19 alone, specifically IFN-y, CXCL10, CCL2, CCL3, CCL4, IL-1RA, IL-10	Co-infected patients had elevated TNF, CCL4, IL-9 compared to COVID-19 only		Patients with co-infection displayed a negative effect on their *Mtb*-specific responses	Co-infected patients had higher IL-1β, TNF, IL-17A. IL-5 compared to *Mtb* infection only.	Higher levels of TNF and IL-9 suggested co-infection and authors speculate it can help discriminate TB-COVID-19 from COVID-19 alone.

*CD4-S: peptide megapool consisting of 253 15-mers overlapping by 10 amino acids, spanning the entire spike protein of the Wuhan-Hu-1 strain.
**Latent Tuberculosis Infection (LTBI).
*** LTBI individuals with SARS-CoV-2 seropositivity.

changes, relative to uninfected control samples, across immune cells, 24 and 96 hours post-infection. Distinct neutrophil and monocyte clustering was observed between the three infection conditions. The strongest synergistic co-infection responses were associated with IFN-γ and TNF pathway enrichment 24 hours post-infection. SARS-CoV-2 infection, in the absence of *Mtb* infection, was associated with enrichment of extrinsic apoptotic signalling, which was negatively regulated by *Mtb* co-infection, resulting in enhanced cell survival in co-infected verses SARS-CoV-2-only infected cells. SARS-CoV-2 also showed unique enrichment of αβ T cell activation and differentiation not seen in *Mtb* infection.

TB vaccination with BCG and protection against SARS-CoV-2

The TB vaccine *Mycobacterium bovis* BCG is known to induce both cellular and humoral immunity in vaccinated individuals (52). The rationale for the potential beneficial effects of BCG in the context of SARS-CoV-2 infection was proposed to include protection via the induction and improved production of pro-inflammatory cytokines through "trained immunity" (53). BCG is thought to provide enhanced protection and/or vaccine responsiveness against a range of pathogens, including *Candida*

albicans, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, vaccinia virus, *Bordetella pertussis*, and yellow fever virus (54–56); this protection is provided primarily through enhancing monocyte and NK cell production of IL-6, IL-1β, TNF and IFN-γ, and cytokine-induced antigen-specific memory T and B cell activation. BCG enhances innate cytokine production to non-specific pathogens through epigenetic modification and chromatin relaxation at the promoters of these genes, facilitating faster and enhanced cytokine production (57).

There were several suggestions early in the COVID-19 pandemic of epidemiological evidence that prior BCG vaccination correlated with protection against COVID-19 (58), although the evidence became quite mixed as the pandemic progressed (59). Several randomised control trials (RCTs) were set up to test the efficacy of BCG to prevent or decrease the severity of COVID-19 but overall little evidence to support the use of BCG for this purpose has emerged. (A list of all BCG strains used in each of the references is provided in Supplementary Text 2).

A phase III multicentre RCT testing a genetically modified BCG vaccine VPM1002 suggested a prophylactic effect against the development of severe disease in the elderly (60). Another RCT in the elderly reported a reduced rate of new infections after vaccination with standard BCG (61), whereas a larger RCT in the elderly reported no effect on the incidence of disease but noted improved cytokine responses to viral infection (62). An RCT conducted in high-risk adults in India reported that standard BCG reduced the incidence and severity of COVID-19 (63), while a multi-dose BCG phase II/III in diabetic adults claimed an efficacy of 92% for preventing COVID-19 with this regimen (64). Most RCT were conducted in healthcare workers who were among those with the highest risk of exposure to and infection with SARS-CoV-2: an RCT in Brazil reported that re-vaccination with BCG Moscow did not lead to statistically significant reduction in COVID-19 incidence (65), while RCTs conducted in Poland (66), the Netherlands (67) and South Africa (68) also reported no benefit in healthcare workers. A study using samples collected from an Australian RCT investigating the BCG Denmark vaccine in healthcare workers preliminarily reported modulation of cytokines IL-6, TNF and IL-10 and CD4+ and CD8+ T cells upon *ex vivo* stimulation of PBMC, suggesting that this may protect against severe COVID-19 (69), but the same trial recently reported no prevention or reduction in severity of COVID-19 (70). A meta-analysis conducted using these trials revealed no decrease in incidence or hospitalisation from COVID-19 (71).

Experimental models of *Mtb* and SARS-CoV-2 co-infection and BCG vaccination

Animal studies evaluating immunological responses can contribute to our understanding of host-pathogen interactions and interactions between multiple pathogens within the same host. As summarised in Table 3, Rosas Meija et al. (72) studied mice and the effects of *Mtb* infection on the immune response to SARS-CoV-2. They used human *ACE2* transgenic mice that were chronically infected with *Mtb* and found these mice to be resistant to secondary infection with SARS-CoV-2. The authors speculated this might be due to the proinflammatory lung environments created by *Mtb* that are not conducive to SARS-CoV-2 proliferation. Furthermore, SARS-CoV-2 infection did not affect *Mtb* burden in their experiments.

Hilligan et al. (73) also studied human *ACE2* transgenic mice to demonstrate that intravenous, but not subcutaneous, inoculation with BCG protected them against lethal challenge with SARS-CoV-2, associated with reduced cytokine production, less tissue pathology and decreased inflammatory cell recruitment, and that was only partially due to the significantly reduced viral load. They speculated that this protection was associated with changes in the composition and function of the pulmonary cellular compartment, likely induced by BCG, providing an experimental model for understanding how a host's resistance might be promoted by non-specific stimulation of the pulmonary immune response. The protective benefits in this model are in contrast to the lack of clinical efficacy found in RCTs (71). Such discordance may suggest mouse models of *Mtb*/SARS-CoV-2 co-infection may not reflect the course

TABLE 3 Murine studies.

Name of study	Major findings	Specific findings
Rosas Mejia et al. (72)	Mice with *Mtb* infection were not susceptible to the consequences of SARS-CoV-2 disease.	*Mtb*-infected mice did not show an increased burden of TB in lung tissues, as well as no difference in liver or spleen after being challenged with SARS-CoV-2, when compared to mice who were SARS-CoV-2 negative.
Hiligan et al. (73)	Intravenous BCG injection protects mice against lethal challenges with SARS-CoV-2.	- Less tissue pathology - Decreased inflammatory cell, and cytokine production. (Not only due to associated reduced viral load)
Mambelli et al. (74)	Using rBCG expressing domains of SARS-CoV-2 nucleocapsid and spike proteins in mice, one dose of rBCG-ChD6 boosted with the recombinant nucleocapsid and spike chimera (rChimera) elicited the highest anti-Chimera total IgG and IgG2c Ab titres with neutralising activity against SARS-CoV-2, compared with control groups.	This vaccination regimen: - induced IFN-γ and IL-6 production in spleen cells - decreased viral load in lungs (after SARS-CoV-2 challenge) - No viable virus detected in mice - Decreased lung pathology when compared with control groups.

of human co-infection or could be due to differences in the route of BCG vaccination, as seen in the mouse study discussed above where only the IV route of BCG administration induced protection against a lethal dose of SARS-CoV-2. However, apart from the route of administration (intravenous vs subcutaneous), other factors such as the type of BCG strain or the genetic background of the mouse, might also contribute.

More recently, Mambelli et al. (74) constructed a recombinant BCG (rBCG) that expressed domains of the SARS-CoV-2 nucleocapsid and spike proteins (termed rBCG-ChD6). Using *ACE2* transgenic mice, they found that a single dose of rBCG-ChD6 boosted with the recombinant nucleocapsid and spike chimera (rChimera) adjuvanted with alum, resulted in the highest anti-Chimera total IgG and IgG2c Ab titres with neutralising activity against SARS-CoV-2 (specifically the Wuhan strain), compared to their control groups. Furthermore, following SARS-CoV-2 challenge, this vaccination regimen induced IFN-γ and IL-6 production in spleen cells and reduced viral load in the lungs. Moreover, no viable virus was detected in mice immunised with rBCG-ChD6 boosted with rChimera, which was associated with decreased lung pathology when compared with control groups. This study showed the possibility of a prime-boost immunisation system based on an rBCG expressing a chimeric protein derived from SARS-CoV-2.

Mouse models offer numerous useful immunological tools and can be genetically modified. Among mouse strains, the C3HeB/FeJ mouse is the only strain reproducing the pathophysiology of TB, with comparable granuloma encapsulation (75). Although not discussed here, other models, like hamsters and ferrets, and Non-Human Primates (NHP) are also incredibly useful when investigating human pathologies.

Conclusion and consequences

Diversion of healthcare services during the COVID-19 pandemic undoubtedly had an adverse effect on the ongoing TB epidemic. Acute COVID-19 and TB can be coincident and the occurrence of such co-infections in areas of high TB prevalence may have been underestimated. Previous or current TB is a risk factor for death from SARS-CoV-2. *Ex vivo* studies of blood cells in acutely infected humans suggest the T cell response to *Mtb* may be modulated by SARS-CoV-2: conversely coincident TB may impair immune responses to SARS-CoV-2 and exacerbate inflammatory responses through enhanced innate and adaptive immune activation. Despite animal studies and epidemiological evidence pointing to potential protection against SARS-CoV-2 by BCG, efficacy has not been borne out in several large-scale clinical evaluations. Further studies of the long-term consequences of SARS-CoV-2 infection on the immune response in, and outcome of latent TB are warranted.

Author contributions

PB: Conceptualisation, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. KW: Writing – original draft, Writing – review & editing, Investigation. DS: Writing – original draft, Writing – review & editing, Investigation. RW: Writing – review & editing, Investigation. AC: Writing – review & editing, Investigation. RJW: Conceptualisation, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1254206/full#supplementary-material

References

1. WHO. *Global tuberculosis report 2022* (2022). Available at: https://wwwwhoint/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022.

2. WHO. Available at: https://covid19.who.int/.

3. Hyams C, Challen R, Marlow R, Nguyen J, Begier E, Southern J, et al. Severity of Omicron (B.1.1.529) and Delta (B.1.617.2) SARS-CoV-2 infection among hospitalised adults: a prospective cohort study in Bristol, United Kingdom. *Lancet Regional Health - Europe* (2023) 25:100556. doi: 10.1016/j.lanepe.2022.100556

4. Pai M, Kasaeva T, Swaminathan S. Covid-19's devastating effect on tuberculosis care — A path to recovery. *N Engl J Med* (2022) 386(16):1490–3. doi: 10.1056/NEJMp2118145

5. Benade M, Long L, Meyer-Rath G, Miot J, Evans D, Tucker J-M, et al. Reduction in initiations of drug-sensitive tuberculosis treatment in South Africa during the COVID-19 pandemic: Analysis of retrospective, facility-level data. *PloS Global Public Health* (2022) 2(10):e0000559. doi: 10.1371/journal.pgph.0000559

6. Sanduzzi Zamparelli S, Mormile M, Sanduzzi Zamparelli A, Guarino A, Parrella R, Bocchino M. Clinical impact of COVID-19 on tuberculosis. *Infez Med* (2022) 30 (4):495–500. doi: 10.53854/liim-3004-3

7. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. *Nat Med* (2020) 26(7):1017–32. doi: 10.1038/s41591-020-0968-3

8. Jassat W, Cohen C, Tempia S, Masha M, Goldstein S, Kufa T, et al. Risk factors for COVID-19-related in-hospital mortality in a high HIV and tuberculosis prevalence setting in South Africa: a cohort study. *Lancet HIV* (2021) 8(9):e554–e67. doi: 10.1016/S2352-3018(21)00151-X

9. Mwananyanda L, Gill CJ, MacLeod W, Kwenda G, Pieciak R, Mupila Z, et al. Covid-19 deaths in Africa: prospective systematic postmortem surveillance study. *BMJ* (2021) 372:n334. doi: 10.1136/bmj.n334

10. Stochino C, Villa S, Zucchi P, Parravicini P, Gori A, Raviglione MC. Clinical characteristics of COVID-19 and active tuberculosis co-infection in an Italian reference hospital. *Eur Respir J* (2020) 56(1). doi: 10.1183/13993003.01708-2020

11. van der Zalm MM, Lishman J, Verhagen LM, Redfern A, Smit L, Barday M, et al. Clinical experience with severe acute respiratory syndrome coronavirus 2-related illness in children: hospital experience in Cape Town, South Africa. *Clin Infect Dis* (2021) 72(12):e938–e44. doi: 10.1093/cid/ciaa1666

12. Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases SA. Risk factors for coronavirus disease 2019 (COVID-19) death in a population cohort study from the western cape province, South Africa. *Clin Infect Dis* (2021) 73(7):e2005–e15. doi: 10.1093/cid/ciaa1198

13. Collins LF, Moran CA, Oliver NT, Moanna A, Lahiri CD, Colasanti JA, et al. Clinical characteristics, comorbidities and outcomes among persons with HIV hospitalized with coronavirus disease 2019 in Atlanta, Georgia. *AIDS* (2020) 34 (12):1789–94. doi: 10.1097/QAD.0000000000002632

14. Motta I, Centis R, D'Ambrosio L, Garcia-Garcia JM, Goletti D, Gualano G, et al. Tuberculosis, COVID-19 and migrants: Preliminary analysis of deaths occurring in 69 patients from two cohorts. *Pulmonology* (2020) 26(4):233–40. doi: 10.1016/j.pulmoe.2020.05.002

15. Chen Y, Wang Y, Fleming J, Yu Y, Gu Y, Liu C, et al. Active or latent tuberculosis increases susceptibility to COVID-19 and disease severity. *medRxiv* (2020) 2020:3.

16. du Bruyn E, Stek C, Daroowala R, Said-Hartley Q, Hsiao M, Schafer G, et al. Effects of tuberculosis and/or HIV-1 infection on COVID-19 presentation and immune response in Africa. *Nat Commun* (2023) 14(1):188. doi: 10.1038/s41467-022-35689-1

17. Wang Y, Feng R, Xu J, Hou H, Feng H, Yang H. An updated meta-analysis on the association between tuberculosis and COVID-19 severity and mortality. *J Med Virol* (2021) 93(10):5682–6. doi: 10.1002/jmv.27119

18. Daneshvar P, Hajikhani B, Sameni F, Noorisepehr N, Zare F, Bostanshirin N, et al. COVID-19 and tuberculosis coinfection: An overview of case reports/case series and meta-analysis of prevalence studies. *Heliyon* (2023) 9(2):e13637. doi: 10.1016/j.heliyon.2023.e13637

19. Bradshaw D, Dorrington RE, Laubscher R, Moultrie TA, Groenewald P. Tracking mortality in near to real time provides essential information about the impact of the COVID-19 pandemic in South Africa in 2020. *S Afr Med J* (2021) 111 (8):732–40. doi: 10.7196/SAMJ.2021.v111i8.15809

20. Tadolini M, Codecasa LR, Garcia-Garcia JM, Blanc FX, Borisov S, Alffenaar JW, et al. Active tuberculosis, sequelae and COVID-19 co-infection: first cohort of 49 cases. *Eur Respir J* (2020) 56(1). doi: 10.1183/13993003.02328-2020

21. Walker NF, Meintjes G, Wilkinson RJ. HIV-1 and the immune response to TB. *Future Virol* (2013) 8(1):57–80. doi: 10.2217/fvl.12.123

22. Lerner TR, Borel S, Gutierrez MG. The innate immune response in human tuberculosis. *Cell Microbiol* (2015) 17(9):1277–85. doi: 10.1111/cmi.12480

23. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. *Annu Rev Immunol* (2013) 31:475–527. doi: 10.1146/annurev-immunol-032712-095939

24. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. *Clin Immunol* (2020) 215:108427. doi: 10.1016/j.clim.2020.108427

25. Amraei R, Yin W, Napoleon MA, Suder EL, Berrigan J, Zhao Q, et al. CD209L/L-SIGN and CD209/DC-SIGN act as receptors for SARS-coV-2. *ACS Cent Sci* (2021) 7 (7):1156–65. doi: 10.1021/acscentsci.0c01537

26. Bayati A, Kumar R, Francis V, McPherson PS. SARS-CoV-2 infects cells after viral entry via clathrin-mediated endocytosis. *J Biol Chem* (2021) 296:100306. doi: 10.1016/j.jbc.2021.100306

27. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-coV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* (2020) 181(2):271–80 e8. doi: 10.1016/j.cell.2020.02.052

28. Zhao MM, Yang WL, Yang FY, Zhang L, Huang WJ, Hou W, et al. Cathepsin L plays a key role in SARS-CoV-2 infection in humans and humanized mice and is a promising target for new drug development. *Signal Transduct Target Ther* (2021) 6 (1):134. doi: 10.1038/s41392-021-00558-8

29. Ibrahim IM, Abdelmalek DH, Elshahat ME, Elfiky AA. COVID-19 spike-host cell receptor GRP78 binding site prediction. *J Infect* (2020) 80(5):554–62. doi: 10.1016/j.jinf.2020.02.026

30. Shin W-J, Ha DP, Machida K, Lee AS. The stress-inducible ER chaperone GRP78/BiP is upregulated during SARS-CoV-2 infection and acts as a pro-viral protein. *Nat Commun* (2022) 13(1):6551. doi: 10.1038/s41467-022-34065-3

31. Madden EA, Diamond MS. Host cell-intrinsic innate immune recognition of SARS-CoV-2. *Curr Opin Virol* (2022) 52:30–8. doi: 10.1016/j.coviro.2021.11.002

32. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* (2020) 369(6504):718–24. doi: 10.1126/science.abc6027

33. Delorey TM, Ziegler CGK, Heimberg G, Normand R, Yang Y, Segerstolpe Å, et al. COVID-19 tissue atlases reveal SARS-CoV-2 pathology and cellular targets. *Nature* (2021) 595(7865):107–13. doi: 10.1038/s41586-021-03570-8

34. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med* (2020) 26(6):842–4. doi: 10.1038/s41591-020-0901-9

35. Schulte-Schrepping J, Reusch N, Paclik D, Baßler K, Schlickeiser S, Zhang B, et al. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. *Cell* (2020) 182(6):1419–40.e23. doi: 10.1016/j.cell.2020.08.001

36. Carissimo G, Xu W, Kwok I, Abdad MY, Chan Y-H, Fong S-W, et al. Whole blood immunophenotyping uncovers immature neutrophil-to-VD2 T-cell ratio as an early marker for severe COVID-19. *Nat Commun* (2020) 11(1):5243. doi: 10.1038/s41467-020-19080-6

37. Wu D, Yang XO. TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib. *J Microbiol Immunol Infect* (2020) 53(3):368–70. doi: 10.1016/j.jmii.2020.03.005

38. Witkowski M, Tizian C, Ferreira-Gomes M, Niemeyer D, Jones TC, Heinrich F, et al. Untimely TGFβ responses in COVID-19 limit antiviral functions of NK cells. *Nature* (2021) 600(7888):295–301. doi: 10.1038/s41586-021-04142-6

39. Wu C, Chen X, Cai Y, Ja X, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Internal Med* (2020) 180(7):934–43. doi: 10.1001/jamainternmed.2020.0994

40. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang Y-Q, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct Target Ther* (2020) 5(1):33. doi: 10.1038/s41392-020-0148-4

41. Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, et al. Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. *J Transl Med* (2020) 18(1):206. doi: 10.1186/s12967-020-02374-0

42. Panteleev AV, Nikitina IY, Burmistrova IA, Kosmiadi GA, Radaeva TV, Amansahedov RB, et al. Severe tuberculosis in humans correlates best with neutrophil abundance and lymphocyte deficiency and does not correlate with antigen-specific CD4 T-cell response. *Front Immunol* (2017) 8. doi: 10.3389/fimmu.2017.00963

43. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* (2020) 395(10223):497–506. doi: 10.1016/S0140-6736(20)30183-5

44. Ward JD, Cornaby C, Schmitz JL. Indeterminate quantiFERON gold plus results reveal deficient interferon gamma responses in severely ill COVID-19 patients. *J Clin Microbiol* (2021) 59(10):e00811–21. doi: 10.1128/JCM.00811-21

45. Riou C, du Bruyn E, Stek C, Daroowala R, Goliath RT, Abrahams F, et al. Relationship of SARS-CoV-2-specific CD4 response to COVID-19 severity and impact of HIV-1 and tuberculosis coinfection. *J Clin Invest* (2021) 131(12). doi: 10.1172/JCI149125

46. Rajamanickam A, Kumar NP, Padmapriyadarsini C, Nancy A, Selvaraj N, Karunanithi K, et al. Latent tuberculosis co-infection is associated with heightened levels of humoral, cytokine and acute phase responses in seropositive SARS-CoV-2 infection. *J Infect* (2021) 83(3):339–46. doi: 10.1016/j.jinf.2021.07.029

47. Rajamanickam A, Pavan Kumar N, Chandrasekaran P, Nancy A, Bhavani PK, Selvaraj N, et al. Effect of SARS-CoV-2 seropositivity on antigen - specific cytokine and

chemokine responses in latent tuberculosis. *Cytokine* (2022) 150:155785. doi: 10.1016/j.cyto.2021.155785

48. Petrone L, Petruccioli E, Vanini V, Cuzzi G, Gualano G, Vittozzi P, et al. Coinfection of tuberculosis and COVID-19 limits the ability to in *vitro* respond to SARS-CoV-2. *Int J Infect Dis* (2021) 113 Suppl 1:S82–S7. doi: 10.1016/j.ijid.2021.02.090

49. Najafi-Fard S, Aiello A, Navarra A, Cuzzi G, Vanini V, Migliori GB, et al. Characterization of the immune impairment of patients with tuberculosis and COVID-19 coinfection. *Int J Infect Dis* (2023) 130(Suppl 1):S34–S42. doi: 10.1016/j.ijid.2023.03.021

50. Sheerin D, Abhimanyu, Peton N, Vo W, Allison CC, Wang X, et al. Immunopathogenic overlap between COVID-19 and tuberculosis identified from transcriptomic meta-analysis and human macrophage infection. *iScience* (2022) 25 (6):104464. doi: 10.1016/j.isci.2022.104464

51. Sheerin D, Phan TK, Eriksson EM, Consortium CP, Coussens AK. Distinct and overlapping immunological responses to SARS-CoV-2 and Mycobacterium tuberculosis identified by single-cell RNA-seq of co-infected whole blood. *medRxiv* (2023), 23290499. doi: 10.1101/2023.05.24.23290499

52. Dennehy M, Williamson A-L. Factors influencing the immune response to foreign antigen expressed in recombinant BCG vaccines. *Vaccine* (2005) 23(10):1209–24. doi: 10.1016/j.vaccine.2004.08.039

53. O'Neill LAJ, Netea MG. BCG-induced trained immunity: can it offer protection against COVID-19? *Nat Rev Immunol* (2020) 20(6):335–7. doi: 10.1038/s41577-020-0337-y

54. Moorlag SJCFM, Arts RJW, van Crevel R, Netea MG. Non-specific effects of BCG vaccine on viral infections. *Clin Microbiol Infect* (2019) 25(12):1473–8. doi: 10.1016/j.cmi.2019.04.020

55. Covián C, Fernández-Fierro A, Retamal-Díaz A, Díaz FE, Vasquez AE, Lay MK, et al. BCG-induced cross-protection and development of trained immunity: implication for vaccine design. *Front Immunol* (2019) 10. doi: 10.3389/fimmu.2019.02806

56. Arts RJW, Moorlag SJCFM, Novakovic B, Li Y, Wang S-Y, Oosting M, et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. *Cell Host Microbe* (2018) 23(1):89–100.e5. doi: 10.1016/j.chom.2017.12.010

57. Mantovani A, Netea MG. Trained innate immunity, epigenetics, and covid-19. *N Engl J Med* (2020) 383(11):1078–80. doi: 10.1056/NEJMcibr2011679

58. Escobar LE, Molina-Cruz A, Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). *Proc Natl Acad Sci* (2020) 117 (30):17720–6. doi: 10.1073/pnas.2008410117

59. Fu W, Ho P-C, Liu C-L, Tzeng K-T, Nayeem N, Moore JS, et al. Reconcile the debate over protective effects of BCG vaccine against COVID-19. *Sci Rep* (2021) 11 (1):8356. doi: 10.1038/s41598-021-87731-9

60. Blossey AM, Brückner S, May M, Parzmair GP, Sharma H, Shaligram U, et al. VPM1002 as prophylaxis against severe respiratory tract infections including coronavirus disease 2019 in the elderly: A phase 3 randomized, double-blind, placebo-controlled, multicenter clinical study. *Clin Infect Dis* (2022) 76(7):1304–10. doi: 10.1093/cid/ciac881

61. Tsilika M, Taks E, Dolianitis K, Kotsaki A, Leventogiannis K, Damoulari C, et al. ACTIVATE-2: A double-blind randomized trial of BCG vaccination against COVID-19 in individuals at risk. *Front Immunol* (2022) 13. doi: 10.3389/fimmu.2022.873067

62. Moorlag SJCFM, Taks E, ten Doesschate T, van der Vaart TW, Janssen AB, Müller L, et al. Efficacy of BCG vaccination against respiratory tract infections in older adults during the coronavirus disease 2019 pandemic. *Clin Infect Dis* (2022) 75(1): e938–e46. doi: 10.1093/cid/ciac182

63. Sinha S, Ajayababu A, Thukral H, Gupta S, Guha SK, Basu A, et al. Efficacy of bacillus calmette–guérin (BCG) vaccination in reducing the incidence and severity of COVID-19 in high-risk population (BRIC): a phase III, multi-centre, quadruple-blind randomised control trial. *Infect Dis Ther* (2022) 11(6):2205–17. doi: 10.1007/s40121-022-00703-y

64. Faustman DL, Lee A, Hostetter ER, Aristarkhova A, Ng NC, Shpilsky GF, et al. Multiple BCG vaccinations for the prevention of COVID-19 and other infectious diseases in type 1 diabetes. *Cell Rep Med* (2022) 3(9):100728. doi: 10.1016/j.xcrm.2022.100728

65. dos Anjos LRB, da Costa AC, Cardoso A, Guimarães RA, Rodrigues RL, Ribeiro KM, et al. Efficacy and safety of BCG revaccination with M. bovis BCG moscow to prevent COVID-19 infection in health care workers: A randomized phase II clinical trial. *Front Immunol* (2022) 13. doi: 10.3389/fimmu.2022.841868

66. Czajka H, Zapolnik P, Krzych Ł, Kmiecik W, Stopyra L, Nowakowska A, et al. A Multi-Center, Randomised, Double-Blind, Placebo-Controlled Phase III Clinical Trial Evaluating the Impact of BCG Re-Vaccination on the Incidence and Severity of SARS-CoV-2 Infections among Symptomatic Healthcare Professionals during the COVID-19 Pandemic in Poland-First Results. *Vaccines* (2022) 10(2):314. doi: 10.3390/vaccines10020314

67. ten Doesschate T, van der Vaart TW, Debisarun PA, Taks E, Moorlag SJCFM, Paternotte N, et al. Bacillus Calmette-Guérin vaccine to reduce healthcare worker absenteeism in COVID-19 pandemic, a randomized controlled trial. *Clin Microbiol Infect* (2022) 28(9):1278–85. doi: 10.1016/j.cmi.2022.04.009

68. Upton CM, van Wijk RC, Mockeliunas L, Simonsson USH, McHarry K, van den Hoogen G, et al. Safety and efficacy of BCG re-vaccination in relation to COVID-19 morbidity in healthcare workers: A double-blind, randomised, controlled, phase 3 trial. *eClinicalMedicine* (2022) 48:101414. doi: 10.1016/j.eclinm.2022.101414

69. Messina NL, Germano S, McElroy R, Rudraraju R, Bonnici R, Pittet LF, et al. Off-target effects of bacillus Calmette-Guérin vaccination on immune responses to SARS-CoV-2: implications for protection against severe COVID-19. *Clin Transl Immunol* (2022) 11(4):e1387. doi: 10.1002/cti2.1387

70. Pittet LF, Messina NL, Orsini F, Moore CL, Abruzzo V, Barry S, et al. Randomized trial of BCG vaccine to protect against covid-19 in health care workers. *N Engl J Med* (2023) 388(17):1582–96. doi: 10.1056/NEJMoa2212616

71. Wen J, Liu Q, Tang D, He J-Q. Efficacy of BCG vaccination against COVID-19: systematic review and meta-analysis of randomized controlled trials. *J Clin Med* (2023) 12(3):1154. doi: 10.3390/jcm12031154

72. Rosas Mejia O, Gloag ES, Li J, Ruane-Foster M, Claeys TA, Farkas D, et al. Mice infected with Mycobacterium tuberculosis are resistant to acute disease caused by secondary infection with SARS-CoV-2. *PloS Pathog* (2022) 18(3):e1010093. doi: 10.1371/journal.ppat.1010093

73. Hilligan KL, Namasivayam S, Clancy CS, O'Mard D, Oland SD, Robertson SJ, et al. Intravenous administration of BCG protects mice against lethal SARS-CoV-2 challenge. *J Exp Med* (2022) 219(2). doi: 10.1084/jem.20211862

74. Mambelli F, Marinho FV, Andrade JM, de Araujo A, Abuna RPF, Fabri VMR, et al. Recombinant bacillus calmette-guérin expressing SARS-coV-2 chimeric protein protects K18-hACE2 mice against viral challenge. *J Immunol* (2023) 210(12):1925–37. doi: 10.4049/jimmunol.2200731

75. Harper J, Skerry C, Davis SL, Tasneen R, Weir M, Kramnik I, et al. Mouse model of necrotic tuberculosis granulomas develops hypoxic lesions. *J Infect Dis* (2012) 205 (4):595–602. doi: 10.1093/infdis/jir786

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Tuberculosis and COVID-19 in the elderly: factors driving a higher burden of disease

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Mycobacterium tuberculosis (*M.tb*) and SARS-CoV-2 are both infections that can lead to severe disease in the lower lung. However, these two infections are caused by very different pathogens (*Mycobacterium vs.* virus), they have different mechanisms of pathogenesis and immune response, and differ in how long the infection lasts. Despite the differences, SARS-CoV-2 and *M.tb* share a common feature, which is also frequently observed in other respiratory infections: the burden of disease in the elderly is greater. Here, we discuss possible reasons for the higher burden in older adults, including the effect of co-morbidities, deterioration of the lung environment, auto-immunity, and a reduced antibody response. While the answer is likely to be multifactorial, understanding the main drivers across different infections may allow us to design broader interventions that increase the health-span of older people.

KEYWORDS

SARS-CoV-2, *Mycobacterium tuberculosis*, COVID-19, TB, elderly, immunity, infectious diseases

Introduction

The older adult population (> 60 years old) is projected to double to 2 billion by 2050 (1, 2). Natural lung aging is associated with progressive changes at both the cellular and organ level, including cellular senescence and chronic inflammation among others (3). This causes a decline in lung function and impaired immunological responses (4–7), which would be expected to influence the response to respiratory infections.

Coronavirus disease 2019 (COVID-19) and tuberculosis (TB) are both predominantly respiratory diseases but do not have a great deal in common beyond that. COVID-19 results from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, a virus that in most people persists for a few weeks or less and is cleared by the adaptive immune response. Protection against symptomatic SARS-CoV-2 infection correlates strongly with the levels of neutralizing antibodies against the virus (8). In support of this, the Omicron variant of SARS-CoV-2 was able to extensively re-infect people with pre-existing immunity (9) because it had high-level escape from neutralizing antibodies elicited

by previous infection or vaccination (10). In contrast, TB, caused by *Mycobacterium tuberculosis* (*M.tb*), can persist indefinitely in the infected individual (11, 12). Further, TB generally follows a bimodal age pattern, with higher risk of severe disease in children below 5 years of age and adult individuals of > 30 years old (13, 14), while severe COVID-19 is more common in older adults and pediatric COVID-19 deaths are relatively rare (15). These differences may be due to the fact that the immune responses in TB and COVID-19 are different.

Despite the differences, these two infections share common features: first, both are strongly affected by immunosuppression (e.g. during HIV infection), indicating that their control strongly depends on T cell and/or antibody responses, which are compromised by the CD4 T cell depletion and dysregulation during HIV infection (16–24). Indeed, TB is one of the cardinal diseases leading to the death of people living with HIV (PLWH) in the pre-ART era (25, 26). On the other hand, the most striking effect of HIV co-infection in COVID-19 happens in advanced HIV disease (defined as a CD4 T cell counts of less than 200 cells per microliter), where prolonged SARS-CoV-2 infection can last for months (27–33), leading to extensive SARS-CoV-2 genome evolution. A second common feature, which will be the focus of this review, is the remarkably higher disease burden in the elderly population (34). This is also true for most respiratory infections such as respiratory syncytial virus (35, 36), influenza (37, 38), and even rhinovirus, which is usually a mild upper respiratory tract infection, but can become a more severe lower respiratory infection in the elderly, very young children, or immunocompromised people (39).

Globally, COVID-19 has a mortality rate of about 1% (40), although this is influenced and fluctuates depending on many factors, including phenotypic and genotypic host factors, host immunity, and SARS-CoV-2 variants, among others. The elderly are at a higher risk of having more severe disease, which manifests as a lower respiratory tract infection that may require hospitalization, intensive care, and ventilation. It also results in higher mortality (41–43). The increase in the probability to die from COVID-19 as a function of age is dramatic: relative to the under-55 age group, mortality increases 8-fold in the 55-64 age group and 62-fold in the over 65 age group (43).

In contrast to the 1% mortality rate from COVID-19, mortality from TB disease is roughly a quarter of the TB incidence (44). That is, about a quarter of people diagnosed with TB disease will die. However, most people who are exposed to *M.tb* do not progress to symptomatic disease and instead have subclinical or asymptomatic infection for years. In this case, the infection is controlled by the host immune response (45, 46). In the elderly population, such subclinical or asymptomatic infection has a higher chance to develop into TB disease (47–49). Indeed, more than 90% of TB cases in older individuals result from reactivation of latent TB infection (LTBI) (50). Elderly people that develop TB disease have high mortality, mainly due to treatment failure. A recent report evaluating data from four countries shows that the treatment success rate among people with TB < 65 years old is 82% but decreases among the older age groups to 76% in 65–74 year-olds, 65% in 75–84 year-olds, and 46% in ≥85 year-olds (51).

There are multiple factors that may interact with each other and potentially play a role in the higher disease burden in COVID-19 and TB in the elderly, and their contribution may differ between the two infections. These include age-associated inflammation (inflammaging), a less effective immune response due to immunosenescense, and a highly oxidized lung environment (Figure 1). Although observed less frequently, other factors such as an age-related increase in autoantibodies (autoimmunity) may play a role in higher COVID-19 severity in the elderly. In addition, increasing numbers of people living with comorbidities in the elderly population may be particularly important. These factors tend to arise at different times along the life span (Figure 1). In the next sections, we outline examples for each of these factors, including how they may exacerbate COVID-19 and TB in older individuals.

Inflammaging and immunosenescence

The process of aging is associated with a decline in immune functions marked by immunosenescence, resulting in increased susceptibility to autoimmunity, malignancies, and infectious diseases (4, 5, 52–58). Immunosenescence is relatively well characterized in the adaptive immune system. Age-related

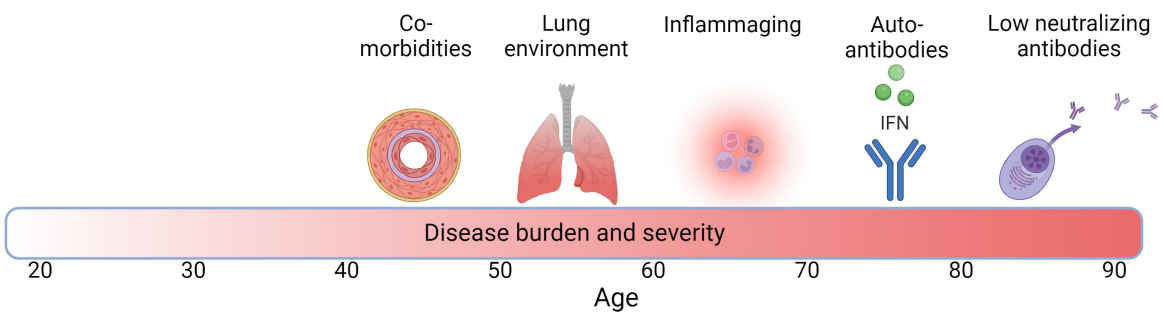

FIGURE 1
Factors associated with increased TB and COVID-19 disease burden with age across the life span. Darker red in bar denotes higher disease burden and severity and numbers denote age. Created with BioRender.com.

adaptive immune dysfunction is related to a lingering level of low-grade inflammation, immune dysfunction, increased number of memory T cells, and the loss ability of T cells to respond to antigens, as well as irreversible T cell loss of proliferation capacity. Interestingly, viral (e.g. SARS-CoV-2) and bacterial (e.g. *M.tb*) infections can also increase the extent of immune senescence, adding to the increased immune dysfunction and inflammation, especially in the elderly population (reviewed in detail in (59)).

Senescence in the innate immune system, where innate immunity is the first response to infection, is less well-characterized. Evidence for macrophage senescence during aging is supported by decreased pro-inflammatory responses of human (60–64) and mouse phagocytes to lipopolysaccharide (LPS) stimulation (65–70), which could be linked to age-related alterations in Toll-Like Receptor (TLR) expression and/or signaling which recognizes pathogen-associated molecular patterns (65, 66, 71–73).

Still, cellular immunosenescence does not fully explain increased circulating pro-inflammatory cytokines seen in elderly people, non-human primates, and old mice (61, 74–76), or the increased expression of pro-inflammatory genes with aging in several organs (77–81). This has led to a second paradigm, termed inflammaging, in which chronic, low-grade inflammation develops with increasing age in tissues that are frequently exposed to innate immune stimulation and oxidative stress (82, 83). Inflammaging occurs in the human lung, with increased numbers of macrophages and neutrophils in the lung alveolar lining fluid (ALF) of elderly individuals, as well as increased levels of IL-6 and IL-8 (84, 85). Specifically, IL-6 is the commonly used biomarker of inflammaging (86). There is also increased p38 MAPK phosphorylation and nuclear localization of NF-κB (87–89), a critical regulator of inflammation. Resident alveolar macrophages are more activated in the elderly (90–92) and have increased production of pro-inflammatory cytokines in response to TLR stimulation (93). Taken together, there is strong evidence that chronic inflammation occurs in the lungs as we age.

How inflammaging affects *M.tb* and SARS-CoV-2 infection is not completely understood. However, in both infections, a balanced immune response is thought to be critical both for infection control and to prevent immune system mediated damage. Tumor necrosis factor (TNF), the upstream activator of the NF-κB system, is elevated in inflammaging. High levels of TNF lead to reduced control of *M.tb* through programmed cell necrosis of activated macrophages via the mitochondrial-lysosomal-endoplasmic reticulum signaling circuit (94–96). Since macrophages are the primary host cells of *M.tb* as well as the most important line of defense against this pathogen, macrophage death in turn increases *M.tb* replication since the bacilli are able to robustly grow in the dead infected cells (97).

Immunosenescence and inflammaging are also suspected to contribute to severe COVID-19 in the elderly as well as to persistence of symptoms following acute disease (98, 99). Severe SARS-CoV-2 infection is characterized by a cytokine storm that, combined with a dysfunctional immune response in the elderly, leads to the accumulation of immune cells in the lungs and overproduction of pro-inflammatory molecules such as IL-6 (a marker of inflammaging), resulting in more tissue damage (100–102). High levels of pro-inflammatory molecules (hyperinflammatory syndrome) promote the survival of neutrophils via decreased apoptosis (103); and persistent increased systemic levels of neutrophils and monocytes in COVID-19 patients are associated with increased disease severity (104). Age-associated dysregulation and senescence of T-cells may also influence the immune response to SARS-CoV-2 (105). As seen in HIV infection, CD4 T cell depletion and dysregulation may lead to the inability to clear SARS-CoV-2 infection, most likely due to the inability to generate antibodies which will effectively neutralize the virus (27). This would be expected since CD4 T cells are critical to facilitate the antibody response to infection (106). Lastly, SARS-CoV-2 infection might also increase chronic inflammation in the elderly, resulting in a higher chance of long-term sequelae even after viral clearance (long-COVID) (107). New therapies targeting age-associated pathways may be critical to reduce COVID-19 mortality and/or long-term sequelae in the aging population (108).

Lung environment in the elderly in the context of TB and COVID-19

Local inflammation and oxidation occur in the aging lung and influences the ALF (109, 110). ALF is a surfactant which reduces surface tension and allows the lung alveoli to expand. It also functions in multiple ways in the innate immune response to lung pathogens. Here we will focus on the ALF as an example of how the lung environment can change with age and its impact on TB and COVID-19.

ALF is generated, secreted, and recycled by alveolar epithelial type II cells (ATII), and is essential for maintaining lung homeostasis (111, 112). ALF in elderly individuals degrades quickly and is not regenerated efficiently because of ATII senescence. In addition, low-grade chronic inflammation in old age is expected to alter ALF component production and activity, due in part to biochemical modifications because of the alveolar oxidation state. We and others have shown that components of human ALF including collectins (which bind pathogen surface oligosaccharides or lipids and mark the pathogen for the innate immune response), surfactant protein (SP)-A and SP-D, homeostatic hydrolytic activities (hydrolases), surfactant lipids, and the complement system are critical elements of the innate immune system during *M.tb* infection (113–115) and play important roles in *M.tb*-phagocyte encounters (116–119). Indeed, SP-A upregulates the expression of the mannose receptor in macrophages, which in turn favors *M.tb* survival within phagocytes. In contrast, SP-D can directly bind *M.tb* clustering bacteria, favoring recognition and uptake by phagocytes driving better control of the infection (110).

In a study defining the molecular composition of ALF in the aging lung, our findings demonstrate that pro-inflammatory cytokines are increased, SP-A and SP-D and complement components are significantly increased but dysfunctional, ALF hydrolases are decreased, and surfactant lipids are oxidized in both mice and humans (109). Further, a recent quantitative proteomic profiling of the lung environment of human adult *vs.*

elderly ALF investigated molecular fingerprints, pathways, and regulatory networks that characterize the alveolar space in old age compared to younger individuals (120). ALF from elderly individuals had significantly increased production of matrix metalloproteinases, markers of cellular senescence, antimicrobials, and proteins of neutrophilic granule origin, among others, suggesting that neutrophils could be potential contributors to the dysregulated alveolar environment with increasing age.

Consistent with reduced ALF functionality with age, *M.tb* exposed to human ALF obtained from older adults showed increased intracellular growth in macrophages and ATIIs (121–123), as well as increased bacterial burden and lung tissue damage in mice (121). In addition, *M.tb* exposed to ALF from healthy 18- to 45-year-old adults upregulated key cell envelope genes associated with amino acid, carbohydrate, and lipid metabolism, as well as genes associated with redox homeostasis and transcriptional regulators, while *M.tb* exposed to ALF from 60+ year-old individuals showed lower transcriptional responses (124). The changes in ALF in aging support the concept that the pulmonary environment can modify mucosal immune responses, thereby increasing the susceptibility to pulmonary infections in the elderly population.

How ALF influences SARS-CoV-2 infection is mostly unknown. However, patients with severe COVID-19 sometimes harbor IgA autoantibodies against pulmonary SP-B and SP-C, blocking the function of the lung surfactant lipid layer and potentially contributing to alveolar collapse and poor oxygenation (125). Other studies indicate that levels of SP-D in blood could be used as a biomarker for COVID-19 severity as a result of the impairment of the pulmonary barrier caused by prolonged inflammation (126). Still, how the levels, status, and function of ALF components in the alveolar environment determine the outcome of *M.tb* and SARS-CoV-2 infection and disease severity of TB and COVID-19, respectively, still needs to be elucidated in detail.

Reduced adaptive immune responses

The adaptive immune response is essential to control both *M.tb* and SARS-CoV-2. CD4 T cells are critical in the orchestration of both the antibody and cellular adaptive immune response to infections (106, 127, 128). For SARS-CoV-2, the strongest correlate of protection against symptomatic infection is the level of pre-existing neutralizing antibody immunity (8, 129). Thus, SARS-CoV-2 neutralizing antibody levels are studied extensively as a function of age. A complication of measuring neutralizing antibody levels after infection is that higher disease severity elicits higher antibody levels (130). However, it is possible to distinguish between neutralizing antibody production capacity and disease severity by measuring neutralizing antibody levels to SARS-CoV-2 post-vaccination, with much of the data coming from mRNA vaccines.

One of the first studies examining the neutralizing antibody response to the Pfizer BNT162b2 mRNA vaccine against SARS-CoV-2 found that the fraction of people with a detectable neutralizing antibody response decreased slowly as a function of age up to the age of 80, with almost all individuals responding to the vaccine. After 80, the probability to elicit a neutralizing antibody response plummeted and was close to zero at 90, although the number of individuals in this part of the age range was small in the study (131). A second study used a cutoff of 55 years for who is elderly and showed substantially lower neutralizing antibody levels in the older age group after the first dose of an mRNA vaccine (132). However, this difference decreased with the second dose. A third study done in Singapore also found that people over 60 had lower neutralizing responses with an mRNA vaccine. However, they showed a strong increase in neutralizing antibodies with a third, booster dose (133). The benefit of a booster dose was recapitulated in a group of over-80-year-olds who did not have an antibody response to the first two doses (134).

The role of neutralizing antibodies in the immune response to *M.tb* is currently unclear. However, the reduced ability to mount an effective neutralizing antibody response may indicate an overall less effective adaptive immune response in the elderly, which would reduce *M.tb* control.

Autoimmunity

An essential component of the initial immune response to both *M.tb* and SARS-CoV-2 is interferon (IFN), which orchestrates the innate immune response to infection. In TB, the type II interferon IFN-γ activates macrophages and enables them to initiate maturation and acidification of the *M.tb*-containing phagosome, as well as other antimicrobial responses (135). The failure of this process to kill the internalized bacilli leads to macrophage death and *M.tb* growth in the dead infected cells (97). Mice deficient in IFN-γ quickly succumb to TB (136, 137).

The role of type I interferons during *M.tb* infection is not completely understood, with some studies reporting a host protective role *vs.* other studies suggesting a detrimental role under different host-*M.tb* encounter settings (138, 139). However, type I interferons including IFN-α are an important component of the innate immune response to SARS-CoV-2 (100), and they are rapidly induced in early stages of the infection (140). Multiple SARS-CoV-2 genes attempt to interfere with IFN (141–144). Individuals with inborn errors in type I IFN immunity are much more prone to severe COVID-19 (145) and mice deficient for type I IFN have reduced activation of CD4 and CD8 T cells and reduced recruitment of monocytes and monocyte-derived macrophages to the lung (146).

Anti-IFN antibodies might block IFN binding to IFN receptors, impairing its antiviral effect (147). There have been sporadic case reports of anti-IFN antibodies increasing susceptibility to mycobacterial infections (148–150) or shown to be elevated at the site of infection in advanced TB patients (151). In contrast, SARS-CoV-2 infection is reported to be more severe in individuals with autoantibodies to type I IFN. In one study, 101 of 987 patients with severe COVID-19 have been found to have these autoantibodies, while none of the 663 individuals with asymptomatic or mild SARS-CoV-2 infection had anti-IFN type I antibodies (152). The

prevalence of anti-IFN type I antibodies was found to be strongly age dependent (153, 154). Autoantibodies neutralizing high concentrations of IFN-α were present in 0.18% of individuals between 18 and 69 years, 1.1% of individuals between 70 and 79 years, and 3.4% of people >80 years of age (154). Autoantibodies are unlikely to completely explain the higher susceptibility of the elderly population to severe COVID-19. However, such autoimmunity may be a contributing factor in a subset of people (155) and an example of an age dependent affect which is highly variable between people of the same age. Also, antibodies to other host proteins are known to increase with age (156). This may potentially add to disease pathology in a similar way.

Comorbidities

An important aspect of the shift towards a global aging population is increasing chronic illness. The top 10 comorbidities associated with the elderly population include hypertension (58%), high cholesterol (47%), arthritis (31%), ischemic heart disease (29%), diabetes (27%), chronic kidney disease (18%), heart failure (14%), depression, Alzheimer disease and dementia (11%) and chronic obstructive pulmonary disease (COPD, 11%) (157). Some of these comorbidities overlap with known risk factors for TB (diabetes) and higher COVID-19 severity (obesity, hypertension, high cholesterol, and diabetes). Thus, a common risk factor for TB and COVID-19 which increases in prevalence in the elderly is diabetes. This is not surprising, as diabetes leads to higher mortality from a range of infectious diseases (158).

Projections suggest that the global incidence of diabetes will double in the next 20 years, with 40% of this estimated to result from the aging population (159, 160). The elderly are at high risk for developing type 2 diabetes due to underlying insulin resistance, impaired pancreatic function, and a higher obesity prevalence linked to changes in body composition and physical inactivity (161, 162). The resulting high blood sugar can cause serious complications such as heart disease, kidney problems, and loss of vision. Furthermore, diabetes in older adults is associated with a higher risk for chronic microvascular and cardiovascular complications and common geriatric syndromes and is linked to higher mortality (163). People with diabetes have altered cytokine release by macrophages and T cells, impaired neutrophil recruitment, and decreased levels of type I interferons as well as reduced numbers of new populations of dendritic cells (DCs) and natural killer (NK) cells (164). Also, the diabetic lung is characterized by structural modifications such as abnormalities in small vessels (alveolar diabetic microangiopathy or microvascular disease) (165), as well as alterations in the interstitial environment (166, 167) and autonomic neuropathy with loss of autonomic innervation in bronchioles (168), which might contribute to adverse outcomes in respiratory diseases (169).

While the interactions between TB and diabetes in the elderly are not completely understood, people with diabetes are 3 times more likely to develop pulmonary TB, especially those with poorly controlled diabetes (170, 171). *In vitro* and *in vivo* studies have found reduced association and uptake of *M.tb* by monocytes from people with diabetes and alveolar macrophages from mice with chronic diabetes, as well as reduced innate immune responses and a persistent systemic hyper-inflammation in TB-diabetic individuals (172–174). Diabetes also promotes TB reactivation due to impaired T cell immunity, specifically because of decreased IFN-γ production by CD4 T cells (175). In addition, cavitary disease (where cavities are abnormal, thick-walled, air-filled spaces in the lung which result when a granuloma encasing *M.tb* liquifies and ruptures) is more frequently observed in elderly TB patients with diabetes than in non-diabetic elderly patients, suggesting that diabetes promotes cavitation in the aging lung parenchyma (176).

In addition, TB might pose a risk of developing diabetes (177). A persistent inflammatory state in response to TB disease might result in secondary metabolic effects such as "stress hyperglycemia", defined as temporary hyperglycemia caused by stress during acute illness (178). It has been suggested that stress hyperglycemia may negatively influence TB treatment outcomes, although this relationship is still poorly understood (178).

Individuals with diabetes are at a higher risk for SARS-CoV-2 severe disease and mortality (34, 179–185). According to an analysis done in the South African population, the hazard ratios for mortality range from 3 to 12 for ≥20 years old public-sector patients, with the mortality risk increasing as blood sugar control decreases. Risk may be lower in other populations, perhaps due to better diabetes control: about 3-fold higher for mortality as reported in a meta-analysis (181). While the worse disease outcome of SARS-CoV-2 infection in diabetics is well established, diabetics are not necessarily at higher risk of infection with SARS-CoV-2 (179), indicating that not all aspects of immunity are equally compromised.

M.tb and SARS-CoV-2 co-infection

Respiratory infections tend to interact in one of two ways. They can synergize, with the cardinal example being *Streptococcus pneumoniae* bacterial infection after influenza virus infection. This happens because the virus causes damage to the mucosal surface, allowing the bacteria to attach better and invade more easily (186). In addition, the type I interferon response to the virus decreases phagocyte function and therefore control of the bacteria by phagocytosis (186). The other possible interaction is antagonism, and usually happens between viruses. This is called super-infection exclusion and occurs because the type I interferon antiviral response trigged by one virus can inhibit other viruses (187). Two studies by independent groups examined experimental SARS-CoV-2/*M.tb* co-infection in K18-hACE2

transgenic mice. Both groups found that SARS-CoV-2 infection did not affect *M.tb* loads or associated pathology. They also observed that *M.tb* infected mice were more resistant to SARS-CoV-2 infection (188, 189). This is consistent with a report that intravenous administration of BCG, a live attenuated TB vaccine developed from *Mycobacterium bovis*, protects mice against lethal SARS-CoV-2 challenge (190). Thus, there is currently no mechanistic basis for synergy between SARS-CoV-2 and *M.tb*. There is still a poor understanding of the pathology and immunological changes associated with *M.tb*/SARS-CoV-2 co-infection (191), as recently reviewed in (192).

In terms of epidemiology, some studies suggest that the dysregulated immunity during *M.tb* infection is associated with increased susceptibility and severity of COVID-19 and *vice versa* (193–198). There is also some evidence suggesting that in the elderly population, TB and COVID-19 may be associated with increased mortality compared to each disease occurring alone (199–201). Mechanisms may include increased lung damage in TB patients with COVID-19, resulting in impaired lung function (202) or higher risk of TB reactivation after COVID-19 infection due to depletion of CD4 T cells and excessive lung fibrosis. Worse outcomes of co-infection may also be because of shared clinical, immunological, and social determinants (203–206), as well as compromised linkage to care for HIV and TB in a pandemic environment (207). In our own South Africa based cohort of SARS-CoV-2 infected individuals, we did not observe a clear enrichment of active TB disease (208) relative to the observed incidence in the South African population (209).

Conclusions and future perspectives

Aging has a negative effect on the outcomes of both SARS-CoV-2 and *M.tb* infection, and may be considered a subtype of immunosuppression/dysregulation which varies widely between individuals of a similar age. This may be because the effect is multi-factorial and involves age-related inflammation (inflammaging) and senescence of immune cell subsets, as reviewed previously (210). It is particularly damaging to the adaptive arm of the immune response which is critical to control both infections. In addition to that, the lung environment itself also changes with age, and many of the changes are associated with the reduced ability of alveolar fluid to perform its innate immune functions. Aging also increases autoimmunity, and in a subset of individuals this may manifest as autoantibodies to immune mediators such as interferons, with the result that innate immunity becomes less effective at reducing pathogen replication. This is an example of how the effects of aging can be heterogeneous. Lastly, co-morbidities such as type II diabetes increase with age, and such co-morbidities, though they do not necessarily increase the chances of infection, are risk factors for more severe disease if infection does occur.

Conversely, SARS-CoV-2 and *M.tb* infections may accelerate age-related processes. For example, *M.tb* infection and TB treatment, as well as long-COVID, might result in cardiovascular complications and induce cardiovascular disease (211), an important comorbidity associated with the older population. Also, SARS-CoV-2 is associated with increased oxidative stress, which also plays a role in the pathogenesis of diabetes (212).

Some of the processes described here are already targets for interventions. For example, the elderly are prioritized for COVID-19 vaccination to compensate for the less effective immune response to SARS-CoV-2 (213). Other interventions, for example better control of diabetes, are available but are not uniformly implemented due to health systems challenges, particularly in low- and middle-income countries (214). Interventions which may increase lung health at a given stage of life are yet little explored, but have the potential to work across pathogens to decrease the effects of infection, which could translate to substantial gains in the health span of aging populations.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. UN. *World population Ageing 2019: Highlights*. (2020).

2. Schneider JL, Rowe JH, Garcia-de-Alba C, Kim CF, Sharpe AH, Haigis MC. The aging lung: Physiology, disease, and immunity. *Cell* (2021) 184(8):1990–2019. doi: 10.1016/j.cell.2021.03.005

3. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. *Cell* (2023) 186(2):243–78. doi: 10.1016/j.cell.2022.11.001

4. Chalise HN. Aging: basic concept. *Am J BioMed Sci Res* (2019) 1:8–10. doi: 10.34297/AJBSR.2019.01.000503

5. Cho SJ, Stout-Delgado HW. Aging and lung disease. *Annu Rev Physiol* (2020) 82:433–59. doi: 10.1146/annurev-physiol-021119-034610

6. Angelidis I, Simon LM, Fernandez IE, Strunz M, Mayr CH, Greiffo FR, et al. An atlas of the aging lung mapped by single cell transcriptomics and deep tissue proteomics. *Nat Commun* (2019) 10(1):963. doi: 10.1038/s41467-019-08831-9

7. Kim J, Heise RL, Reynolds AM, Pidaparti RM. Aging effects on airflow dynamics and lung function in human bronchioles. *PloS One* (2017) 12(8):e0183654. doi: 10.1371/journal.pone.0183654

8. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* (2021) 27:1205–11. doi: 10.1038/s41591-021-01377-8

9. Pulliam JR, van Schalkwyk C, Govender N, von Gottberg A, Cohen C, Groome MJ, et al. Increased risk of SARS-CoV-2 reinfection associated with emergence of Omicron in South Africa. *Science* (2022) 376(6593):eabn4947. doi: 10.1126/science.abn4947

10. Cele S, Jackson L, Khoury DS, Khan K, Moyo-Gwete T, Tegally H, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature* (2021) 602(7898):654–6. doi: 10.1038/s41586-021-04387-1

11. Menzies NA, Swartwood N, Testa C, Malyuta Y, Hill AN, Marks SM, et al. Time since infection and risks of future disease for individuals with *mycobacterium tuberculosis* infection in the United States. *Epidemiology* (2021) 32(1):70–8. doi: 10.1097/EDE.0000000000001271

12. Kiazyk S, Ball TB. Latent tuberculosis infection: An overview. *Can Commun Dis Rep* (2017) 43(3-4):62–6. doi: 10.14745/ccdr.v43i34a01

13. Bunyasi EW, Mulenga H, Luabeya AKK, Shenje J, Mendelsohn SC, Nemes E, et al. Regional changes in tuberculosis disease burden among adolescents in South Africa (2005-2015). *PloS One* (2020) 15(7):e0235206. doi: 10.1371/journal.pone.0235206

14. Iqbal SA, Winston CA, Bardenheier BH, Armstrong LR, Navin TR. Age-period-cohort analyses of tuberculosis incidence rates by nativity, United States, 1996-2016. *Am J Public Health* (2018) 108:S315–S20. doi: 10.2105/AJPH.2018.304687

15. Wang JG, Zhong ZJ, Mo YF, Wang LC, Chen R. Epidemiological features of coronavirus disease 2019 in children: a meta-analysis. *Eur Rev Med Pharmacol Sci* (2021) 25(2):1146–57. doi: 10.26355/eurrev_202101_24685

16. Monroe KM, Yang Z, Johnson JR, Geng X, Doitsh G, Krogan NJ, et al. IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV. *Science* (2014) 343(6169):428–32. doi: 10.1126/science.1243640

17. Doitsh G, Cavrois M, Lassen KG, Zepeda O, Yang Z, Santiago ML, et al. Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. *Cell* (2010) 143(5):789–801. doi: 10.1016/j.cell.2010.11.001

18. Doitsh G, Galloway NL, Geng X, Yang Z, Monroe KM, Zepeda O, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* (2014) 505(7484):509–14. doi: 10.1038/nature12940

19. Galloway NL, Doitsh G, Monroe KM, Yang Z, Muñoz-Arias I, Levy DN, et al. Cell-to-cell transmission of HIV-1 is required to trigger pyroptotic death of lymphoid-tissue-derived CD4 T cells. *Cell Rep* (2015) 12(10):1555–63. doi: 10.1016/j.celrep.2015.08.011

20. Banda NK, Bernier J, Kurahara DK, Kurrle R, Haigwood N, Sekaly RP, et al. Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. *J Exp Med* (1992) 176(4):1099–106. doi: 10.1084/jem.176.4.1099

21. Westendorp MO, Frank R, Ochsenbauer C, Stricker K, Dhein J, Walczak H, et al. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. *Nature* (1995) 375(6531):497–500. doi: 10.1038/375497a0

22. Westendorp MO, Shatrov VA, Schulze-Osthoff K, Frank R, Kraft M, Los M, et al. HIV-1 Tat potentiates TNF-induced NF-kappa B activation and cytotoxicity by altering the cellular redox state. *EMBO J* (1995) 14(3):546–54. doi: 10.1002/j.1460-2075.1995.tb07030.x

23. Zeng M, Haase AT, Schacker TW. Lymphoid tissue structure and HIV-1 infection: life or death for T cells. *Trends Immunol* (2012) 33(6):306–14. doi: 10.1016/j.it.2012.04.002

24. Cooper A, García M, Petrovas C, Yamamoto T, Koup RA, Nabel GJ. HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration. *Nature* (2013) 498(7454):376–9. doi: 10.1038/nature12274

25. Sepkowitz KA, Raffalli J, Riley L, Kiehn TE, Armstrong D. Tuberculosis in the AIDS era. *Clin Microbiol Rev* (1995) 8(2):180–99. doi: 10.1128/CMR.8.2.180

26. Bell LC, Noursadeghi M. Pathogenesis of HIV-1 and *Mycobacterium tuberculosis* co-infection. *Nat Rev Microbiol* (2018) 16(2):80–90. doi: 10.1038/nrmicro.2017.128

27. Cele S, Karim F, Lustig G, San JE, Hermanus T, Tegally H, et al. SARS-CoV-2 prolonged infection during advanced HIV disease evolves extensive immune escape. *Cell Host Microbe* (2022) 30(2):154–162.e5. doi: 10.1016/j.chom.2022.01.005

28. Lustig G, Ganga Y, Rodel H, Tegally H, Jackson L, Cele S, et al. SARS-CoV-2 evolves increased infection elicited cell death and fusion in an immunosuppressed individual. *medRxiv* (2022), 22282673. doi: 10.1101/2022.11.23.22282673

29. Riddell AC, Kele B, Harris K, Bible J, Murphy M, Dakshina S, et al. Generation of novel SARS-CoV-2 variants on B.1.1.7 lineage in three patients with advanced HIV disease. *Clin Infect Dis* (2022) 75(11):2016–8. doi: 10.1093/cid/ciac409

30. Wilkinson SAJ, Richter A, Casey A, Osman H, Mirza JD, Stockton J, et al. Recurrent SARS-CoV-2 mutations in immunodeficient patients. *Virus Evol* (2022) 8(2):veac050. doi: 10.1093/ve/veac050

31. Maponga TG, Jeffries M, Tegally H, Sutherland A, Wilkinson E, Lessells RJ, et al. Persistent SARS-CoV-2 infection with accumulation of mutations in a patient with poorly controlled HIV infection. *Clin Infect Dis* (2022). doi: 10.2139/ssrn.4014499

32. Hoffman SA, Costales C, Sahoo MK, Palanisamy S, Yamamoto F, Huang C, et al. SARS-coV-2 neutralization resistance mutations in patient with HIV/AIDS, california, USA. *Emerging Infect Diseases* (2021) 27(10):2720–3. doi: 10.3201/eid2710.211461

33. Karim F, Moosa MY, Gosnell B, Sandile C, Giandhari J, Pillay S, et al. Persistent SARS-CoV-2 infection and intra-host evolution in association with advanced HIV infection. *medRxiv* (2021). doi: 10.1101/2021.06.03.21258228

34. Boulle A, Davies M-A, Hussey H, Ismail M, Morden E, Vundle Z. Western cape department of health in collaboration with the national institute for communicable diseases SA. Risk factors for coronavirus disease 2019 (COVID-19) death in a population cohort study from the western cape province, South Africa. *Clin Infect Dis* (2020) 73(7):e2005–e15. doi: 10.1093/cid/ciaa1198

35. Savic M, Penders Y, Shi T, Branche A, Pircon JY. Respiratory syncytial virus disease burden in adults aged 60 years and older in high-income countries: A systematic literature review and meta-analysis. *Influenza Other Respir Viruses* (2023) 17(1):e13031. doi: 10.1111/irv.13031

36. Branche AR, Falsey AR. Respiratory syncytial virus infection in older adults: an under-recognized problem. *Drugs Aging* (2015) 32(4):261–9. doi: 10.1007/s40266-015-0258-9

37. Paget J, Spreeuwenberg P, Charu V, Taylor RJ, Iuliano AD, Bresee J, et al. Global mortality associated with seasonal influenza epidemics: New burden estimates and predictors from the GLaMOR Project. *J Glob Health* (2019) 9(2):020421. doi: 10.7189/jogh.09.020421

38. Langer J, Welch VL, Moran MM, Cane A, Lopez SMC, Srivastava A, et al. High clinical burden of influenza disease in adults aged >/= 65 years: can we do better? A systematic literature review. *Adv Ther* (2023) 40(4):1601–27. doi: 10.1007/s12325-023-02432-1

39. Hung IF, Zhang AJ, To KK, Chan JF, Zhu SH, Zhang R, et al. Unexpectedly higher morbidity and mortality of hospitalized elderly patients associated with rhinovirus compared with influenza virus respiratory tract infection. *Int J Mol Sci* (2017) 18(2):259. doi: 10.3390/ijms18020259

40. WHO. *WHO Coronavirus (COVID-19) Dashboard 2023*. Available at: https://covid19.who.int/.

41. O'Driscoll M, Ribeiro Dos Santos G, Wang L, Cummings DAT, Azman AS, Paireau J, et al. Age-specific mortality and immunity patterns of SARS-CoV-2. *Nature* (2021) 590(7844):140–5. doi: 10.1038/s41586-020-2918-0

42. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* (2020) 395(10229):1054–62. doi: 10.1016/S0140-6736(20)30566-3

43. Yanez ND, Weiss NS, Romand JA, Treggiari MM. COVID-19 mortality risk for older men and women. *BMC Public Health* (2020) 20(1):1742. doi: 10.1186/s12889-020-09826-8

44. Dolin PJ, Raviglione MC, Kochi A. Global tuberculosis incidence and mortality during 1990-2000. *Bull World Health Organization* (1994) 72(2):213.

45. Wong EB. It is time to focus on asymptomatic tuberculosis. *Clin Infect Diseases* (2021) 72(12):E1044–E6. doi: 10.1093/cid/ciaa1827

46. Shah M, Dorman SE. Latent tuberculosis infection. *New Engl J Med* (2021) 385(24):2271–80. doi: 10.1056/NEJMcp2108501

47. Li SJ, Li YF, Song WM, Zhang QY, Liu SQ, Xu TT, et al. Population aging and trends of pulmonary tuberculosis incidence in the elderly. *BMC Infect Dis* (2021) 21(1):302. doi: 10.1186/s12879-021-05994-z

48. Piergallini TJ, Turner J. Tuberculosis in the elderly: Why inflammation matters. *Exp Gerontol* (2018) 105:32–9. doi: 10.1016/j.exger.2017.12.021

49. Caraux-Paz P, Diamantis S, de Wazières B, Gallien S. Tuberculosis in the elderly. *J Clin Med* (2021) 10(24):5888. doi: 10.3390/jcm10245888

50. Kim S, Cohen T, Horsburgh CR, Miller JW, Hill AN, Marks SM, et al. Trends, mechanisms, and racial/ethnic differences of tuberculosis incidence in the US-born population aged 50 years or older in the United States. *Clin Infect Dis* (2022) 74 (9):1594–603. doi: 10.1093/cid/ciab668

51. Teo AKJ, Rahevar K, Morishita F, Ang A, Yoshiyama T, Ohkado A, et al. Tuberculosis in older adults: case studies from four countries with rapidly ageing populations in the western pacific region. *BMC Public Health* (2023) 23(1):370. doi: 10.1186/s12889-023-15576-0

52. Janssens JP, Pache JC, Nicod LP. Physiological changes in respiratory function associated with ageing. *Eur Respir J* (1999) 13(1):197–205. doi: 10.1183/09031936.99.14614549

53. Lowery EM, Brubaker AL, Kuhlmann E, Kovacs EJ. The aging lung. *Clin Interv Aging* (2013) 8:1489–96. doi: 10.2147/CIA.S51152

54. Sharma G, Goodwin J. Effect of aging on respiratory system physiology and immunology. *Clin Interv Aging* (2006) 1(3):253–60. doi: 10.2147/ciia.2006.1.3.253

55. Miller RA. The aging immune system: primer and prospectus. *Science* (1996) 273 (5271):70–4. doi: 10.1126/science.273.5271.70

56. Pawelec G, Solana R. Immunosenescence. *Immunol Today* (1997) 18(11):514–6. doi: 10.1016/S0167-5699(97)01145-6

57. Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. *Nat Immunol* (2004) 5(2):133–9. doi: 10.1038/ni1033

58. Pawelec G, Derhovanessian E, Larbi A. Immunosenescence and cancer. *Crit Rev Oncol Hematol* (2010) 75(2):165–72. doi: 10.1016/j.critrevonc.2010.06.012

59. Lee KA, Flores RR, Jang IH, Saathoff A, Robbins PD. Immune senescence, immunosenescence and aging. *Front Aging* (2022) 3:900028. doi: 10.3389/fragi.2022.900028

60. Rink L, Cakman I, Kirchner H. Altered cytokine production in the elderly. *Mech Ageing Dev* (1998) 102(2-3):199–209. doi: 10.1016/S0047-6374(97)00153-X

61. Bruunsgaard H, Andersen-Ranberg K, Hjelmborg J, Pedersen BK, Jeune B. Elevated levels of tumor necrosis factor alpha and mortality in centenarians. *Am J Med* (2003) 115(4):278–83. doi: 10.1016/S0002-9343(03)00329-2

62. van den Biggelaar AH, Huizinga TW, de Craen AJ, Gussekloo J, Heijmans BT, Frolich M, et al. Impaired innate immunity predicts frailty in old age. *Leiden 85-plus study Exp Gerontol* (2004) 39(9):1407–14. doi: 10.1016/j.exger.2004.06.009

63. van Duin D, Mohanty S, Thomas V, Ginter S, Montgomery RR, Fikrig E, et al. Age-associated defect in human TLR-1/2 function. *J Immunol* (2007) 178(2):970–5. doi: 10.4049/jimmunol.178.2.970

64. Nyugen J, Agrawal S, Gollapudi S, Gupta S. Impaired functions of peripheral blood monocyte subpopulations in aged humans. *J Clin Immunol* (2010) 30(6):806–13. doi: 10.1007/s10875-010-9448-8

65. Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. Cutting edge: impaired Toll-like receptor expression and function in aging. *J Immunol* (2002) 169(9):4697–701. doi: 10.4049/jimmunol.169.9.4697

66. Boehmer ED, Goral J, Faunce DE, Kovacs EJ. Age-dependent decrease in Toll-like receptor 4-mediated proinflammatory cytokine production and mitogen-activated protein kinase expression. *J Leukoc Biol* (2004) 75(2):342–9. doi: 10.1189/jlb.0803389

67. Boehmer ED, Meehan MJ, Cutro BT, Kovacs EJ. Aging negatively skews macrophage TLR2- and TLR4-mediated pro-inflammatory responses without affecting the IL-2-stimulated pathway. *Mech Ageing Dev* (2005) 126(12):1305–13. doi: 10.1016/j.mad.2005.07.009

68. Chelvarajan RL, Collins SM, Van Willigen JM, Bondada S. The unresponsiveness of aged mice to polysaccharide antigens is a result of a defect in macrophage function. *J Leukoc Biol* (2005) 77(4):503–12. doi: 10.1189/jlb.0804449

69. Chelvarajan RL, Liu Y, Popa D, Getchell ML, Getchell TV, Stromberg AJ, et al. Molecular basis of age-associated cytokine dysregulation in LPS-stimulated macrophages. *J Leukoc Biol* (2006) 79(6):1314–27. doi: 10.1189/jlb.0106024

70. Armstrong JA, Hart PD. Response of cultured macrophages to Mycobacterium tuberculosis, with observations on fusion of lysosomes with phagosomes. *J Exp Med* (1971) 134(3 Pt 1):713–40. doi: 10.1084/jem.134.3.713

71. Dunston CR, Griffiths HR. The effect of ageing on macrophage Toll-like receptor-mediated responses in the fight against pathogens. *Clin Exp Immunol* (2010) 161(3):407–16. doi: 10.1111/j.1365-2249.2010.04213.x

72. Shaw AC, Panda A, Joshi SR, Qian F, Allore HG, Montgomery RR. Dysregulation of human Toll-like receptor function in aging. *Ageing Res Rev* (2011) 10(3):346–53. doi: 10.1016/j.arr.2010.10.007

73. Hajishengallis G. Too old to fight? Aging and its toll on innate immunity. *Mol Oral Microbiol* (2010) 25(1):25–37. doi: 10.1111/j.2041-1014.2009.00562.x

74. Bruunsgaard H, Ladelund S, Pedersen AN, Schroll M, Jorgensen T, Pedersen BK. Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. *Clin Exp Immunol* (2003) 132(1):24–31. doi: 10.1046/j.1365-2249.2003.02137.x

75. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. *Exp Gerontol* (2004) 39(5):687–99. doi: 10.1016/j.exger.2004.01.009

76. Singh T, Newman AB. Inflammatory markers in population studies of aging. *Ageing Res Rev* (2011) 10(3):319–29. doi: 10.1016/j.arr.2010.11.002

77. Korhonen P, Helenius M, Salminen A. Age-related changes in the regulation of transcription factor NF-kappa B in rat brain. *Neurosci Lett* (1997) 225(1):61–4. doi: 10.1016/S0304-3940(97)00190-0

78. Csiszar A, Ungvari Z, Koller A, Edwards JG, Kaley G. Aging-induced proinflammatory shift in cytokine expression profile in coronary arteries. *FASEB J* (2003) 17(9):1183–5. doi: 10.1096/fj.02-1049fje

79. de Magalhaes JP, Curado J, Church GM. Meta-analysis of age-related gene expression profiles identifies common signatures of aging. *Bioinformatics* (2009) 25 (7):875–81. doi: 10.1093/bioinformatics/btp073

80. Swindell WR. Genes and gene expression modules associated with caloric restriction and aging in the laboratory mouse. *BMC Genomics* (2009) 10:585. doi: 10.1186/1471-2164-10-585

81. Lumeng CN, Liu J, Geletka L, Delaney C, Delproposto J, Desai A, et al. Aging is associated with an increase in T cells and inflammatory macrophages in visceral adipose tissue. *J Immunol* (2011) 187(12):6208–16. doi: 10.4049/jimmunol.1102188

82. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging - An evolutionary perspective on immunosenescence. *Mol Cell Gerontology* (2000) 908:244–54. doi: 10.1111/j.1749-6632.2000.tb06651.x

83. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat Rev Cardiol* (2018) 15(9):505–22. doi: 10.1038/s41569-018-0064-2

84. Meyer KC, Ershler W, Rosenthal NS, Lu XG, Peterson K. Immune dysregulation in the aging human lung. *Am J Respir Crit Care Med* (1996) 153(3):1072–9. doi: 10.1164/ajrccm.153.3.8630547

85. Meyer KC, Rosenthal NS, Soergel P, Peterson K. Neutrophils and low-grade inflammation in the seemingly normal aging human lung. *Mech Ageing Dev* (1998) 104 (2):169–81. doi: 10.1016/S0047-6374(98)00065-7

86. Alberro A, Iribarren-Lopez A, Saenz-Cuesta M, Matheu A, Vergara I, Otaegui D. Inflammaging markers characteristic of advanced age show similar levels with frailty and dependency. *Sci Rep* (2021) 11(1):4358. doi: 10.1038/s41598-021-83991-7

87. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, et al. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev* (2007) 128(1):92–105. doi: 10.1016/j.mad.2006.11.016

88. Salminen A, Ojala J, Kaarniranta K, Kauppinen A. Mitochondrial dysfunction and oxidative stress activate inflammasomes: impact on the aging process and age-related diseases. *Cell Mol Life Sci* (2012) 69(18):2999–3013. doi: 10.1007/s00018-012-0962-0

89. Garcia-Garcia VA, Alameda JP, Page A, Casanova ML. Role of NF-kappaB in ageing and age-related diseases: lessons from genetically modified mouse models. *Cells* (2021) 10(8):1906. doi: 10.3390/cells10081906

90. Lafuse WP, Rajaram MVS, Wu Q, Moliva JI, Torrelles JB, Turner J, et al. Identification of an increased alveolar macrophage subpopulation in old mice that displays unique inflammatory characteristics and is permissive to *mycobacterium tuberculosis* infection. *J Immunol* (2019) 203(8):2252–64. doi: 10.4049/jimmunol.1900495

91. Canan CH, Gokhale NS, Carruthers B, Lafuse WP, Schlesinger LS, Torrelles JB, et al. Characterization of lung inflammation and its impact on macrophage function in aging. *J Leukoc Biol* (2014) 96(3):473–80. doi: 10.1189/jlb.4A0214-093RR

92. Duong L, Radley HG, Lee B, Dye DE, Pixley FJ, Grounds MD, et al. Macrophage function in the elderly and impact on injury repair and cancer. *Immun Ageing* (2021) 18(1):4. doi: 10.1186/s12979-021-00215-2

93. Kohut ML, SenChina DS, Madden KS, Martin AE, Felten DL, Moynihan JA. Age effects on macrophage function vary by tissue site, nature of stimulant, and exercise behavior. *Exp Gerontol* (2004) 39(9):1347–60. doi: 10.1016/j.exger.2004.07.001

94. Clay H, Volkman HE, Ramakrishnan L. Tumor necrosis factor signaling mediates resistance to mycobacteria by inhibiting bacterial growth and macrophage death. *Immunity* (2008) 29(2):283–94. doi: 10.1016/j.immuni.2008.06.011

95. Roca FJ, Ramakrishnan L. TNF dually mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. *Cell* (2013) 153(3):521–34. doi: 10.1016/j.cell.2013.03.022

96. Roca FJ, Whitworth LJ, Redmond S, Jones AA, Ramakrishnan L. TNF induces pathogenic programmed macrophage necrosis in tuberculosis through a mitochondrial-lysosomal-endoplasmic reticulum circuit. *Cell* (2019) 178(6):1344–61.e11. doi: 10.1016/j.cell.2019.08.004

97. Mahamed D, Boulle M, Ganga Y, Mc Arthur C, Skroch S, Oom L, et al. Intracellular growth of Mycobacterium tuberculosis after macrophage cell death leads to serial killing of host cells. *Elife* (2017) 6:e22028. doi: 10.7554/eLife.28205

98. Muller L, Di Benedetto S. How immunosenescence and inflammaging may contribute to hyperinflammatory syndrome in COVID-19. *Int J Mol Sci* (2021) 22 (22):12539. doi: 10.3390/ijms222212539

99. Tizazu AM, Mengist HM, Demeke G. Aging, inflammaging and immunosenescence as risk factors of severe COVID-19. *Immun Ageing* (2022) 19 (1):53. doi: 10.1186/s12979-022-00309-5

100. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced host response to SARS-coV-2 drives development of COVID-19. *Cell* (2020) 181(5):1036–45.e9. doi: 10.1016/j.cell.2020.04.026

101. Brabek J, Jakubek M, Vellieux F, Novotny J, Kolar M, Lacina L, et al. Interleukin-6: molecule in the intersection of cancer, ageing and COVID-19. *Int J Mol Sci* (2020) 21(21):7937. doi: 10.3390/ijms21217937

102. Mojtabavi H, Saghazadeh A, Rezaei N. Interleukin-6 and severe COVID-19: a systematic review and meta-analysis. *Eur Cytokine Netw* (2020) 31(2):44–9. doi: 10.1684/ecn.2020.0448

103. Asensi V, Valle E, Meana A, Fierer J, Celada A, Alvarez V, et al. *In vivo* interleukin-6 protects neutrophils from apoptosis in osteomyelitis. *Infect Immun* (2004) 72(7):3823–8. doi: 10.1128/IAI.72.7.3823-3828.2004

104. Kuri-Cervantes L, Pampena MB, Meng W, Rosenfeld AM, Ittner CAG, Weisman AR, et al. Comprehensive mapping of immune perturbations associated with severe COVID-19. *Sci Immunol* (2020) 5(49):eabd7114. doi: 10.1126/sciimmunol.abd7114

105. Pietrobon AJ, Teixeira FME, Sato MN. I mmunosenescence and inflammaging: risk factors of severe COVID-19 in older people. *Front Immunol* (2020) 11:579220. doi: 10.3389/fimmu.2020.579220

106. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T cell responses to SARS-coV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* (2020) 181(7):1489–501.e15. doi: 10.1016/j.cell.2020.05.015

107. Bektas A, Schurman SH, Franceschi C, Ferrucci L. A public health perspective of aging: do hyper-inflammatory syndromes such as COVID-19, SARS, ARDS, cytokine storm syndrome, and post-ICU syndrome accelerate short- and long-term inflammaging? *Immun Ageing* (2020) 17(1):23. doi: 10.1186/s12979-020-00196-8

108. Bartleson JM, Radenkovic D, Covarrubias AJ, Furman D, Winer DA, Verdin E. SARS-CoV-2, COVID-19 and the aging immune system. *Nat Aging* (2021) 1(9):769–82. doi: 10.1038/s43587-021-00114-7

109. Moliva JI, Rajaram MV, Sidiki S, Sasindran SJ, Guirado E, Pan XJ, et al. Molecular composition of the alveolar lining fluid in the aging lung. *Age (Dordr)* (2014) 36(3):9633. doi: 10.1007/s11357-014-9633-4

110. Torrelles JB, Schlesinger LS. Integrating lung physiology, immunology, and tuberculosis. *Trends Microbiol* (2017) 25(8):688–97. doi: 10.1016/j.tim.2017.03.007

111. Notter RH. *Lung surfactants: Basic science and clinical applications*. NY, US: Marcel Dekker (2000) p. 1–444.

112. Canadas O, Olmeda B, Alonso A, Perez-Gil J. Lipid-protein and protein-protein interactions in the pulmonary surfactant system and their role in lung homeostasis. *Int J Mol Sci* (2020) 21(10):3708. doi: 10.3390/ijms21103708

113. Gaynor CD, McCormack FX, Voelker DR, McGowan SE, Schlesinger LS. Pulmonary surfactant protein A mediates enhanced phagocytosis of *Mycobacterium tuberculosis* by a direct interaction with human macrophages. *J Immunol* (1995) 155 (11):5343–51. doi: 10.4049/jimmunol.155.11.5343

114. Ferguson JS, Voelker DR, McCormack FX, Schlesinger LS. Surfactant protein D binds to *Mycobacterium tuberculosis* bacilli and lipoarabinomannan via carbohydrate-lectin interactions resulting in reduced phagocytosis of the bacteria by macrophages. *J Immunol* (1999) 163(1):312–21. doi: 10.4049/jimmunol.163.1.312

115. Ferguson JS, Martin JL, Azad AK, McCarthy TR, Kang PB, Voelker DR, et al. Surfactant protein D increases fusion of *Mycobacterium tuberculosis*-containing phagosomes with lysosomes in human macrophages. *Infect Immun* (2006) 74 (12):7005–9. doi: 10.1128/IAI.01402-06

116. Schlesinger LS, Bellinger-Kawahara CG, Payne NR, Horwitz MA. Phagocytosis of *Mycobacterium tuberculosis* is mediated by human monocyte complement receptors and complement component C3. *J Immunol* (1990) 144(7):2771–80. doi: 10.4049/jimmunol.144.7.2771

117. Ferguson JS, Weis JJ, Martin JL, Schlesinger LS. Complement protein C3 binding to *Mycobacterium tuberculosis* is initiated by the classical pathway in human bronchoalveolar lavage fluid. *Infect Immun* (2004) 72(5):2564–73. doi: 10.1128/IAI.72.5.2564-2573.2004

118. Thacker VV, Dhar N, Sharma K, Barrile R, Karalis K. McKinney JD. A lung-on-chip model of early Mycobacterium tuberculosis infection reveals an essential role for alveolar epithelial cells in controlling bacterial growth. *Elife* (2020) 9:e59961. doi: 10.7554/eLife.59961

119. Hsieh MH, Ou CY, Hsieh WY, Kao HF, Lee SW, Wang JY, et al. Functional analysis of genetic variations in surfactant protein D in mycobacterial infection and their association with tuberculosis. *Front Immunol* (2018) 9. doi: 10.3389/fimmu.2018.01543

120. Garcia-Vilanova A, Olmo-Fontanez AM, Moliva JI, Allue-Guardia A, Singh H, Merritt RE, et al. The aging human lung mucosa: A proteomics study. *J Gerontol A Biol Sci Med Sci* (2022) 77(10):1969–74. doi: 10.1093/gerona/glac091

121. Moliva JI, Duncan MA, Olmo-Fontanez A, Akhter A, Arnett E, Scordo JM, et al. The lung mucosa environment in the elderly increases host susceptibility to *mycobacterium tuberculosis* infection. *J Infect Dis* (2019) 220(3):514–23. doi: 10.1093/infdis/jiz138

122. Scordo JM, Olmo-Fontanez AM, Kelley HV, Sidiki S, Arcos J, Akhter A, et al. The human lung mucosa drives differential *Mycobacterium tuberculosis* infection outcome in the alveolar epithelium. *Mucosal Immunol* (2019) 12(3):795–804. doi: 10.1038/s41385-019-0156-2

123. Olmo-Fontánez AM, Scordo JM, Garcia-Vilanova A, Maselli DJ, Peters JI, Restrepo BI, et al. Human alveolar lining fluid from the elderly promotes *Mycobacterium tuberculosis* growth in alveolar epithelial cells and bacterial translocation into the cytosol. *bioRxiv* (2021), 443884. doi: 10.1101/2021.05.12.443884

124. Allue-Guardia A, Garcia-Vilanova A, Olmo-Fontanez AM, Peters J, Maselli DJ, Wang Y, et al. Host- and Age-Dependent Transcriptional Changes in *Mycobacterium tuberculosis* Cell Envelope Biosynthesis Genes after Exposure to Human Alveolar Lining Fluid. *Int J Mol Sci* (2022) 23(2):983. doi: 10.3390/ijms23020983

125. Sinnberg T, Lichtensteiger C, Ali OH, Pop OT, Jochum AK, Risch L, et al. Pulmonary surfactant proteins are inhibited by immunoglobulin A autoantibodies in severe COVID-19. *Am J Respir Crit Care Med* (2023) 207(1):38–49. doi: 10.1164/rccm.202201-0011OC

126. Salvioni L, Testa F, Sulejmani A, Pepe F, Lovaglio PG, Berta P, et al. (SP-D) as a biomarker of SARS-CoV-2 infection. *Clin Chim Acta* (2022) 537:140–5. doi: 10.1016/j.cca.2022.10.013

127. Kervevan J, Chakrabarti LA. Role of CD4+ T cells in the control of viral infections: recent advances and open questions. *Int J Mol Sci* (2021) 22(2):523. doi: 10.3390/ijms22020523

128. Morgan J, Muskat K, Tippalagama R, Sette A, Burel J, Arlehamn CSL. Classical CD4 T cells as the cornerstone of antimycobacterial immunity. *Immunol Rev* (2021) 301(1):10–29. doi: 10.1111/imr.12963

129. Earle KA, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, et al. Evidence for antibody as a protective correlate for COVID-19 vaccines. *Vaccine* (2021) 39(32):4423–8. doi: 10.1016/j.vaccine.2021.05.063

130. Garcia-Beltran WF, Lam EC, Astudillo MG, Yang D, Miller TE, Feldman J, et al. COVID-19-neutralizing antibodies predict disease severity and survival. *Cell* (2021) 184(2):476–88.e11. doi: 10.1016/j.cell.2020.12.015

131. Collier DA, Ferreira I, Kotagiri P, Datir RP, Lim EY, Touizer E, et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature* (2021) 596(7872):417–22. doi: 10.1038/s41586-021-03739-1

132. Jergović M, Uhrlaub JL, Watanabe M, Bradshaw CM, White LM, LaFleur BJ, et al. Competent immune responses to SARS-CoV-2 variants in older adults following two doses of mRNA vaccination. *Nat Commun* (2022) 13(1):2891. doi: 10.1038/s41467-022-30617-9

133. Renia L, Goh YS, Rouers A, Le Bert N, Chia WN, Chavatte JM, et al. Lower vaccine-acquired immunity in the elderly population following two-dose BNT162b2 vaccination is alleviated by a third vaccine dose. *Nat Commun* (2022) 13(1):4615. doi: 10.1038/s41467-022-32312-1

134. Romero-Olmedo AJ, Schulz AR, Hochstätter S, Das Gupta D, Virta I, Hirseland H, et al. Induction of robust cellular and humoral immunity against SARS-CoV-2 after a third dose of BNT162b2 vaccine in previously unresponsive older adults. *Nat Microbiol* (2022) 7(2):195–9. doi: 10.1038/s41564-021-01046-z

135. Huang L, Nazarova EV, Russell DG. *Mycobacterium tuberculosis*: bacterial fitness within the host macrophage. *Microbiol spectrum* (2019) 7(2):7. doi: 10.1128/microbiolspec.BAI-0001-2019

136. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. *J Exp Med* (1993) 178(6):2249–54. doi: 10.1084/jem.178.6.2249

137. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J Exp Med* (1993) 178(6):2243–7. doi: 10.1084/jem.178.6.2243

138. Moreira-Teixeira L, Mayer-Barber K, Sher A, O'Garra A. Type I interferons in tuberculosis: Foe and occasionally friend. *J Exp Med* (2018) 215(5):1273–85. doi: 10.1084/jem.20180325

139. Mundra A, Yegiazaryan A, Karsian H, Alsaigh D, Bonavida V, Frame M, et al. Pathogenicity of type I interferons in *mycobacterium tuberculosis*. *Int J Mol Sci* (2023) 24(4):3919. doi: 10.3390/ijms24043919

140. Aiello A, Grossi A, Meschi S, Meledandri M, Vanini V, Petrone L, et al. Coordinated innate and T-cell immune responses in mild COVID-19 patients from household contacts of COVID-19 cases during the first pandemic wave. *Front Immunol* (2022) 13:920227. doi: 10.3389/fimmu.2022.920227

141. Mantlo E, Bukreyeva N, Maruyama J, Paessler S, Huang C. Antiviral activities of type I interferons to SARS-CoV-2 infection. *Antiviral Res* (2020) 179:104811. doi: 10.1016/j.antiviral.2020.104811

142. Yuen C-K, Lam J-Y, Wong W-M, Mak L-F, Wang X, Chu H, et al. SARS-CoV-2 nsp13, nsp14, nsp15 and orf6 function as potent interferon antagonists. *Emerging Microbes infections* (2020) 9(1):1418–28. doi: 10.1080/22221751.2020.1780953

143. Lei X, Dong X, Ma R, Wang W, Xiao X, Tian Z, et al. Activation and evasion of type I interferon responses by SARS-CoV-2. *Nat Commun* (2020) 11(1):3810. doi: 10.1038/s41467-020-17665-9

144. Xia H, Cao Z, Xie X, Zhang X, Chen JY-C, Wang H, et al. Evasion of type I interferon by SARS-CoV-2. *Cell Rep* (2020) 33(1):108234. doi: 10.1016/j.celrep.2020.108234

145. Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science* (2020) 370(6515):eabd4570. doi: 10.1126/science.abd4570

146. Israelow B, Song E, Mao T, Lu P, Meir A, Liu F, et al. Mouse model of SARS-CoV-2 reveals inflammatory role of type I interferon signaling. *J Exp Med* (2020) 217 (12):e20201241. doi: 10.1084/jem.20201241

147. Chen LF, Yang CD, Cheng XB. Anti-interferon autoantibodies in adult-onset immunodeficiency syndrome and severe COVID-19 infection. *Front Immunol* (2021) 12:788368. doi: 10.3389/fimmu.2021.788368

148. Döffinger R, Helbert MR, Barcenas-Morales G, Yang K, Dupuis S, Ceron-Gutierrez L, et al. Autoantibodies to interferon-gamma in a patient with selective susceptibility to mycobacterial infection and organ-specific autoimmunity. *Clin Infect Dis* (2004) 38(1):e10–4. doi: 10.1086/380453

149. Höflich C, Sabat R, Rosseau S, Temmesfeld B, Slevogt H, Döcke WD, et al. Naturally occurring anti-IFN-gamma autoantibody and severe infections with *Mycobacterium cheloneae* and Burkholderia cocovenenans. *Blood* (2004) 103(2):673–5. doi: 10.1182/blood-2003-04-1065

150. Kampmann B, Hemingway C, Stephens A, Davidson R, Goodsall A, Anderson S, et al. Acquired predisposition to mycobacterial disease due to autoantibodies to IFN-gamma. *J Clin Invest* (2005) 115(9):2480–8. doi: 10.1172/JCI19316

151. Madariaga L, Amurrio C, Martin G, Garcia-Cebrian F, Bicandi J, Lardelli P, et al. Detection of anti-interferon-gamma autoantibodies in subjects infected by Mycobacterium tuberculosis. *Int J Tuberc Lung Dis* (1998) 2(1):62–8.

152. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* (2020) 370(6515):eabd4585. doi: 10.1126/science.abd4585

153. Manry J, Bastard P, Gervais A, Le Voyer T, Rosain J, Philippot Q, et al. The risk of COVID-19 death is much greater and age dependent with type I IFN autoantibodies. *Proc Natl Acad Sci U S A* (2022) 119(21):e2200413119. doi: 10.1073/pnas.2200413119

154. Bastard P, Gervais A, Le Voyer T, Rosain J, Philippot Q, Manry J, et al. Autoantibodies neutralizing type I IFNs are present in ~4% of uninfected individuals over 70 years old and account for ~20% of COVID-19 deaths. *Sci Immunol* (2021) 6 (62):eabl4340. doi: 10.1126/sciimmunol.abl4340

155. Zhao TV, Sato Y, Goronzy JJ, Weyand CM. T-cell aging-associated phenotypes in autoimmune disease. *Front Aging* (2022) 3:867950. doi: 10.3389/fragi.2022.867950

156. Wang M, Yin X, Zhang S, Mao C, Cao N, Yang X, et al. Autoantibodies against AT1 receptor contribute to vascular aging and endothelial cell senescence. *Aging Dis* (2019) 10(5):1012–25. doi: 10.14336/AD.2018.0919

157. Divo MJ, Martinez CH, Mannino DM. Ageing and the epidemiology of multimorbidity. *Eur Respir J* (2014) 44(4):1055–68. doi: 10.1183/09031936.00059814

158. Zoppini G, Fedeli U, Schievano E, Dauriz M, Targher G, Bonora E, et al. Mortality from infectious diseases in diabetes. *Nutrition Metab Cardiovasc Diseases* (2018) 28(5):444–50. doi: 10.1016/j.numecd.2017.12.007

159. Boyle JP, Thompson TJ, Gregg EW, Barker LE, Williamson DF. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. *Popul Health Metr* (2010) 8:29. doi: 10.1186/1478-7954-8-29

160. WHO. *Global report on diabetes*. (2016).

161. Batsis JA, Zagaria AB. Addressing obesity in aging patients. *Med Clin North Am* (2018) 102(1):65–85. doi: 10.1016/j.mcna.2017.08.007

162. Tuduri E, Soriano S, Almagro L, Montanya E, Alonso-Magdalena P, Nadal A, et al. The pancreatic beta-cell in ageing: Implications in age-related diabetes. *Ageing Res Rev* (2022) 80:101674. doi: 10.1016/j.arr.2022.101674

163. Bellary S, Kyrou I, Brown JE, Bailey CJ. Type 2 diabetes mellitus in older adults: clinical considerations and management. *Nat Rev Endocrinol* (2021) 17(9):534–48. doi: 10.1038/s41574-021-00512-2

164. Sen S, Chakraborty R, Kalita P, Pathak MP. Diabetes mellitus and COVID-19: Understanding the association in light of current evidence. *World J Clin Cases* (2021) 9 (28):8327–39. doi: 10.12998/wjcc.v9.i28.8327

165. Zhang L, Jiang F, Xie Y, Mo Y, Zhang X, Liu C. Diabetic endothelial microangiopathy and pulmonary dysfunction. *Front Endocrinol (Lausanne)* (2023) 14:1073878. doi: 10.3389/fendo.2023.1073878

166. Foster DJ, Ravikumar P, Bellotto DJ, Unger RH, Hsia CC. Fatty diabetic lung: altered alveolar structure and surfactant protein expression. *Am J Physiol Lung Cell Mol Physiol* (2010) 298(3):L392–403. doi: 10.1152/ajplung.00041.2009

167. Marunaka Y. The Proposal of Molecular Mechanisms of Weak Organic Acids Intake-Induced Improvement of Insulin Resistance in Diabetes Mellitus via Elevation of Interstitial Fluid pH. *Int J Mol Sci* (2018) 19(10):3244. doi: 10.3390/ijms19103244

168. Sharma JK, Rohatgi A, Sharma D. Diabetic autonomic neuropathy: a clinical update. *J R Coll Physicians Edinb* (2020) 50(3):269–73. doi: 10.4997/jrcpe.2020.310

169. Whyte MB, Vas P, Heiss C, Feher MD. The contribution of diabetic micro-angiopathy to adverse outcomes in COVID-19. *Diabetes Res Clin Pract* (2020) 164:108217. doi: 10.1016/j.diabres.2020.108217

170. Restrepo BI. Diabetes and tuberculosis. *Microbiol Spectr* (2016) 4(6):10.1128/microbiolspec.TNMI7-0023-2016. doi: 10.1128/microbiolspec.TNMI7-0023-2016

171. Ngo MD, Bartlett S, Ronacher K. Diabetes-associated susceptibility to tuberculosis: contribution of hyperglycemia vs. Dyslipidemia. *Microorganisms* (2021) 9(11):2282. doi: 10.3390/microorganisms9112282

172. Vallerskog T, Martens GW, Kornfeld H. Diabetic mice display a delayed adaptive immune response to Mycobacterium tuberculosis. *J Immunol* (2010) 184 (11):6275–82. doi: 10.4049/jimmunol.1000304

173. Kumar NP, Fukutani KF, Shruthi BS, Alves T, Silveira-Mattos PS, Rocha MS, et al. Persistent inflammation during anti-tuberculosis treatment with diabetes comorbidity. *Elife* (2019) 8:e46477. doi: 10.7554/eLife.46477

174. Kumar Nathella P, Babu S. Influence of diabetes mellitus on immunity to human tuberculosis. *Immunology* (2017) 152(1):13–24. doi: 10.1111/imm.12762

175. Jali MV, Kavital A, Hiremath MB. Challenges of diabetes in elderly TB patients. *Indian J Tuberc* (2022) 69 Suppl 2:S264–S6. doi: 10.1016/j.ijtb.2022.10.017

176. Perez-Guzman C, Vargas MH. Diabetes, aging, and tuberculosis. *Lung India* (2011) 28(3):191–2. doi: 10.4103/0970-2113.83976

177. Menon S, Rossi R, Dusabimana A, Zdraveska N, Bhattacharyya S, Francis J. The epidemiology of tuberculosis-associated hyperglycemia in individuals newly screened for type 2 diabetes mellitus: systematic review and meta-analysis. *BMC Infect Dis* (2020) 20(1):937. doi: 10.1186/s12879-020-05512-7

178. Magee MJ, Salindri AD, Kyaw NTT, Auld SC, Haw JS, Umpierrez GE. Stress hyperglycemia in patients with tuberculosis disease: epidemiology and clinical implications. *Curr Diabetes Rep* (2018) 18(9):71. doi: 10.1007/s11892-018-1036-y

179. Fadini G, Morieri M, Longato E, Avogaro DA. Prevalence and impact of diabetes among people infected with SARS-CoV-2. *J Endocrinological Invest* (2020) 43:867–9. doi: 10.1007/s40618-020-01236-2

180. Zhang Y, Li H, Zhang J, Cao Y, Zhao X, Yu N, et al. The clinical characteristics and outcomes of patients with diabetes and secondary hyperglycaemia with coronavirus disease 2019: A single-centre, retrospective, observational study in Wuhan. *Diabetes Obes Metab* (2020) 22(8):1443–54. doi: 10.1111/dom.14086

181. Roncon L, Zuin M, Rigatelli G, Zuliani G. Diabetic patients with COVID-19 infection are at higher risk of ICU admission and poor short-term outcome. *J Clin Virol* (2020) 127:104354. doi: 10.1016/j.jcv.2020.104354

182. Guo L, Shi Z, Zhang Y, Wang C, Do Vale Moreira NC, Zuo H, et al. Comorbid diabetes and the risk of disease severity or death among 8807 COVID-19 patients in China: A meta-analysis. *Diabetes Res Clin Pract* (2020) 166:108346. doi: 10.1016/j.diabres.2020.108346

183. Ou M, Zhu J, Ji P, Li H, Zhong Z, Li B, et al. Risk factors of severe cases with COVID-19: a meta-analysis. *Epidemiol Infect* (2020) 148:e175. doi: 10.1017/S095026882000179X

184. Chang MC, Park Y-K, Kim B-O, Park D. Risk factors for disease progression in COVID-19 patients. *BMC Infect diseases* (2020) 20:1–6. doi: 10.1186/s12879-020-05144-x

185. Singh AK, Gupta R, Ghosh A, Misra A. Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations. *Diabetes Metab Syndrome: Clin Res Rev* (2020) 14(4):303–10. doi: 10.1016/j.dsx.2020.04.004

186. Bosch AA, Biesbroek G, Trzcinski K, Sanders EA, Bogaert D. Viral and bacterial interactions in the upper respiratory tract. *PloS Pathog* (2013) 9(1):e1003057. doi: 10.1371/journal.ppat.1003057

187. Piret J, Boivin G. Viral interference between respiratory viruses. *Emerging Infect Diseases* (2022) 28(2):273. doi: 10.3201/eid2802.211727

188. Rosas Mejia O, Gloag ES, Li J, Ruane-Foster M, Claeys TA, Farkas D, et al. Mice infected with *Mycobacterium tuberculosis* are resistant to acute disease caused by secondary infection with SARS-CoV-2. *PloS Pathog* (2022) 18(3):e1010093. doi: 10.1371/journal.ppat.1010093

189. Baker PJ, Amaral E P, Castro E, Bohrer AC, Torres-Juárez F, Jordan CM, et al. Co-infection of mice with SARS-CoV-2 and Mycobacterium tuberculosis limits early viral replication but does not affect mycobacterial loads. *Front Immunol* (2023) 14:1240419. doi: 10.3389/fimmu.2023.1240419

190. Hilligan KL, Namasivayam S, Clancy CS, O'Mard D, Oland SD, Robertson SJ, et al. Intravenous administration of BCG protects mice against lethal SARS-CoV-2 challenge. *J Exp Med* (2022) 219(2):e20211862. doi: 10.1084/jem.20211862

191. Flores-Lovon K, Ortiz-Saavedra B, Cueva-Chicana LA, Aperrigue-Lira S, Montes-Madariaga ES, Soriano-Moreno DR, et al. Immune responses in COVID-19 and tuberculosis coinfection: A scoping review. *Front Immunol* (2022) 13:992743. doi: 10.3389/fimmu.2022.992743

192. Aiello A, Najafi-Fard S, Goletti D. Initial immune response after exposure to Mycobacterium tuberculosis or to SARS-COV-2: similarities and differences. *Front Immunol* (2023) 14. doi: 10.3389/fimmu.2023.1244556

193. Luke E, Swafford K, Shirazi G, Venketaraman V. TB and COVID-19: an exploration of the characteristics and resulting complications of co-infection. *Front Biosci (Schol Ed)* (2022) 14(1):6. doi: 10.31083/j.fbs1401006

194. Shah T, Shah Z, Yasmeen N, Baloch Z, Xia X. Pathogenesis of SARS-coV-2 and *mycobacterium tuberculosis* coinfection. *Front Immunol* (2022) 13:909011. doi: 10.3389/fimmu.2022.909011

195. Petrone L, Petruccioli E, Vanini V, Cuzzi G, Gualano G, Vittozzi P, et al. Coinfection of tuberculosis and COVID-19 limits the ability to in *vitro* respond to SARS-CoV-2. *Int J Infect Dis* (2021) 113 Suppl 1:S82–S7. doi: 10.1016/j.ijid.2021.02.090

196. Sheerin D, Abhimanyu, Peton N, Vo W, Allison CC, Wang X. Immunopathogenic overlap between COVID-19 and tuberculosis identified from transcriptomic meta-analysis and human macrophage infection. *Iscience* (2022) 25 (6):104464. doi: 10.1016/j.isci.2022.104464

197. Riou C, du Bruyn E, Stek C, Daroowala R, Goliath RT, Abrahams F, et al. Relationship of SARS-CoV-2-specific CD4 response to COVID-19 severity and impact of HIV-1 and tuberculosis coinfection. *J Clin Invest* (2021) 131(12):e149125. doi: 10.1172/JCI149125

198. Najafi-Fard S, Aiello A, Navarra A, Cuzzi G, Vanini V, Migliori GB, et al. Characterization of the immune impairment of patients with tuberculosis and COVID-19 coinfection. *Int J Infect Dis* (2023) 130 Suppl 1:S34–42. doi: 10.1016/j.ijid.2023.03.021

199. Nabity SA, Han E, Lowenthal P, Henry H, Okoye N, Chakrabarty M, et al. Sociodemographic characteristics, comorbidities, and mortality among persons diagnosed with tuberculosis and COVID-19 in close succession in california, 2020. *JAMA Network Open* (2021) 4(12):e2136853. doi: 10.1001/jamanetworkopen.2021.36853

200. Kim B, Kang YA, Lee J. Heterogeneous impact of Covid-19 response on tuberculosis burden by age group. *Sci Rep* (2022) 12(1):13773. doi: 10.1038/s41598-022-18135-6

201. Group TC-GS. Tuberculosis and COVID-19 co-infection: description of the global cohort. *Eur Respir J* (2022) 59(3):2102538. doi: 10.1183/13993003.02538-2021

202. Gupta N, Ish P, Gupta A, Malhotra N, Caminero JA, Singla R, et al. A profile of a retrospective cohort of 22 patients with COVID-19 and active/treated tuberculosis. *Eur Respir J* (2020) 56(5):2003408. doi: 10.1183/13993003.03408-2020

203. Elziny MM, Ghazy A, Elfert KA, Aboukamar M. Case report: development of miliary pulmonary tuberculosis in a patient with peritoneal tuberculosis after COVID-19 upper respiratory tract infection. *Am J Trop Med Hyg* (2021) 104(5):1792–5. doi: 10.4269/ajtmh.20-1156

204. Visca D, Ong CWM, Tiberi S, Centis R, D'Ambrosio L, Chen B, et al. Tuberculosis and COVID-19 interaction: A review of biological, clinical and public health effects. *Pulmonology* (2021) 27(2):151–65. doi: 10.1016/j.pulmoe.2020.12.012

205. Yang H, Lu S. COVID-19 and tuberculosis. *J Transl Int Med* (2020) 8(2):59–65. doi: 10.2478/jtim-2020-0010

206. Daneshvar P, Hajikhani B, Sameni F, Noorisepehr N, Zare F, Bostanshirin N, et al. COVID-19 and tuberculosis coinfection: An overview of case reports/case series and meta-analysis of prevalence studies. *Heliyon* (2023) 9(2):e13637. doi: 10.1016/j.heliyon.2023.e13637

207. Norwood J, Kheshti A, Shepherd BE, Rebeiro PF, Ahonkhai A, Kelly S, et al. The impact of COVID-19 on the HIV care continuum in a large urban southern clinic. *AIDS Behav* (2022) 26(8):2825–9. doi: 10.1007/s10461-022-03615-7

208. Karim F, Gazy I, Cele S, Zungu Y, Krause R, Bernstein M, et al. HIV status alters disease severity and immune cell responses in beta variant SARS-CoV-2 infection wave. *Elife* (2021) 10:e67397. doi: 10.7554/eLife.67397

209. Conan N, Simons E, Ohler L, Mbatha M, van Cutsem G, Huerga H. Prevalence of TB and health-seeking behaviour. *Int J Tuberc Lung Dis* (2022) 26(5):463–5. doi: 10.5588/ijtld.22.0001

210. Grifoni A, Alonzi T, Alter G, Noonan DM, Landay AL, Albini A, et al. Impact of aging on immunity in the context of COVID-19, HIV, and tuberculosis. *Front Immunol* (2023) 14:1146704. doi: 10.3389/fimmu.2023.1146704

211. Marcu DTM, Adam CA, Mitu F, Cumpat C, Aursulesei Onofrei V, Zabara ML, et al. Cardiovascular involvement in tuberculosis: from pathophysiology to diagnosis and complications-A narrative review. *Diagnostics (Basel)* (2023) 13(3):432. doi: 10.3390/diagnostics13030432

212. Styskal J, Van Remmen H, Richardson A, Salmon AB. Oxidative stress and diabetes: what can we learn about insulin resistance from antioxidant mutant mouse models? *Free Radic Biol Med* (2012) 52(1):46–58. doi: 10.1016/j.freeradbiomed.2011.10.441

213. Nomoto H, Hayakawa K, Ohmagari N. Impact of prioritized vaccinations for the elderly on the COVID-19 pandemic in Japan. *Glob Health Med* (2022) 4(2):129–32. doi: 10.35772/ghm.2022.01015

214. Motala AA, Mbanya JC, Ramaiya K, Pirie FJ, Ekoru K. Type 2 diabetes mellitus in sub-Saharan Africa: challenges and opportunities. *Nat Rev Endocrinology* (2022) 18 (4):219–29. doi: 10.1038/s41574-021-00613-y

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Vaccine development against tuberculosis before and after Covid-19

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Coronavirus disease (Covid-19) has not only shaped awareness of the impact of infectious diseases on global health. It has also provided instructive lessons for better prevention strategies against new and current infectious diseases of major importance. Tuberculosis (TB) is a major current health threat caused by *Mycobacterium tuberculosis* (Mtb) which has claimed more lives than any other pathogen over the last few centuries. Hence, better intervention measures, notably novel vaccines, are urgently needed to accomplish the goal of the World Health Organization to end TB by 2030. This article describes how the research and development of TB vaccines can benefit from recent developments in the Covid-19 vaccine pipeline from research to clinical development and outlines how the field of TB research can pursue its own approaches. It begins with a brief discussion of major vaccine platforms in general terms followed by a short description of the most widely applied Covid-19 vaccines. Next, different vaccination regimes and particular hurdles for TB vaccine research and development are described. This specifically considers the complex immune mechanisms underlying protection and pathology in TB which involve innate as well as acquired immune mechanisms and strongly depend on fine tuning the response. A brief description of the TB vaccine candidates that have entered clinical trials follows. Finally, it discusses how experiences from Covid-19 vaccine research, development, and rollout can and have been applied to the TB vaccine pipeline, emphasizing similarities and dissimilarities.

KEYWORDS

tuberculosis, COVID-19, vaccines, correlate of protection, protective antigen, prevention of disease, prevention of infection, prevention of recurrence

1 Introduction

The Severe Acquired Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) has had an unprecedented impact on our understanding and awareness of the continuous threat of emerging infectious diseases. Coronavirus disease 2019 (Covid-19), caused the death of more than seven million individuals (1, 2) The World Health Organization (WHO) has

estimated mortality rates to be approximately 15 million deaths over three years (3). In addition, Covid-19 led to a sudden increase in incidences of numerous other communicable and non-communicable diseases (4). One disease that was been profoundly affected is tuberculosis (TB) (5). This was due to multiple factors, notably the disruption of laboratory services, shortages of drug supply, and deviation of funding and personnel to diagnosis and care of Covid-19 patients. During the height of the Covid-19 crisis, TB morbidity and mortality increased for the first time in the 21st century to 10-11 million new cases and 1.6 million deaths in 2021 (5). It has been estimated that over the last 200 years, TB has been the cause of one billion deaths averaging annual mortality in the order of five million deaths over 200 years, similar to the estimated five million annual deaths caused by Covid-19 over three years (5–9). It is now clear that TB patients after successful therapy can develop post-TB, which not only affects the lungs but can also lead to other disabilities, notably neurological and cardiac impairments. The Covid-19 crisis led to reduced case findings and therapy for TB. This coincidence will likely lead to the increased appearance of post-TB. Thus, the consequences of the Covid-19 TB syndemic will have a much greater impact on health and consequently on economic losses in the years to come (10, 11). Yet, to my knowledge, there is no direct information regarding the impact of Covid-19 on post-TB.

As soon as the pandemic potential of SARS-CoV-2 became apparent, multiple efforts were undertaken to develop and deploy Covid-19 vaccines at unprecedented speed (6). Research and development (R&D) of Covid-19 vaccines could build on knowledge gathered in the aftermath of the emergence of SARS-CoV-1 and the Middle East Respiratory Syndrome (MERS) virus, even though these coronaviruses had been brought under control by conventional public health measures (12). Through these efforts, it became clear that the Spike protein mediates virus attachment to and entry into host cells and that blocking the viral attachment by neutralizing antibodies represents a key protective mechanism (13).

Supported by virtually unlimited funding, the research and development (R&D) of SARS-CoV-2 vaccines was pursued at accelerated speed through remarkable collaborations between scientific communities across continents (14). Furthermore, adoptive trial design, streamlined regulatory processes, expedited regulatory review and rapid emergency use approval made vaccine rollout possible within less than one year (15). Complemented by scaled-up manufacturing capacities, millions of lives could be saved. The mRNA encapsulated in lipid nanoparticles (LNP) turned out the most efficacious vaccine platform (16). Although this platform was a new aspect of the vaccine portfolio, its manufacturing could be scaled up rapidly. In total, more than 13 billion doses were deployed in record time, nearly fulfilling the demands of the industrialized world. This scenario, however, was overshadowed by inequitable access to vaccines in low- and middle-income countries (17).

Vaccine R&D in general has benefited from the example of the Covid-19 vaccine pipeline in several instances. First, virtually unlimited investment into novel vaccines in the very beginning does not only save lives but also generates a return on investment (14, 18, 19). A study in New York provides an illustrative example by showing that 10 US$ was saved for every 1 US$ invested in vaccination against Covid-19 (19). Second, adoptive trials combining safety and efficacy assessments are feasible (20). Third, accelerated regulatory processes as well as provisional authorization for emergency use act as accelerators. Fourth, vaccine rollout at a large scale in high-income countries proved that logistic, manufacturing, and deployment hurdles can be overcome. Fifth, the speed of vaccine development can be markedly accelerated by sharing data and samples. Sixth, vaccines need to be made available across continents including low- and middle-income countries (17). As a corollary, R&D centers as well as manufacturing facilities, complemented by an infrastructure that guarantees adequate education, trust, and expertise in the global south are needed to ensure a robust supply chain for equitable access to vaccines (17, 21).

Applying the lessons learned in recent vaccine R&D will enable a more rapid response against future emerging diseases with pandemic potential. This will also promote vaccine R&D against diseases that already pose an enormous threat and for which efficacious vaccines are not yet available, such as Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS), malaria, Hepatitis C, Dengue, and TB. There will be no strategy that fits all; thus, specific modifications are critical for each vaccination strategy under development. It is also unclear whether the new mRNA : LNP vaccine type, which was so successful in the case of Covid-19, can be applied to infectious diseases that are chronic and controlled by complex cell-mediated rather than humoral immunity, such as TB.

This article briefly discusses the major vaccine platforms in general terms (section 2), summarizes the major Covid-19 vaccines (section 3), and reviews immunity in TB (section 4). It then discusses different vaccination regimes and hurdles for vaccine R&D relevant to TB (section 5) before describing the pipeline of TB vaccines in clinical trials in more detail (section 6). The final sections examine which lessons from Covid-19 vaccine R&D could benefit the TB vaccine portfolio and what approach TB research needs to undertake (sections 7 and 8).

2 Major vaccine platforms

Vaccines can be divided into subunit vaccines or whole-cell vaccines (22). To ensure induction of adequate immunity, major subunit vaccine platforms comprise: (i) well-defined antigen(s) formulated in adjuvant, (ii) mRNA encoding such antigen(s) and packaged in LNP (mRNA : LNP), or (iii) bacterial or viral vectors expressing such antigen(s). Whole-cell vaccines are either inactivated non-viable or attenuated viable vaccines. They more or less comprise all antigens of the pathogen independent of their role in protective immunity.

2.1 Subunit vaccines

The most successful subunit vaccines target pathogens that are primarily controlled by neutralizing antibodies (22). These types of

vaccines depend on one or a few protective antigen(s) that either cause disease directly or are critical for the establishment of stable infection, e.g. by mediating entry into host cells. These include antiviral vaccines (e.g. Hepatitis B), antitoxin vaccines (diphtheria and tetanus), or conjugate vaccines (pneumococci). Further improvement can be accomplished by generating virus-like particles in which the protective antigen forms structured particles resembling the viral pathogen. First-generation adjuvants, notably aluminum salts primarily stimulate the production of neutralizing antibodies.

More recent advances have led to the creation of adjuvant formulations that also stimulate cell-mediated immune responses including CD4 and CD8 T cells (23–26). These novel adjuvants include surface-active components such as saponins (e.g. QS21, an active compound from the bark of Quillaja Saponaria), ligands for pattern recognition receptors, notably toll-like receptors (TLRs), and aqueous and oleaginous formulations that ensure continuous antigen release over prolonged periods of time. The choice of TLR ligands depends on the type of pathogen targeted by the specific vaccine, e.g. TLR-7/TLR-8 ligands for viral and TLR-9 ligands for bacterial pathogens. Examples of T- cell stimulating adjuvants are $AS01_E$ (adjuvant system 01_E) and ISCOM (immune stimulating complex) based adjuvants (23–25). Alternatively, recombinant viral vectors expressing vaccine antigen(s) have been generated, which are mostly replication-deficient (27–31). The recently licensed Ebola vaccination scheme is based on a prime/boost scheme comprising adenovirus (Ad) 26 and Modified Vaccinia Ankara (MVA) virus as vectors, both expressing Ebola antigen (32). Another example is the chimpanzee adenovirus Oxford (ChAdOx) vector expressing the Spike protein of SARS-CoV-2 against Covid-19 (Vaxzevria by Oxford/AstraZeneca).

The major breakthrough in mRNA : LNP vaccine development was the encoding of modified Spike protein (mRNA: LNP) (16, 33, 34). These vaccines exploit Methyl-Pseudouridine modifications of mRNA leading to superior vaccine efficacy compared to unmodified mRNA. The higher efficacy of modified mRNA over unmodified mRNA is likely due to the more rapid inactivation by an innate immune response (35). Principally, LNP are composed of long-chain fatty acids, cholesterol, and polyethylene glycol. The latter may be substituted by polysarcosine with a lower risk of adverse events. In short, LNP (i) protect RNA from rapid degradation; (ii) facilitate introduction into host cells; and (iii) provide a certain degree of adjuvanticity.

2.2 Whole cell vaccines

Whole cell vaccines are preferred when protective antigens do not exist or have not been identified. They are given in inactivated form or as attenuated live vaccines. Several inactivated vaccines have been successfully deployed for viral infections such as the inactivated vaccines against Hepatitis A, Polio (Salk vaccine), and Influenza. Yet, only a few inactivated vaccines have been introduced for control of bacterial infections such as the Cholera vaccine. To improve the protective immune response, adjuvants may be required. In contrast, attenuated viable vaccines generally get by without adjuvant. Attenuated vaccines have been most successfully deployed against viral pathogens including measles, mumps, rubella, or polio (Sabin vaccine). The most widely distributed attenuated vaccine against a bacterial pathogen, Bacille-Calmette-Guérin (BCG), targets TB, but with limited success (36).

3 A short primer on Covid-19 vaccines

Roughly one year after the introduction of the first vaccines against Covid-19, numerous vaccines had been rolled out in different regions of the globe and more than 11 vaccines have been granted emergency use listing (EUL) by the WHO (37). These include inactivated whole cell vaccines, viral-vectored vaccines, protein: adjuvant vaccines, and mRNA : LNP vaccines. Some of these vaccines had been approved in a large number of states, notably for emergency use; others had received approval in only a few countries. In the following, a brief description of the most widely used vaccines granted EUL by the WHO is provided (Figure 1).

The inactivated whole cell vaccines Covilo and CoronaVac by Sinopharm or Sinovac, respectively, had been approved rapidly in China and received permission for emergency use in other countries (38, 39). Both vaccines had been inactivated with beta-propiolactone and formulated in alum salt as adjuvant to stimulate neutralizing antibodies (40). These vaccines probably possess low T cell stimulatory activity because of the exclusive use of alum and they would likely benefit from a T cell stimulating adjuvant. In contrast, the inactivated vaccine VLA2001 of Valneva is formulated in alum salt plus CpG as a TLR-9 agonist, thereby stimulating both humoral and cellular immune responses (41). Similarly, Covaxin from Bharat Biotech contains inactivated SARS-CoV-2 formulated in alum adsorbed TLR-7/TLR-8 agonist (42).

Ad has been the preferred vector system for the expression of the Spike protein. These include human Ad26 and Ad5 as well as the chimpanzee Ad, ChAdOx (30). These Ad serotypes were chosen to avoid rapid inactivation of the carrier by pre-existing antibodies induced by circulating Ad serotypes. The prevalence of Ad26 and Ad5 in humans is low and ChAdOx does not circulate in humans. To avoid the generation of novel virus particles in the immunized host, the Ad vectors have been rendered non-replicative. The ChAdOx vaccine (Vaxzevria from Oxford AstraZeneca, COVISHIELD from Serum Institute of India) given as homologous prime/boost, has been broadly deployed (42, 43). The Ad26-based vaccine JCOVDEN from Janssen (Johnson & Johnson) and the Ad5-based vaccine Convidecia from CanSino Biologics are considered single shot vaccines (44, 45).

The protein:adjuvant vaccine (Nuvaxovid from Novavax, COVOVAX from Serum Institute of India) had received emergency use in several countries (40, 46). This vaccine is composed of protein nanoparticles (similar to virus-like particles) incorporated in the Matrix-M adjuvant containing saponin and based on ISCOM.

Within less than a year, the mRNA : LNP vaccines turned out to be most efficacious with the frontrunners produced by Pfizer/BioNTech (Comirnaty) and Moderna (Spikevax) (34, 47, 48). The mRNA : LNP vaccines comprise a modified mRNA encoding part of the Spike protein as an antigen. The mRNA : LNP vaccines do not only stimulate neutralizing antibodies but also T cell responses that recognize conserved epitopes in the Spike protein, which are

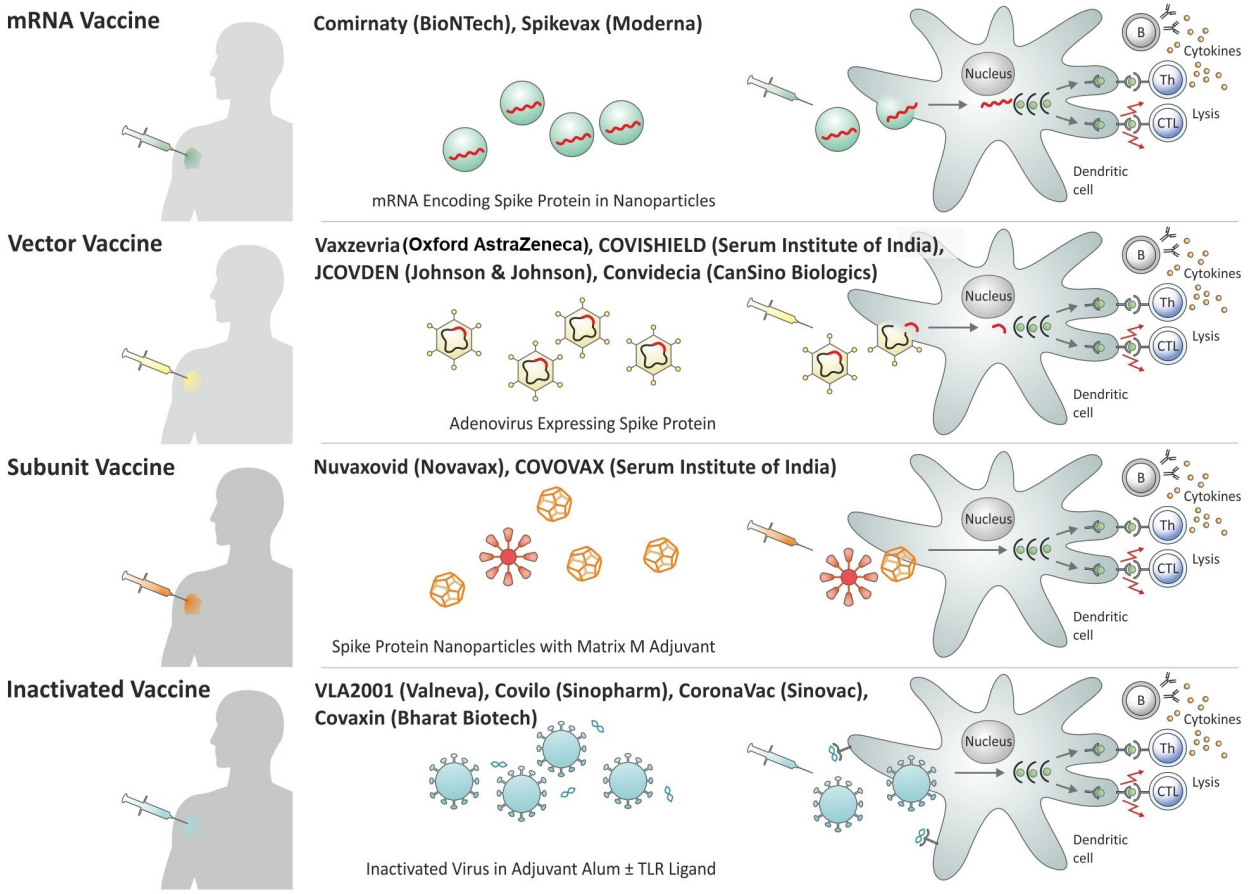

FIGURE 1
Overview of Covid-19 vaccines granted Emergency Use Listing (EUL) by the World Health Organization (WHO) by platform.

broadly shared with various coronaviruses including circulating viruses and novel variants of SARS-CoV-2. Hence, they elicit protective immunity against severe disease even in cases in which the highly specific antibodies fail to adequately neutralize new mutations in the Spike protein.

In summary, the major lessons learned from the R&D of the Covid-19 vaccines can be summarized as follows:

- Neutralizing antibodies specific for the receptor binding domain (RBD) within the Spike protein directed at the angiotensin converting enzyme 2 (ACE-2) receptor reduce infection by blocking attachment to and entry into host cells of SARS-CoV2 (49). Because of the intracellular lifestyle of Mtb, which primarily resides in macrophages, neutralizing antibodies against protective antigens do not exist in TB (see 4). This represents the Achilles' heel of TB vaccine development. These neutralizing antibodies are highly specific and hence cause immune pressure favoring viral mutations to evade protective immunity. Selection of such mutated strains can rapidly lead to the emergence and spreading of novel strains which render available vaccines partially ineffective.
- Aside from neutralizing antibodies, non-neutralizing antibodies, and T lymphocytes specific for epitopes located outside of the RBD of the Spike protein are being generated (50–52). Non-neutralizing antibodies contribute to protection via additional effector mechanisms, notably complement activation, attraction of inflammatory cells, and arming of NK cells for antibody-dependent cellular cytotoxicity (ADCC).
- T lymphocytes directed at conserved epitopes in the Spike protein can contribute to protection at later stages, notably through lysis of infected cells, which ultimately blocks viral replication (53, 54). Aside from these direct effector functions, mostly executed by CD8 T cells with cytolytic activity (cytolytic T lymphocytes, CTL), CD4 helper T cells (Th cells) are activated. Neutralizing antibodies depend on Th2 cells, whereas non-neutralizing antibodies require help from both Th1 and Th2 cells. Th1 cells are also required for activation of CTL and mononuclear phagocytes and Th17 cells can attract inflammatory cells to the site of viral replication.

The development of effective Covid-19 vaccines was an outstanding success story. Yet, in the long-term, a universal pan-corona vaccine providing long-term protection would be extremely valuable. Such next generation vaccines should induce an immune response comprising:

- neutralizing antibodies to the Spike RBD;
- trained immunity for rapid dampening of infection (55);
- broadly reactive antibodies for conserved epitopes with low selection advantage (50, 51, 56);
- CD4 and CD8 T cells to conserved Spike epitopes and perhaps other viral components with low selection advantage;
- additionally, unconventional T cells such as mucosal-associated invariant T (MAIT) cells should be considered.

Figure 2 schematically summarizes protective immunity elicited by SARS-CoV-2 infection and by mRNA : LNP vaccines against Covid-19.

4 Immunity in TB: protection and pathology

Establishment of infection (Figure 3): TB is primarily a disease of the lung that also serves as the main port of entry for the causative agent, *Mycobacterium tuberculosis* (Mtb) (57, 58). TB is transmitted via aerosols, coughed up by a patient with active TB although other modes of transmission are possible. Pathogens transmitted via the aerogenic route enter the lung alveoli within small aerosol particles which provide some shield for Mtb. Bacteria are engulfed by alveolar macrophages, tissue-resident mononuclear

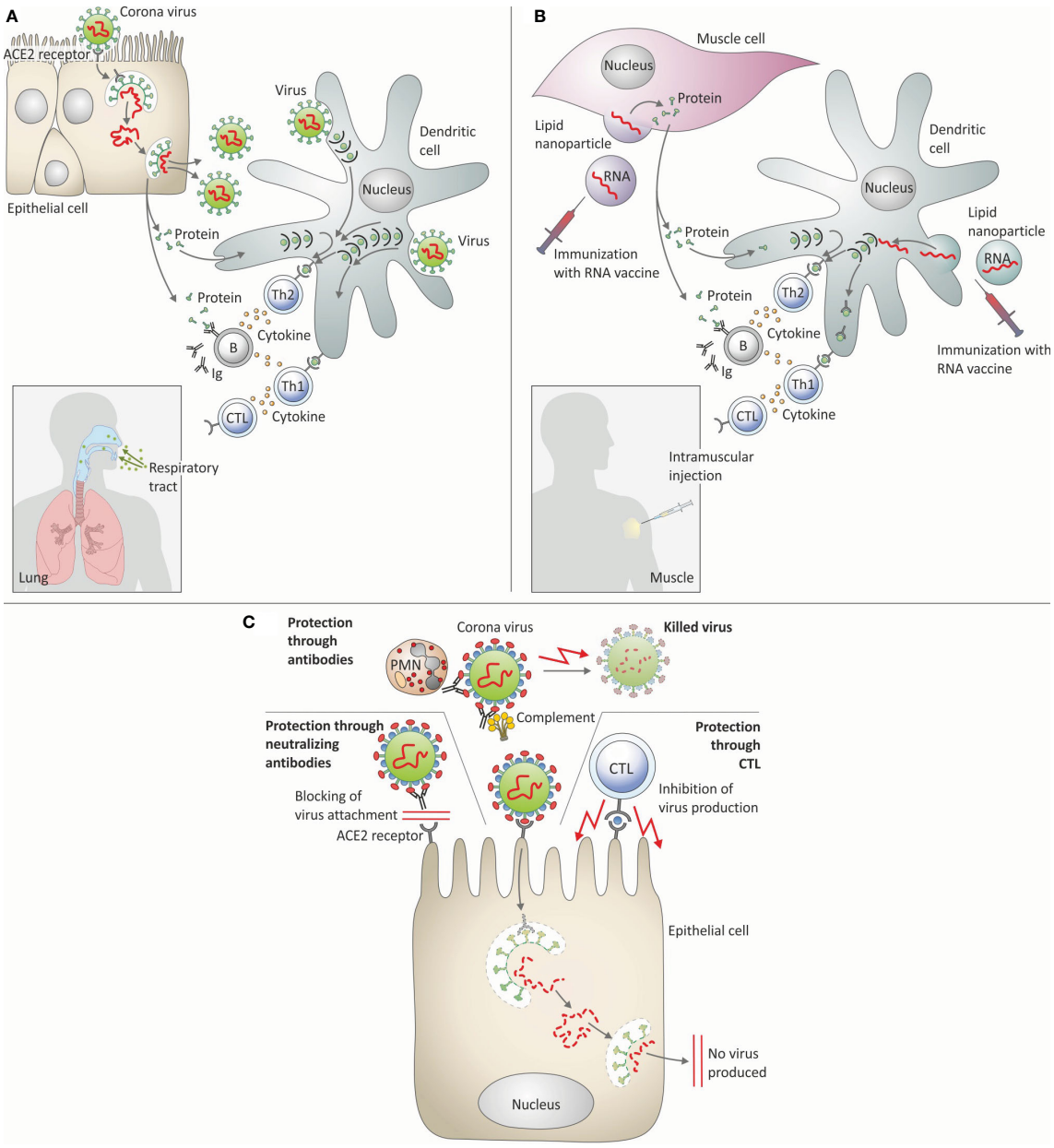

FIGURE 2
Immune response elicited by SARS-CoV-2 infection and by mRNA : LNP vaccination. **(A)** Infection with SARS-CoV-2. **(B)** Immunization with mRNA: LNP. **(C)** Protective immune response against SARS-Cov-2. ACE-2 receptor, Angiotensin converting enzyme 2 receptor; B, B cells; CTL, Cytolytic T lymphocytes; Ig, Immunoglobulin; PMN, Polymorphonuclear neutrophils; Th1, T helper 1 cells; Th2, T helper 2 cells.

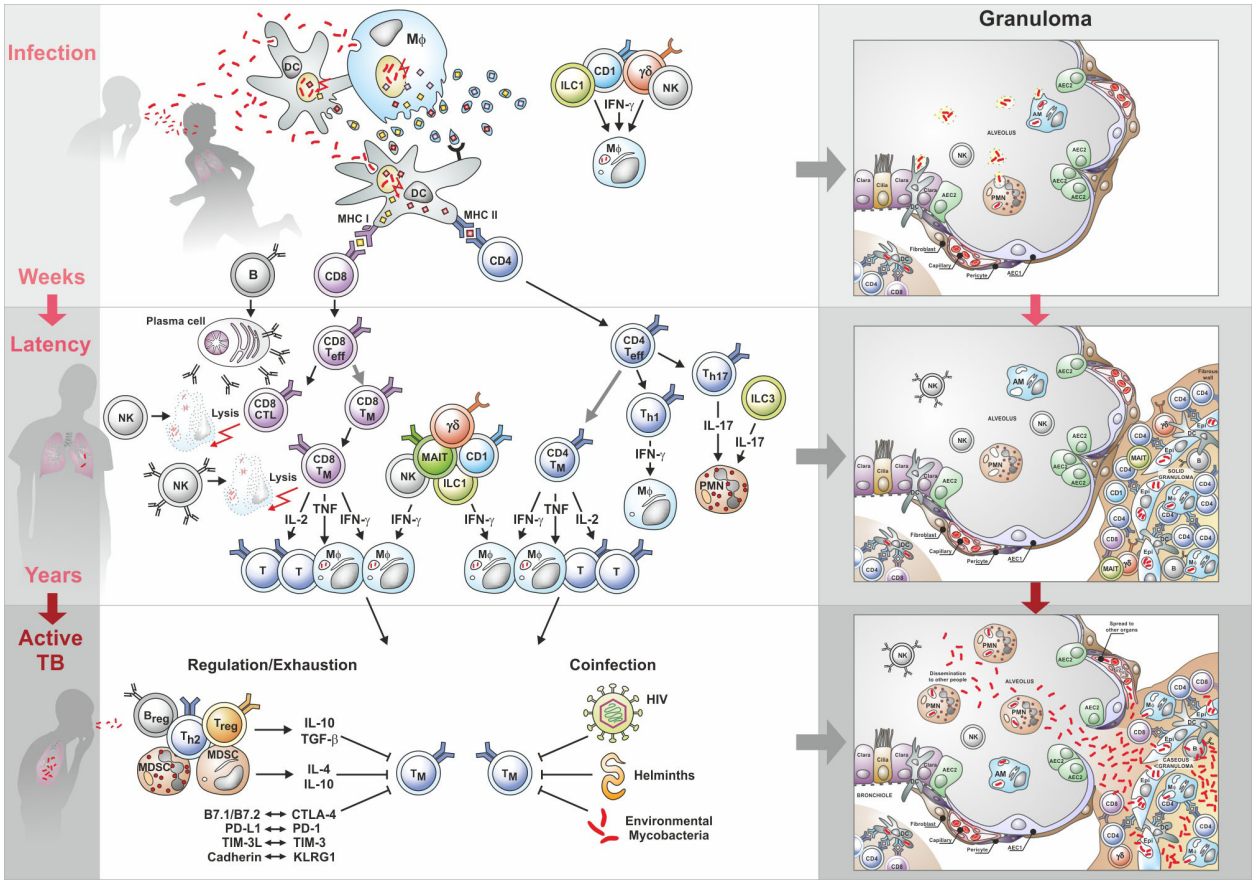

FIGURE 3
Immunity to tuberculosis (TB): from infection to active disease. Upper part shows cell interactions induced by infection with Mtb; the middle section describes interactions during latency; the lower part describes cell interactions underlying active TB. Characteristic granuloma stages are depicted on the right side. AEC, Alveolar epithelial cells; AM, Alveolar macrophages; B, B cells; CD4, CD4 T helper cells; CD8, CD8 T helper cells; CTL, Cytotoxic T lymphocytes; CTLA-4, Cytotoxic T-lymphocyte-associated protein-4; Epi, Epithelial cell; γ/δ, Gamma/delta cells; IFN-γ, Interferon-γ; ILC, Innate lymphoid cells; IL, Interleukin; KLRG1, Killer cell lectin-like receptor G1; MAIT, Mucosal-associated invariant T cells; MDSC, Myeloid derived suppressor cells; MHC, Major histocompatibility complex; Mφ, Macrophage; NK, Natural killer cells; PD-1, Programmed cell death protein 1; PD1-L, Ligand for PD1; PMN. Polymorphonuclear neutrophils; T, T cells; Teff, T effector cells; Treg, T regulatory cells; TGF, Transforming growth factor; Th1, T helper 1 cells; Th2, T helper 2 cells; Th17, T helper 17 cells; TIM-3, T-cell immunoglobulin and mucin domain-containing protein 3; TIM-3L, Ligand for T-cell immunoglobulin and mucin domain-containing protein 3; TM, Memory T cells; TNF, Tumor necrosis factor.

phagocytes with the capacity for self-renewal. In addition, notably after the onset of inflammation and attracted by chemokines and other attractants, neutrophils, and monocytes enter alveoli from the blood circulation, which are capable of engulfing Mtb (59). The pathogen is transported to different sites of the lung parenchyma by mononuclear phagocytes. At the site of Mtb deposition, granulomas begin to develop independently from each other (60, 61). Some Mtb may be killed by the phagocytes soon after infection, notably in individuals who have been immunized with BCG and/or carry latent TB infection (LTBI). In this situation, macrophages could develop trained immunity based on epigenetic changes (62). Evidence for the participation of natural killer (NK) cells in early defense against Mtb has been presented (63). These NK cells are rapidly attracted to the Mtb-infected lung. It is a matter of discussion whether early infection control can lead to sterile eradication in so-called non-converters (see 5.1).

Initiation of the acquired immune response (Figure 3): Interstitial dendritic cells (DC) transport Mtb to draining lymph nodes and chemokines attract additional DC as well as T lymphocytes and B lymphocytes into specialized structures in the lymph node, where the acquired immune response is activated (64–66). CD4 T cells of Th1 type, which produce multiple cytokines, are considered of critical importance (67). The role of CD4 T cells in controlling TB is probably best illustrated by the aggravated outcome of HIV-Mtb coinfection (68). HIV impairs CD4 T cells and people living with HIV (PLWH) are highly susceptible to TB (69). Interferon-γ (IFN-γ) and tumor necrosis factor α (TNFα) are of major importance as they activate mononuclear phagocytes directly (70, 71). The critical role of TNFα in controlling Mtb in infected individuals became obvious when patients with rheumatoid arthritis treated with anti-TNF monoclonal antibodies frequently progressed to active TB (72). IL-2 could contribute to protection by activating other lymphocyte subsets, notably CD8 T cells. In addition to Th1 cells, also Th17 producing cells are considered important, notably at the early stage of infection (73, 74). CD4 T cells are restricted by the major histocompatibility

complex class II (MHC II) and hence are primarily focused on macrophages and DC. CD8 T lymphocytes contribute to protection via the secretion of IFN-γ and TNFα. In addition, they also directly attack infected host cells by means of perforin and granzyme. Moreover, human CD8 T cells produce granulysin which has been shown to directly kill Mtb (75–77). Because of their MHC I restriction, CD8 T cells possess a much broader target spectrum than CD4 T cells (78, 79). Hence, they monitor virtually all nucleated cells, e.g. epithelial cells surrounding alveoli, which can harbor Mtb.

Unconventional T cells are potential contributors to defense against Mtb including (80, 81):

- γ/δ T cells which recognize so-called phospho-antigens and have been found to produce IL-17 (74, 80, 82, 83);
- CD1-restricted T cells which recognize glycolipids prevalent in Mtb and are potent cytokine producers (84);
- MAIT cells which recognize non-peptide antigens and are prevalent in the respiratory tract (74, 78, 85, 86).

These populations are considered donor-unrestricted since they recognize non-peptidic epitopes in the context of unconventional presentation molecules that lack the heterogeneity of canonical MHC molecules that restrict conventional T cell responses (87). The innate lymphoid cells (ILC) are characterized by the absence of T cell receptor (TCR) and hence do not recognize antigens at all (88, 89). They are present in mucosal surfaces and at tissue sites such as the lung and likely participate in the early defense against Mtb. Similar to Th lymphocytes, ILC segregates into subtypes according to their cytokine profile. Hence, by producing IFN-γ and TNFα or IL-17, ILC contributes to protective immunity in TB, notably during the early stages. NK cells can be viewed as ILC since they lack the TCR and are of lymphoid origin (63). Yet, they are not tissue resident and circulate through the blood stream. In conclusion, the role of conventional T cells in TB is well accepted, whereas the participation of unconventional T cells and ILC remains less well understood.

B lymphocytes first play a role in TB by regulating immune responses, mostly by means of cytokines (90, 91). Second, they are the cellular source of antibodies (92). Antibodies can support protective immunity by facilitating phagocytosis, formation of phagosome/lysosome fusion, and stimulation of reactive oxygen and nitrogen intermediates (92–96). Indeed, evidence had already been presented in the 1970s that antibodies mediating the uptake of Mtb through the FcR promote phagosome/lysosome fusion for bactericidal activities (97, 98). Another role of antibodies in TB is the arming of NK cells. Evidence has been presented that NK cells can kill infected cells via ADCC (63).

Immunity during LTBI (Figure 3): The description of the different cell populations should not be interpreted to mean that these cells act independently; rather, they crosstalk with each other and it is this complex interplay between the different cells of the innate and acquired arm of immunity and their secretion products (notably cytokines, chemokines, and antibodies), which results in protective immunity capable of containing Mtb and thus preventing progression to active TB (99–102). At the risk of oversimplification, fine-tuned immunity controls the infection and at the same time keeps inflammation at a minimum. This is the case with LTBI which affects one quarter of the world population. Maladapted immunity fails to control infection and inflammation, thereby allowing progression to active TB (Figure 3). This complex immune response is highly sensitive to perturbations. Notably, in the absence of correlates of protection (see 5.5.2), the mechanisms underlying effective host control in 90% of individuals infected with Mtb and progression to active TB disease in 10% of these remain elusive. Notably, it is unclear whether this failure is due to exhaustion or active downregulation of the protective immune components (Figure 3). Obviously, efficacious vaccines against TB need to induce a fine-tuned immune balance (58, 103, 104).

Because Mtb interferes with the buildup of protective immunity, it takes several weeks before granulomatous lesions develop into solid granulomas that contain Mtb. Within these granulomas, different populations of Mtb-specific lymphocytes, mononuclear phagocytes, DC, and other cell types exist in a well-organized structure. T lymphocytes will develop into memory T cells, which segregate into effector memory T cells, central memory T cells, and resident memory T cells (105–107). Resident memory T cells seem to be of particular importance (108). Active granulomas can induce the formation of lymphoid follicles in their vicinity, which participate in the orchestration of the solid granuloma (99, 109, 110).

Progression to necrotic and caseous granulomas (Figure 3): A maladapted immune response promotes the transition of solid granulomas to necrotic and then caseous granulomas (59, 103, 104). This progression from LTBI to active TB disease can occur months to years after infection. The maladaptation may be caused by exogenous factors such as coinfection with HIV or helminths or through endogenous factors, which can be summarized as suppression and exhaustion. The latter mechanisms are still incompletely understood. It is likely that suppressive mononuclear phagocytes, the myeloid derived suppressor cells (MDSC), regulatory B cells, and regulatory T cells contribute to the transition into necrotic/caseous granulomas (90, 111–113). Moreover, evidence has been presented for the role of checkpoint control in TB, e.g. through interactions between programmed cell death protein 1 (PD-1) and ligand for PD-1 (PDL-1) or between T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) and ligand of TIM-3 (TIM-3L) (114, 115). Evidence suggests that blocking checkpoint control in TB causes excessive immunity characterized by elevated TNF-α production further emphasizing the importance of fine-tuned immunity in TB control and of maladapted immunity as a critical factor of active TB disease (116). The deteriorating immune response in the granulomas causes marked cell destruction, leading to loss of structure and function. In parallel, the lack of granuloma structure allows access of Mtb to capillaries that facilitate transmission to other organs in the body and to alveoli, which promote spread into the environment. At this stage, patients suffer from active TB and are contagious.

During their residence in the solid granuloma, Mtb organisms are mostly in a dormant stage, i.e. they show low to absent metabolic and replicative activity (64, 117, 118). Once the

immune response worsens and caseous granulomas develop Mtb 'wakes up' and transits into a replicative and metabolic active stage. The cellular detritus in the caseous granuloma favors Mtb growth. The switch from dormant to active Mtb could have consequences for the selection of vaccine antigens (117, 119). Vaccines targeting the prevention of infection (PoI) in naïve individuals could benefit from active Mtb antigens, whereas vaccines targeting the prevention of disease (PoD) in LTBI would exploit dormant Mtb antigens. Consistent with this, a preponderance of dormancy associated antigens has been identified in individuals with LTBI (120).

Vaccines for both PoI (reinfection) and PoD in individuals with LTBI will require both types of antigens.

The granuloma landscape in the lung is heterogeneous (61, 99). During the early stages of LTBI, lesions of different developmental stages coexist which will then mature into solid granulomas (121). During progression to active TB, granulomas transit into the necrotic and then caseous stage. Hence, during incipient/pre-clinical TB, necrotic lesions emerge. This seems to induce an increased inflammatory response which can be determined in the blood by means of transcriptomic and metabolomic biosignatures, which can be harnessed for prognosis of active TB (122–126).

5 Vaccination strategies

Figure 4 describes the major stages from infection to disease in TB, which serve as targets for intervention by TB vaccines currently under clinical assessment. In the following, different vaccination strategies relevant to TB control, and the value of correlates and surrogates of protection, which are still missing for TB, but availability for Covid-19 will be discussed (119).

5.1 Prevention of what?

Principally, vaccines induce a protective immune response against the targeted pathogen with different outcomes that are not mutually exclusive. These are: (i) PoI, (ii) PoD, (iii) prevention of transmission (PoT), and (iv) prevention of recurrence (PoR).

- PoI also leads to PoD and PoT. Infection is best diagnosed by detecting the pathogen or its components. This is feasible as long as the pathogen or its components are present in body sites that are easily accessible, e.g. sputum, urine, or blood. In the case of TB, detection of Mtb or its components generally fails in individuals with LTBI. These individuals are healthy but considered infected with Mtb. Even in patients with active TB, detection of Mtb by sputum microscopy can be missed due to insufficient sensitivity. Diagnosis of LTBI is mostly performed indirectly by measuring the cellular immune response, e.g. by so-called IFN-γ release assays (IGRA) which measure IFN-γ release from white blood cells after antigen-specific stimulation (127–130). Individuals with LTBI have been termed

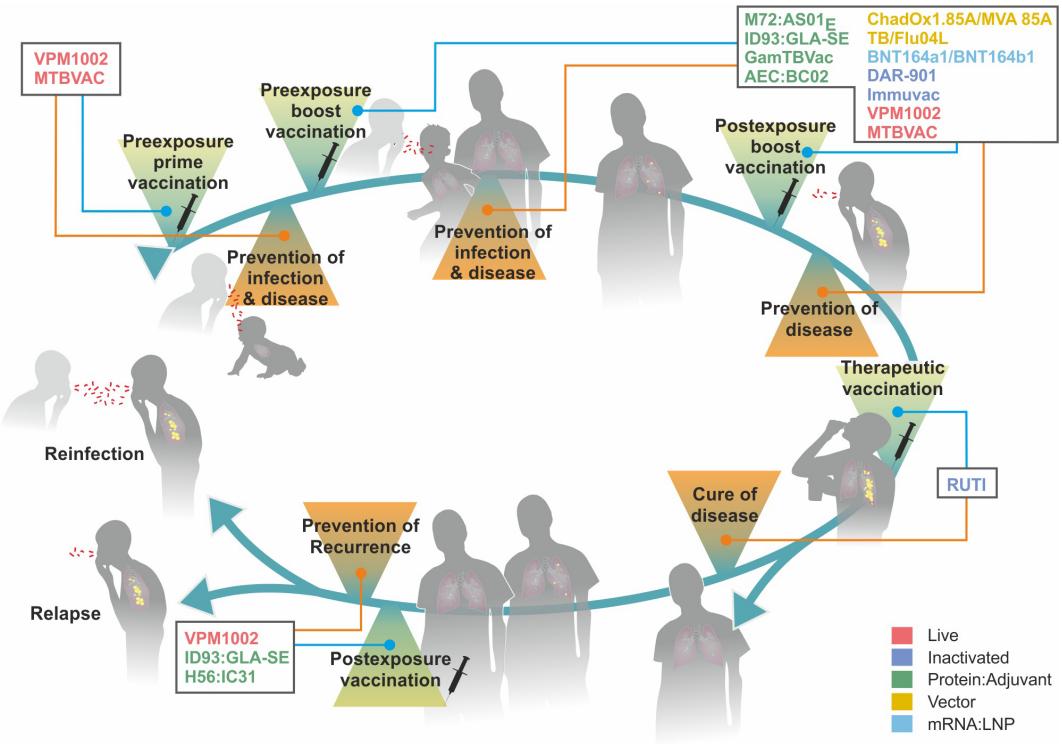

FIGURE 4
Major stages from infection to active disease in tuberculosis (TB) and target points for different vaccine types. Colors indicate different vaccine platforms. For further details on vaccine types, see Figure 5.

converters. Note that about 10% of close contacts of TB patients do not respond by IGRA and accordingly have been termed nonconverters or resistors (119, 121, 131–133). It is unclear whether nonconverters are true resistors that have cleared or prevented infection or are false nonconverters, which harbor Mtb, but fail to generate an immune response that is measured by IGRA. PoI is mostly accomplished by preventing the pathogen from establishing itself in the host. Frequently, PoI is based on rapid eradication of the pathogen after short-term infection and hence should be more precisely defined as prevention of stable infection. In summary, the precise determination of PoI as a clinical endpoint poses challenges in TB (134). Hence, delineation of the underlying immune mechanisms could provide guidelines for the design of vaccines that target PoI.

- PoD needs to be further subdivided according to the severity of disease, i.e. mild disease, severe disease (hospitalization, intensive care unit), and lethal disease. In the case of Covid-19, vaccines only induce partial PoI but are highly effective in preventing severe disease and lethality. In naïve individuals, PoD is a consequence of PoI. In already infected individuals, PoD can be achieved by pathogen eradication during LTBI or by preventing the pathogen from causing disease, e.g. by its containment in an innocuous stage through maintenance of LTBI. Although Mtb infection is thought to last lifelong, so-called reverters have been described, i.e. individuals who reverted from $IGRA^+$ to $IGRA^-$ remaining negative over long periods of time (133, 135). The underlying mechanisms remain elusive and false $IGRA^-$ due to desensitization cannot be excluded. Given that this reversion reflects the eradication of Mtb, information on the underlying mechanisms could provide helpful guidelines for vaccines aimed at sterilizing PoD.
- PoT is a consequence of PoI and PoD since both directly impact the transmission of Mtb. Although LTBI has long been considered non-contagious, more recent evidence suggests that it can be a major source of transmission. Transmission during LTBI likely occurs during the sub-clinical stage (136–139). Future vaccination strategies need to consider whether vaccines aimed at PoD induce sufficient immune control to prevent Mtb transmission by healthy individuals with sub-clinical TB.
- PoT by itself can serve as a target for future vaccination strategies, notably if vaccine-induced PoD only achieves prevention of severe disease, allowing infection and mild disease.
- PoR targets reinfection or relapse (140). Some individuals who have been cured of TB remain susceptible to reinfection since protective immunity induced by natural Mtb infection is insufficient. In addition, a few Mtb microorganisms may persist even after drug treatment and then cause relapse. PoR is considered a valid target for vaccination. However, it is unlikely that post-TB lung damage is tractable by vaccination.

5.2 Preventive and therapeutic vaccination

The major scope of vaccines is to prevent healthy individuals from developing the disease. Yet, therapeutic vaccination in adjunct to chemotherapy is being considered, notably for TB patients suffering from multi- or extensively resistant TB (140). Frequently, vaccines for PoR are grouped as therapeutic vaccines even though recurrence can be caused by reinfection.

5.3 Pre- and post-exposure vaccination

By definition, vaccines targeting PoI are administered pre-exposure with the pathogen. Complete PoI also causes PoD and PoT; incomplete PoI may ameliorate disease and transmission. As Covid-19 vaccination campaigns have shown, partial PoI reduces viral load and pathogenicity resulting in efficacious PoD, notably by reducing disease severity. This is likely due to a direct quantitative relationship between viral load and virulence. In the case of TB, such a quantitative relationship is less likely, and partial PoI may delay, but not prevent progression to active TB disease. Theoretically, two options exist that are difficult to differentiate mechanistically. A TB vaccine could either induce PoD or cause containment of Mtb resulting in long-term LTBI. Generally, post-exposure vaccination aims at (i) sterile pathogen eradication before progression to active disease or (ii) long-term maintenance of LTBI. Secondary infection of an individual with LTBI can further complicate the situation.

5.4 Prime/boost

Vaccines may need a booster if the prime immunization is insufficient or wanes over time. Even though only little evidence exists, it is often assumed that heterologous prime/boost schemes induce stronger effects, either because the two vaccines cause different immune responses or comprise different antigens. Both effects are considered beneficial if they complement each other. In the case of TB, most vaccine candidates are considered boosters for BCG primed individuals, and only a few as prime vaccines instead of BCG (119).

5.5 Surrogates and correlates of protection

5.5.1 Surrogates

A surrogate of protection (SoP) elicited by vaccination is defined as a biologic parameter that in a clinical phase III efficacy trial statistically correlates with vaccine-induced protection (141–143). Typically, SoP is determined by a comparison between the vaccine and the placebo group. SoP, notably if they can be easily determined, facilitates early determination of vaccine effectiveness prior to clinical outcome. Neutralizing antibodies against Spike protein of SARS-CoV-2 are SoP, whereas for TB SoP have not been identified.

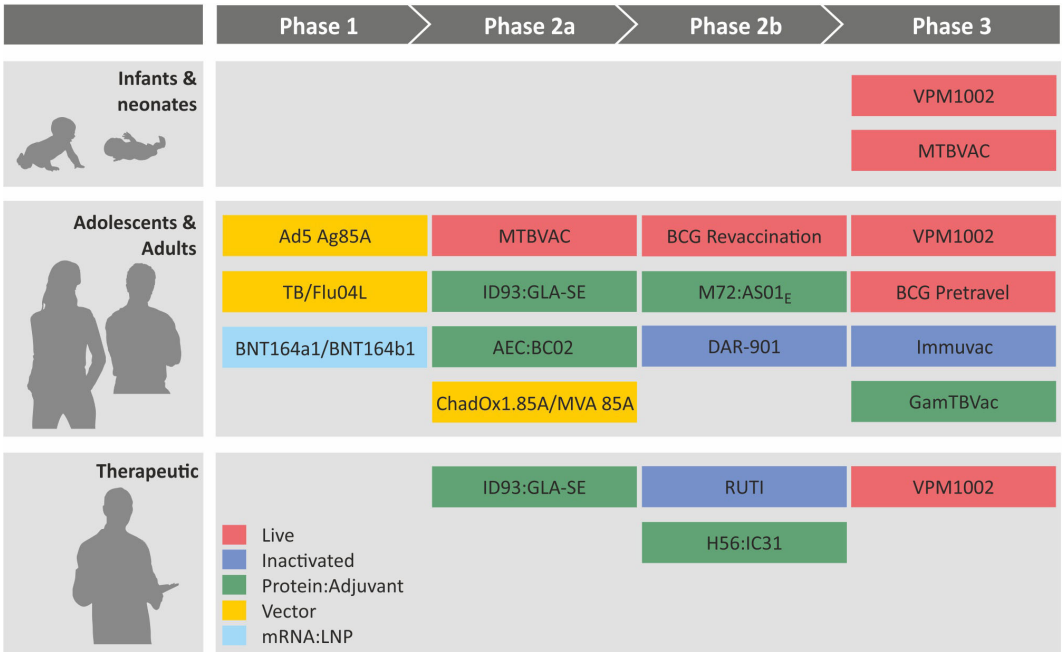

FIGURE 5
Clinical pipeline of major tuberculosis vaccines from Phase I to Phase III. Colors indicate different vaccine platforms. For further details, see article discussion.

5.5.2 Correlates

A correlate of protection (CoP) elicited by vaccination is defined as a biological mechanism, typically an immune mechanism that is induced by immunization and serves as an indicator of protective immunity (142–146). CoP is statistically related to vaccine-induced protection. Comparison of immunized vs. unimmunized (control) individuals will be confounded by the fact that the majority of controls will not develop disease. Given that a vaccine induces protection in some, but not all vaccinees, a comparison of the two immunized groups, i.e. protected vs. unprotected vaccinees provides a strong basis for the definition of a robust CoP. CoP can be used for the definition of surrogate endpoints, i.e. an endpoint that precedes or can be more easily measured than the clinically defined endpoint.

Two groups of CoP need to be distinguished, including direct and indirect CoP. Moreover, CoP induced by infection need not necessarily be identical to CoP induced by vaccination. This is particularly relevant in situations where natural infection does not cause complete protection as in TB. Global gene expression profiling of blood cells and metabolomic analyses of serum led to the design of biosignatures that can potentially predict the progression from LTBI to active TB disease (122–126). Such biosignatures are composed of biomarkers that may or may not be causally linked to the sustenance of LTBI or progression to active TB disease. Biosignature studies on the progression from LTBI to active TB disease as well as on differences between responders and non-responders (see 5.1) can provide important guidelines for the characterization of vaccine-induced CoP. Biosignature studies have provided evidence for a sub-population with sub-clinical TB amongst the LTBI population. Increasing epidemiologic evidence suggests that this healthy sub-population serves as a source of Mtb transmission (136–139).

More recently, biomarkers associated with specific immune responses have been employed for the prediction of TB disease. First, efforts are being made to characterize Mtb-specific antibody profiles comprising Ig isotypes with unique FcR types to identify individuals that progress from LTBI to active TB (91, 93, 147–149). Antibody isotypes related to protection could become promising targets for future vaccines. Second, the identification of Mtb-specific TCR repertoires associated with the outcome of LTBI have been characterized (150). In this study, three groups could be identified based on similarities in TCR sequences: The first group was associated with progression to disease, the second group with maintenance of LTBI, and the third group did not show any association with disease progression or control. Antigenic epitopes related to each group could be identified. PE13, a variable antigen present in both Mtb and BCG as well as CFP10, a cognate of the region of difference (RD)-1 present in Mtb and absent in BCG, were characteristic for infection control. Reciprocally, EspA which is associated with CFP10 was associated with TB progression. Numerous antigens were present in both controllers and progressors, suggesting that they had no direct impact on the course of infection. It should be noted that these analyses focused on CD4 T cells without characterization of their cytokine profile. Future studies extending to functional characterization and other T cell populations, notably CD8 T cells could provide important guidelines for the identification of antigens to be included in, or omitted from, future subunit vaccine candidates. In the long run, an association of functional activities and antigen specificities of B cells and T cells could become important tools for the design of next-generation vaccine candidates.

5.5.2.1 Indirect CoP

Indirect CoP are induced by vaccination either directly and independently from direct CoP, or they are indirect sequelae of direct CoP. They correlate with but are not causally linked to, protection. An example of such indirect CoP is provided by the list of the biomarkers included in biosignatures which potentially predict progression from LTBI to active TB disease (see above).

5.5.2.2 Direct CoP

Direct CoP have also been termed absolute or mechanistic CoP. A direct CoP is not only related to but also responsible for protection. An instructive example of such a causal link is neutralizing antibodies directed against so-called protective antigens, e.g. the Spike protein of SARS-CoV-2 (see also Figure 2). An increase in neutralizing antibody titers against the RBD of the Spike protein is not only directly related to the strength of vaccine-induced immunity, but can also be harnessed for measuring vaccine efficacy. Advantageously, neutralizing antibodies express both relevant functions (blocking of viral entry into host cells) and relevant specificity (targeting the RBD of the Spike protein). Accordingly, the omission of the Spike protein from a vaccine against Covid-19 will fail to produce a protective immune response mediated by neutralizing antibodies. Neutralizing antibodies that prevent Mtb from infection do not exist because Mtb is engulfed by mononuclear phagocytes through a variety of active uptake mechanisms involving numerous receptors. Antigen-specific CD4 T cells are directly involved in protective immunity against TB. Yet, their biological functions depend on mediators such as cytokines and chemokines that act on other immune cells, e.g. stimulation of CD8 T cells to express cytolytic activity, B cells to secrete antibodies or mononuclear phagocytes to express bacteriostatic or bactericidal activity. Moreover, antigen specificity of T cells need not be directly linked to T cell function. In TB immunity that sustains LTBI and therefore prevents active TB disease may differ from immunity that prevents infection or causes sterile eradication of Mtb.

5.5.3 Relevance to vaccine development

For discussion here, it is clear that rational vaccine development will enormously benefit from the identification of direct CoP (151). The most straightforward CoP are antigen-specific antibodies with neutralizing activity, which cause PoI by preventing pathogen entry into host cells. This activity becomes more complex in situations, in which antibodies contribute to protection via FcR-mediated effector functions of antibodies, such as complement activation, opsonization, or ADCC. The most complex situation arises when infection is primarily controlled by cell-mediated immunity. First, the measurement of antigen specificity of T cells is more challenging, and second, the function of T cells is ultimately mediated by effector molecules or effector cells. CD8 T cells, which contribute to protection as CTL are relatively straightforward because they act directly via their 'own' effector molecules (e.g. granzyme, granulysin, perforin). The most complex situation arises for CD4 T cells, which primarily act through soluble mediators such as chemokines and cytokines to stimulate other cells to perform effector mechanisms including mononuclear phagocytes, granulocytes, B cells, and CD8 T cells.

6 TB vaccine candidates

6.1 BCG: from early testing to most recent trials

BCG is one of the most widely used vaccines globally only exceeded by Covid-19 vaccines. Some four billion doses have been rolled out since its first human use in the 1920s (36, 152). The term BCG stems from the two developers, Albert Calmette and Camille Guérin, who succeeded in attenuating *M. bovis*, the causative agent of bovine TB by passaging it > 230 times *in vitro* using ox bile to promote attenuation (153). This vaccine has been designed for the prevention of TB in neonates with a high risk of progression to severe extrapulmonary disease including miliary TB due to dissemination of Mtb to diverse organs. BCG accomplishes this goal, at least in part, but fails to protect against pulmonary TB, notably in adolescents and adults (154). The vaccine was originally administered in three doses given orally and this regimen was later changed to intradermal administration of a single dose. In some countries, revaccination with BCG has been performed, notably in neonates and infants lacking signs of vaccine take and sometimes also in adolescents and adults. Generally, however, BCG boosters are not recommended because of the potential risk of adverse events.

A recent study assessed BCG revaccination of adolescents and adults without signs of Mtb infection (see Figure 5). This study (NCT02075203) found ca. 45% protection against stable Mtb infection indicated by IGRA (155). It is noteworthy that stable, but not transient, infection was prevented by BCG revaccination. It has been argued, therefore, that in the BCG immunized group, Mtb was able to establish itself for a short time period, but was subsequently eliminated by mononuclear phagocytes expressing trained immunity (see 4) (62). Consistently, BCG revaccination caused significant protection against upper respiratory viral infections over controls (note that lower respiratory infections were not observed in either group). A larger confirmatory trial with BCG is underway (NCT04152161). Another study with BCG, which is currently in phase III, assesses the value of BCG for pre-travel vaccination (see Figure 5). The clinical endpoint is PoI in healthy adult travelers from low incidence countries who are at risk of exposure to Mtb in high burden countries (NCT04453293). Completion of this study is expected in 2025.

6.2 Experimental BCG studies as the first step towards CoP

A breakthrough study in which non-human primates (NHP) had been immunized with BCG by intravenous administration resulted in the sterile eradication of Mtb in the majority of animals. Whilst this way of administration is hampered by the

risk of adverse events, it provides proof of concept that sterilizing immunity can be induced even though the underlying mechanisms have to be fully revealed (156). This model was harnessed for the identification of immune mechanisms and biosignatures relevant to protective immunity. By using a lower dose of BCG given intravenously, sterile protection could be induced in about half of the experimental animals. Comparison of protected and unprotected NHP revealed that an abundance of polyfunctional T cells that co-express TNF together with IFN-γ or with IL-17 as well as an abundance of NK cells correlated with protection two months after immunization and before challenge with Mtb (157). In parallel, blood transcriptional correlates were determined. Biosignatures determined two days after immunization correlated with the pulmonary immune responses measured after one to two months (see above) and could predict protection against Mtb challenge after six months (158). These modules included type I IFN as well as Rag-I-like signaling pathways. These studies in NHP both on the cellular and transcriptional level provide the first evidence that vaccine-induced CoP can be defined. As a caveat, it should be noted, however, that the transcriptional module can be affected by viral infections occurring during vaccine trials which induce similar biosignatures. Furthermore, in adolescents and adults, a high proportion of individuals already have LTBI demanding post-exposure vaccination with Mtb which may differ from the pre-exposure situation studied in NHP. Finally, it needs to be clarified whether the gene expression profiles represent direct or indirect CoP. Direct CoP could be harnessed for further refinement of novel vaccine candidates.

6.3 Vaccine candidates in clinical trials

Currently, more than a dozen vaccine candidates against TB are progressing through the clinical trial pipeline and have advanced to different stages, phase I, phase II, or phase III (Figure 5). These include five protein adjuvant vaccines and three viral vectored vaccines as cognates of subunit vaccine candidates as well as three inactivated and two attenuated vaccines as members of whole cell vaccine candidates. Most recently mRNA : LNP vaccines have entered phase I safety assessment as the latest addition to the group of subunit vaccine candidates.

6.3.1 Subunit vaccines

6.3.1.1 Protein:adjuvants

- **H56:IC31** is a fusion protein of three antigens (ESAT-6, a prominent Mtb antigen in the RD-1 region + Ag85B, a member of the Ag85 family of mycolyl-transferases + Rv2660c, a dormancy antigen) in the IC31 adjuvant (cationic peptide + TLR9 agonist) (159). It has successfully completed several phase I trials for safety and immunogenicity and is currently being tested in a clinical phase II trial (NCT03512249) for therapeutic purposes (PoR).
- **ID93:GLA-SE** is based on a fusion protein of four antigens (Rv2608, a PPE family member + Rv3619, a virulence factor + Rv3620, another virulence factor + Rv1813, a dormancy antigen) in the GLA-SE adjuvant (an oil-in-water emulsion + TLR4 agonist) (160). It is considered for preventive and therapeutic purposes (PoR) and has successfully completed a phase IIa trial (NCT03806686).
- **AEC : BC02** comprises three antigens (Ag85B, a member of the Ag85 family of mycolyl-transferases + ESAT-6 + CFP10, two important antigens in the RD-1 region) in the BC02 adjuvant (TLR-9 agonist CpG in alum) (28). It has reached a phase II clinical trial (NCT05284812).
- **GamTBvac** is composed of ESAT-6 + CFP10 (two important antigens in the RD-1 region) + Ag85A (a member of the Ag85 family of mycolyl-transferases) with a modified Dextran-binding domain formulated with the TLR-9 agonist CpG as adjuvant (161). This vaccine candidate has entered a phase III trial for the prevention of TB in adolescents and adults (NCT04975737). Completion is expected in 2025.
- **M72:AS01$_E$** comprises a fusion protein of two antigens (Rv1196, a PPE family member + Rv0125, a peptidase) in the AS01$_E$ (liposome + TLR4 agonist). The M72:AS01$_E$ showed ca. 54% protection against progression to active TB from LTBI in a phase IIb prevention trial (162, 163). In this trial (NCT01755598), M72:AS01$_E$ was given as a post-exposure boost vaccine in adults and adolescents with LTBI who had been BCG primed as infants. This vaccine is planned for a larger phase II/III trial to validate its protective efficacy in PLWH (NCT04556981).
- Two similar mRNA : LNP vaccines against TB have entered the clinical trial pipeline. BNT164a1 and BNT164b1 encoding multiple Mtb antigens of undisclosed identity are in phase I trials in BCG-vaccinated HIV-negative individuals (NCT 05547464) and in IGRA-negative, BCG naïve individuals (NCT 05537038). Thus, the two mRNA : LNP vaccine candidates are considered both as a prime vaccine in Mtb-uninfected and BCG-unvaccinated individuals and as a boost vaccine in BCG-immunized, Mtb-infected (LTBI) and naïve individuals.

6.3.1.2 Viral vectors

- **Ad5Ag85A** is based on a nonreplicating Ad vector expressing Ag85A (a member of the Ag85 mycolyl-transferase family) (164). It has completed a phase I trial for safety and immunogenicity after aerosol inhalation (NCT02337270). Work with this vaccine candidate has been discontinued.
- **TB/Flu04L** comprises a non-replicating influenza virus as a vector expressing Ag85A (a member of the Ag85 mycolyl-transferase family) and ESAT-6 (a prominent Mtb antigen in the RD-1 region) (31). It has successfully completed a

phase I clinical trial for safety after intranasal and sublingual administration (NCT03017378).

- **ChAdOx1.85A/MVA** is given in a heterologous prime/boost scheme, where ChAdOx serves as the prime and MVA as a boost. Both vectors express Ag85 and thus differ in the vector, not in the antigen (165). This heterologous vaccination regimen has recently entered a phase IIa trial (NCT03681860). The ChAdOx1.85A vaccine has also completed a comparative phase I trial for aerosol versus intramuscular vaccination (NCT04121494). Previously, a completed phase IIb trial with MVA85A alone had failed to provide evidence for protective efficacy (NCT00480558) (166–168).

6.3.2 Whole cell vaccines

6.3.2.1 Inactivated

- **RUTI** which is exclusively targeted for the therapy of TB, notably multidrug-resistant (MDR) or extensively drug-resistant (XDR)-TB in adjunct to chemotherapy has reached phase IIb stage (NCT04919239). RUTI is a killed and detoxified Mtb preparation in liposome suspension (169).
- **DAR-901** has been tested for the prevention of TB in adolescents and adults. A phase IIb trial has been completed without evidence for protective efficacy (NCT02712424). This vaccine comprises a killed *M. obuense* preparation (170).
- **Immuvac**, the most advanced inactivated vaccine is based on killed M. *indicus pranii* (171). This vaccine provided some evidence for therapeutic protection when given in adjunct to chemotherapy (NCT00265226). It is currently tested head-to-head with VPM1002 (CTRI/2019/01/017026) in a phase III trial for PoD with estimated completion in 2024.

6.3.2.2 Attenuated

- **MTBVAC** is a viable vaccine candidate which is tested for TB prevention in infants and adolescents/adults. It has reached a phase III trial in infants (NCT04975178), and completion is expected in 2029. It is a live Mtb vaccine candidate that had been attenuated by genetic deletion of two independent loci that regulate more than 100 genes in Mtb (172).
- **VPM1002** is an improved BCG vaccine candidate, in which the urease C gene has been replaced by the listeriolysin gene (152, 173). It is currently undergoing three phase 3 trials: (i) PoI and PoD in neonates in comparison to BCG (NCT04351685) with expected completion by 2025; (ii) PoD in adolescent and adult household contacts of recently diagnosed TB patients head-to-head with Immuvac (CTRI/2019/01/017026) with estimated completion in 2024; (iii) PoR in individuals who had completed TB chemotherapy (NCT03152903) with estimated completion in 2024.

6.4 Concluding remarks on TB vaccine candidates

Globally an estimated 1.7 billion individuals live with LTBI, of whom approximately 10% develop active disease, the majority within the first year, but others after decades. Therefore, vaccines need to be considered for pre- and post-Mtb exposure and for induction of long-lasting protection not only in individuals who develop active TB within less than 12 months but also in those who become ill much later.

As outlined in the above review, the genome of Mtb comprises some 4000 protein-encoding genes, which in principle could all be target antigens for vaccine-induced immunity (174). Several of these antigens are regulated, with some proteins being more abundant during active TB disease and others during LTBI. Post-exposure vaccination of individuals with LTBI carrying dormant Mtb, therefore, may depend on antigens different from those in a vaccine that prevents infection with active Mtb.

In contrast to SARS-CoV-2, where neutralizing antibodies are of critical importance, evidence is missing as to whether neutralizing antibodies are generated in TB. Accordingly, protective antigens are absent. Increasing evidence suggests a role for non-neutralizing antibodies in protective immunity which activate different effector functions. Moreover, T cells are essential for protection and pathology and strong evidence exists that a fine-tuned balance between innate and acquired immune cells is critical for protective immunity. Based on these features neither surrogates nor direct correlates of protection against TB have been identified thus far.

7 Lessons from Covid-19 for TB vaccine R&D

The devastating health crisis created by Covid-19 provided pivotal lessons for future epidemic, endemic, and pandemic control measures at all levels including vaccine R&D for TB. Lessons of general relevance include the need for stronger healthcare systems, improved infection control measures, better preparedness and resilience to emerging and existing health threats, and better public health education.

More specific guidelines from the Covid-19 crisis that are relevant for TB vaccine R&D include, financial support for TB vaccine R&D is of critical importance. At present, support is still insufficient despite a slight increase over the last decade, costing approximately $1 billion US dollars per year (175). The immediate support by public, philanthropic, and private partners for Covid-19 vaccine R&D up to $100 billion US dollars (14). This unprecedented funding demonstrates the impact that early financial investment can have on vaccine R&D for health. It has to be voiced more clearly that in the long run, investment in TB vaccine

R&D will pay back (176). The current financial burden of TB has been estimated in the order of $100 billion US dollars annually. Hence, public-philanthropic-private partnerships should be formed if the industry hesitates to invest in TB vaccines because of assumed low profit. A recent example of a philanthropic-private partnership is the handing over of the TB vaccine, M72:AS01$_E$, for phase II/III clinical trial testing from GlaxoSmithKline to the Bill and Melinda Gates Foundation and the Wellcome Trust (177).

The global response to the Covid-19 crisis fostered a stronger collaborative spirit among researchers from both public and private entities, which profoundly accelerated vaccine development. This lesson should be applied to improve joint research and resource mobilization for TB vaccine R&D. A recent example is the head-to-head phase III clinical trial performed by the Indian Council of Medical Research to compare protection against TB by the attenuated vaccine, VPM1002, and the inactivated vaccine, Immuvac (178). In a similar vein, late stage vaccine trials should not only aim to provide information on the vaccine candidate under trial but also to generate information for the informed design of next-generation vaccine candidates.

The transition from preclinical to clinical studies has frequently been termed the 'valley of death' due to the many obstacles that can occur. During the Covid-19 crisis, the regulatory processes for vaccines were markedly expedited by streamlined regulatory processes to mitigate such obstacles, at least partially. TB vaccine R&D could similarly benefit from streamlined regulatory processes without any curtailment in safety and efficacy standards (20). Related to this, adaptive clinical trial design can further contribute to accelerated clinical vaccine testing (15, 134). Both strategies can speed up the clinical development pipeline without compromising safety and efficacy standards.

Once a better efficacy and/or safety profile for a novel TB vaccine over BCG has been established, vaccine manufacturing capacity will become a critical factor (179). Hence, appropriate manufacturing capacities need to be established early on: at the latest, in parallel to a phase III vaccine trial. Since this is best accomplished by facilities with high manufacturing capacity meeting global demands, appropriate partnerships need to be established and investment into expanded manufacturing capabilities needs to be mobilized. The Covid-19 pandemic provides lessons, some of which should be followed and others modified or avoided. A positive example is the agreement between the startup company BioNTech and the big pharma company Pfizer to develop, test, and deploy Comirnaty as fast as possible. On the other hand, the COVAX enterprise ultimately failed to achieve equitable vaccine distribution across the globe (180, 181).

Another important aspect of this topic is equitable access to TB vaccines at low cost, which needs to be guaranteed for low- and middle-income countries, not the least because they face the highest TB burden (17, 182). One step towards this could be the establishment of vaccine manufacturing capacities in regions where TB vaccines are needed most. This strategy includes not only manufacturing capacities but also strong educational and training activities to ensure successful TB vaccine production and deployment from local manufacturers (21). The largest vaccine manufacturer by dose is the Serum Institute of India Pvt. Ltd., which is based in India, a country with a high prevalence of TB. For regions without manufacturing capacities, the founding of WHO mRNA vaccine hubs on the African continent provides precedent from the Covid-19 field for this strategy (183).

Covid-19 vaccination campaigns have highlighted the importance of robust surveillance and monitoring systems that track the effectiveness and adverse events of the newly deployed vaccines. These lessons need to be adopted in modified form for TB from rollout to long-term surveillance of vaccines, notably since long-term protection is essential for TB control.

The impact of Covid-19 vaccination programs has saved millions of lives. Yet, in a small proportion of the global population, in both the North and the South, vaccine hesitancy and even aggressive vaccine opposition arose (184). Many diverse reasons account for vaccine hesitancy and denial including distrust of traditional political authorities (185). Hence, multidimensional approaches will be required to mitigate these challenges (186, 187). Successful TB vaccine rollout needs to be accompanied by a build-up in public trust through engaging and educating communities that suffer from high TB burden and addressing their concerns.

A major game changer emerged during the Covid-19 vaccine crisis, namely the creation of viral-vectored and mRNA : LNP vaccines as novel vaccine platforms. Whilst vector-based vaccines have already been included in the TB vaccine R&D portfolio, mRNA : LNP vaccines represent a novelty. This versatile platform must be included in the TB vaccine R&D pipeline. A major vaccine developer, BioNTech, already started a phase I safety trial for mRNA : LNP vaccines that encode various TB antigens. Assuming that subunit vaccines covering a small number of antigens can target Mtb with sufficient efficiency to provide long-term protection it is likely that mRNA : LNP vaccines can become major players for TB control.

8 Conclusion

TB has been around for centuries, claiming more than a billion lives (9). Despite its threat, the TB crisis has remained largely silent. Indeed, TB morbidity and mortality have been on the decline over recent decades; yet this decline is far too meager and alone it will not enable us to reach the goal of ending TB by 2030 as proposed by the Stop TB Partnership and the WHO (188, 189). This decline even reversed with the emergence of SARS-CoV-2 with an estimated 10-11 million people acquiring active TB and 1.6 million people dying. In 2018, a High-Level Meeting of the United Nations (UN) General Assembly made a strong commitment to end TB by 2030 (190–192). To achieve the goal, the UN is committed to creating "an environment conducive to research and development for new tools for TB". Accordingly, the commitment was made "to mobilize sufficient and sustainable financing with the aim of increasing overall global investments to 2 billion US dollars […] in funding annually for tuberculosis research" (191). This noble goal was interrupted by Covid-19. Hence, in 2023, a second High-Level Meeting on TB will be convened by the UN (193).

A strong commitment to ending TB is urgently needed. Otherwise, the WHO goal of reducing TB incidence by 50% and the numbers of TB deaths by 75% between 2015 and 2025 will be missed, notably because by 2021 only 10% reduction in TB

incidence and 5.9% reduction in TB deaths had been accomplished. In 2023, 30,000 people develop active TB every day and approximately 4,200 of them will die of this disease. By 2050, four million deaths will occur leading to an economic loss of $13 billion US Dollars (192). Better intervention measures are urgently needed and TB vaccines play a major role in this endeavor.

As has been discussed in this review, TB vaccine R&D cannot replicate the success story of Covid-19 vaccine R&D. Yet, by building on the experience gathered during the Covid-19 pandemic, the conditions for TB can be changed for the better. In the aftermath of the Covid-19 crisis, a working group had been established under the leadership of E.J. Sirleaf, former President of Liberia, and H. Clark, former Prime Minister of New Zealand, on 'How an Outbreak Became a Pandemic' under the ethos, 'Covid-19: Make it the Last Pandemic' (7, 8). Their concluding comment stated that their "message for change is clear: no more pandemic. If we fail to take this goal seriously, we will condemn the world to successive catastrophes". They go on to outline how the demands of this task are "large and challenging, but the price is even larger and more rewarding. With so many lives at stake, now is the time to resolve ". This call to prevent the next pandemic can be rephrased as task for future control of the ongoing TB pandemic: the "message for change is clear: No more TB. If we fail to take this goal seriously, we will condemn the world to continued catastrophes. The ask is large and challenging, but the price is even larger and more rewarding. With so many lives at stake, now is the time to resolve".

Author contributions

SK: Conceptualization, Writing – original draft.

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Conflict of interest

SK is the coinventor of the TB vaccine, VPM1002, and coholder of a patent licensed to Vakzine Projekt Management GmbH, Hannover, Germany, and sub-licensed to the Serum Institute of India PVT. Ltd., Pune, India. The vaccine is currently undergoing phase III efficacy trial testing. SK is also coholder of a patent for TB biomarkers.

Publisher's note

References

1. World Health Organization. *COVID-19 dashboard* (2020). Available at: https://covid19.who.int/.

2. Mathieu E, Ritchie H, Rodés-Guirao L, Appel C, Giattino C, Hasell J, et al. Coronavirus pandemic (COVID-19). *Our World Data* (2020).

3. Msemburi W, Karlinsky A, Knutson V, Aleshin-Guendel S, Chatterji S, Wakefield J. The WHO estimates of excess mortality associated with the COVID-19 pandemic. *Nature* (2022) 613:130–7. doi: 10.1038/s41586-022-05522-2

4. Hogan AB, Jewell BL, Sherrard-Smith E, Vesga JF, Watson OJ, Whittaker C, et al. Potential impact of the COVID-19 pandemic on HIV, tuberculosis, and malaria in low-income and middle-income countries: a modelling study. *Lancet Glob Health* (2020) 8: e1132–41. doi: 10.1016/S2214-109X(20)30288-6

5. World Health Organization. *Global tuberculosis report* (2022). Available at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022.

6. World Health Organization. *Coronavirus disease (COVID-19) pandemic*. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.

7. *How an outbreak became a pandemic: The defining moments of the COVID-19 pandemic. The Independent Panel for Pandemic Preparedness and Response* (2021). Available at: https://theindependentpanel.org/wp-content/uploads/2021/05/How-an-outbreak-became-a-pandemic_final.pdf.

8. *COVID-19: Make it the Last Pandemic. The Independent Panel for Pandemic Preparedness and Response* (2021). Available at: https://theindependentpanel.org/wp-content/uploads/2021/05/COVID-19-Make-it-the-Last-Pandemic_final.pdf.

9. Paulson T. Epidemiology: A mortal foe. *Nature* (2013) 502:S2–3. doi: 10.1038/502S2a

10. Ivanova O, Hoffmann VS, Lange C, Hoelscher M, Rachow A. Post-tuberculosis lung impairment: systematic review and meta-analysis of spirometry data from 14 621 people. *Eur Respir Rev* (2023) 32:220221. doi: 10.1183/16000617.0221-2022

11. Nightingale R, Carlin F, Meghji J, McMullen K, Evans D, van der Zalm MM, et al. Post-TB health and wellbeing. *Int J Tuberc Lung Dis* (2023) 27:248–83. doi: 10.5588/ijtld.22.0514

12. Ayouba A, Thaurignac G, Morquin D, Tuaillon E, Raulino R, Nkuba A, et al. Multiplex detection and dynamics of IgG antibodies to SARS-CoV2 and the highly pathogenic human coronaviruses SARS-CoV and MERS-CoV. *J Clin Virol* (2020) 129:104521. doi: 10.1016/j.jcv.2020.104521

13. Hu B, Guo H, Zhou P, Shi Z-L. Characteristics of SARS-coV-2 and COVID-19. *Nat Rev Microbiol* (2021) 19:141–54. doi: 10.1038/s41579-020-00459-7

14. Florio M, Gamba S, Pancotti C. Mapping of long-term public and private investments in the development of Covid-19 vaccines. *Eur Parliament COVI Committee* (2023) 1–95. doi: 10.2861/481388

15. Corey L, Miner MD. Accelerating clinical trial development in vaccinology: COVID-19 and beyond. *Curr Opin Immunol* (2022) 76:102206. doi: 10.1016/j.coi.2022.102206

16. Edwards DK, Carfi A. Messenger ribonucleic acid vaccines against infectious diseases: current concepts and future prospects. *Curr Opin Immunol* (2022) 77:102214. doi: 10.1016/j.coi.2022.102214

17. Alakija A. Leveraging lessons from the COVID-19 pandemic to strengthen low-income and middle-income country preparedness for future global health threats. *Lancet Infect Dis* (2023) 23(8):e310-e317. doi: 10.1016/S1473-3099(23)00279-7

18. Agarwal R, Reed T. *Finance vaccine equity: funding for day-zero of the next pandemic IMF working paper no. 2022/099* (2022). Available at: https://ssrn.com/abstract=4129564.

19. Sah P, Vilches TN, Moghadas SM, Pandey A, Gondi S, Schneider EC, et al. Return on investment of the COVID-19 vaccination campaign in new york city. *JAMA Netw Open* (2022) 5:e2243127. doi: 10.1001/jamanetworkopen.2022.43127

20. Scheppler L, De Clercq N, McGoldrick M, Dias J. Regulatory Harmonization and Streamlining of Clinical Trial Applications globally should lead to faster clinical development and earlier access to life-saving vaccines. *Vaccine* (2021) 39:790–6. doi: 10.1016/j.vaccine.2020.11.077

21. Kana BD, Arbuthnot P, Botwe BK, Choonara YE, Hassan F, Louzir H, et al. Opportunities and challenges of leveraging COVID-19 vaccine innovation and technologies for developing sustainable vaccine manufacturing capabilities in Africa. *Lancet Infect Dis* (2023) 23:e288–300. doi: 10.1016/S1473-3099(22)00878-7

22. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol* (2021) 21:83–100. doi: 10.1038/s41577-020-00479-7

23. Chauhan N, Tiwari S, Iype T, Jain U. An overview of adjuvants utilized in prophylactic vaccine formulation as immunomodulators. *Expert Rev Vaccines* (2017) 16:491–502. doi: 10.1080/14760584.2017.1306440

24. Didierlaurent AM, Laupèze B, Di Pasquale A, Hergli N, Collignon C, Garçon N. Adjuvant system AS01: helping to overcome the challenges of modern vaccines. *Expert Rev Vaccines* (2017) 16:55–63. doi: 10.1080/14760584.2016.1213632

25. Stertman L, Palm A-KE, Zarnegar B, Carow B, Lunderius Andersson C, Magnusson SE, et al. The Matrix-M™ adjuvant: A critical component of vaccines for the 21st century. *Hum Vaccin Immunother* (2023) 19:2189885. doi: 10.1080/21645515.2023.2189885

26. Turley JL, Lavelle EC. Resolving adjuvant mode of action to enhance vaccine efficacy. *Curr Opin Immunol* (2022) 77:102229. doi: 10.1016/j.coi.2022.102229

27. Hu Z, Lu S-H, Lowrie DB, Fan X-Y. Research advances for virus-vectored tuberculosis vaccines and latest findings on tuberculosis vaccine development. *Front Immunol* (2022) 13:895020. doi: 10.3389/fimmu.2022.895020

28. Lu J, Guo X, Wang C, Du W, Shen X, Su C, et al. Therapeutic effect of subunit vaccine AEC/BC02 on mycobacterium tuberculosis post-chemotherapy relapse using a latent infection murine model. *Vaccines* (2022) 10:825. doi: 10.3390/vaccines10050825

29. McCann N, O'Connor D, Lambe T, Pollard AJ. Viral vector vaccines. *Curr Opin Immunol* (2022) 77:102210. doi: 10.1016/j.coi.2022.102210

30. Jacob-Dolan C, Barouch DH. COVID-19 vaccines: adenoviral vectors. *Ann Rev Med* (2022) 73:41–54. doi: 10.1146/annurev-med-012621-102252

31. Buzitskaya Z, Stosman K, Khairullin B, Kassenov M, Nurpeisova A, Abylai Sansyzbay A, et al. A new intranasal influenza vector-based vaccine TB/FLU-04L against tuberculosis: preclinical safety studies. *Drug Res* (2022) 72:255–8. doi: 10.1055/a-1785-3936

32. Kieh M, Richert L, Beavogui AH, Grund B, Leigh B, D'Ortenzio E, et al. Randomized trial of vaccines for zaire ebola virus disease. *N Engl J Med* (2022) 387:2411–24. doi: 10.1056/NEJMoa2200072

33. Hald Albertsen C, Kulkarni JA, Witzigmann D, Lind M, Petersson K, Simonsen JB. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. *Adv Drug Deliv Rev* (2022) 188:114416. doi: 10.1016/j.addr.2022.114416

34. Hogan MJ, Pardi N. mRNA vaccines in the COVID-19 pandemic and beyond. *Annu Rev Med* (2022) 73:17–39. doi: 10.1146/annurev-med-042420-112725

35. Verbeke R, Hogan MJ, Loré K, Pardi N. Innate immune mechanisms of mRNA vaccines. *Immunity* (2022) 55:1993–2005. doi: 10.1016/j.immuni.2022.10.014

36. Lange C, Aaby P, Behr MA, Donald PR, Kaufmann SHE, Netea MG, et al. 100 years of Mycobacterium bovis bacille Calmette-Guérin. *Lancet Infect Dis* (2022) 22:e2–e12. doi: 10.1016/S1473-3099(21)00403-5

37. World Health Organization. *Emergency use listing (EUL)*. Available at: https://www.who.int/teams/regulation-prequalification/eul.

38. Bao Y, He L, Miao B, Zhong Z, Lu G, Bai Y, et al. BBIBP-CorV vaccination accelerates anti-viral antibody responses in heterologous Omicron infection: A retrospective observation study in Shanghai. *Vaccine* (2023) 41:3258–65. doi: 10.1016/j.vaccine.2023.03.070

39. Ralise AEG, Camargo TM, Marson FAL. Phase 4 clinical trials in the era of the Coronavirus Disease (COVID-19) pandemic and their importance to optimize the COVID-19 vaccination. *Hum Vaccin Immunother* (2023) 19:2234784. doi: 10.1080/21645515.2023.2234784

40. Hotez PJ, Bottazzi ME. Whole inactivated virus and protein-based COVID-19 vaccines. *Ann Rev Med* (2022) 73:55–64. doi: 10.1146/annurev-med-042420-113212

41. Taucher C, Lazarus R, Dellago H, Maurer G, Weisova P, Corbic-Ramljak I, et al. Safety and immunogenicity against ancestral, Delta and Omicron virus variants following a booster dose of an inactivated whole-virus COVID-19 vaccine (VLA2001): Interim analysis of an open-label extension of the randomized, controlled, phase 3 COV-COMPARE trial. *J Infect* (2023) 87(3):242–54. doi: 10.1016/j.jinf.2023.06.022

42. Rose W, Raju R, Babji S, George A, Madhavan R, Leander Xavier JV, et al. Immunogenicity and safety of homologous and heterologous booster vaccination of ChAdOx1 nCoV-19 (COVISHIELD™) and BBV152 (COVAXIN®): a non-inferiority phase 4, participant and observer-blinded, randomised study. *Lancet Reg Health Southeast Asia* (2023) 12:100141. doi: 10.1016/j.lansea.2023.100141

43. Madhi SA, Kwatra G, Richardson SI, Koen AL, Baillie V, Cutland CL, et al. Durability of ChAdOx1 nCoV-19 (AZD1222) vaccine and hybrid humoral immunity against variants including omicron BA.1 and BA.4 6 months after vaccination (COV005): a *post-hoc* analysis of a randomised, phase 1b–2a trial. *Lancet Infect Dis* (2023) 23:295–306. doi: 10.1016/S1473-3099(22)00596-5

44. Ferrara F, Mancaniello C, Varriale A, Sorrentino S, Zovi A, Nava E, et al. COVID-19 mRNA vaccines: A retrospective observational pharmacovigilance study. *Clin Drug Investig* (2022) 42:1065–74. doi: 10.1007/s40261-022-01216-9

45. Jin P, Guo X, Chen W, Ma S, Pan H, Dai L, et al. Safety and immunogenicity of heterologous boost immunization with an adenovirus type-5-vectored and protein-subunit-based COVID-19 vaccine (Convidecia/ZF2001): A randomized, observer-blinded, placebo-controlled trial. *PloS Med* (2022) 19:e1003953. doi: 10.1371/journal.pmed.1003953

46. Underwood E, Dunkle LM, Madhi SA, Gay CL, Heath PT, Kotloff KL, et al. Safety, efficacy, and immunogenicity of the NVX-CoV2373 vaccine. *Expert Rev Vaccines* (2023) 22:501–17. doi: 10.1080/14760584.2023.2218913

47. Ku JH, Sy LS, Qian L, Ackerson BK, Luo Y, Tubert JE, et al. Effectiveness of a fourth dose of mRNA-1273 against COVID-19 among older adults in the United States: Interim results from an observational cohort study. *Vaccine* (2023) 41:4212–9. doi: 10.1016/j.vaccine.2023.06.016

48. Moreira ED, Kitchin N, Xu X, Dychter SS, Lockhart S, Gurtman A, et al. Safety and efficacy of a third dose of BNT162b2 covid-19 vaccine. *N Engl J Med* (2022) 386:1910–21. doi: 10.1056/NEJMoa2200674

49. Pérez-Then E, Lucas C, Monteiro VS, Miric M, Brache V, Cochon L, et al. Neutralizing antibodies against the SARS-CoV-2 Delta and Omicron variants following heterologous CoronaVac plus BNT162b2 booster vaccination. *Nat Med* (2022) 28:481–5. doi: 10.1038/s41591-022-01705-6

50. Murray SM, Ansari AM, Frater J, Klenerman P, Dunachie S, Barnes E, et al. The impact of pre-existing cross-reactive immunity on SARS-CoV-2 infection and vaccine responses. *Nat Rev Immunol* (2022) 23:304–16. doi: 10.1038/s41577-022-00809-x

51. Jacobs JL, Haidar G, Mellors JW. COVID-19: challenges of viral variants. *Annu Rev Med* (2023) 74:31–53. doi: 10.1146/annurev-med-042921-020956

52. Chen Y, Zhao X, Zhou H, Zhu H, Jiang S, Wang P. Broadly neutralizing antibodies to SARS-CoV-2 and other human coronaviruses. *Nat Rev Immunol* (2023) 23:189–99. doi: 10.1038/s41577-022-00784-3

53. Sette A, Sidney J, Crotty S. T cell responses to SARS-coV-2. *Ann Rev Immunol* (2023) 41:343–73. doi: 10.1146/annurev-immunol-101721-061120

54. Wherry EJ, Barouch DH. T cell immunity to COVID-19 vaccines. *Science* (2022) 377:821–2. doi: 10.1126/science.add2897

55. Netea MG, Ziogas A, Benn CS, Giamarellos-Bourboulis EJ, Joosten LAB, Arditi M, et al. The role of trained immunity in COVID-19: Lessons for the next pandemic. *Cell Host Microbe* (2023) 31:890–901. doi: 10.1016/j.chom.2023.05.004

56. Mackin SR, Desai P, Whitener BM, Karl CE, Liu M, Baric RS, et al. Fc-γR-dependent antibody effector functions are required for vaccine-mediated protection against antigen-shifted variants of SARS-CoV-2. *Nat Microbiol* (2023) 8:569–80. doi: 10.1038/s41564-023-01359-1

57. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, et al. Tuberculosis. *Nat Rev Dis Primers* (2016) 2:16076. doi: 10.1038/nrdp.2016.76

58. Reece ST, Kaufmann SHE. Host defenses to intracellular bacteria. In: Rich RR, Fleisher TA, Harry W. Schroeder J, Weyand CM, Corry DB, Puck JM, editors. *Clinical immunology. Principles and practice*. Elsevier (2023). p. 331–46.

59. Dorhoi A, Kaufmann SHE. Pathology and immune reactivity: understanding multidimensionality in pulmonary tuberculosis. *Semin Immunopathol* (2016) 38:153–66. doi: 10.1007/s00281-015-0531-3

60. Cohen SB, Gern BH, Urdahl KB. The tuberculous granuloma and preexisting immunity. *Annu Rev Immunol* (2022) 40:589–614. doi: 10.1146/annurev-immunol-093019-125148

61. Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, Ioerger T, et al. Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. *Nat Med* (2014) 20:75–9. doi: 10.1038/nm.3412

62. Koeken V, Verrall AJ, Netea MG, Hill PC, van Crevel R. Trained innate immunity and resistance to Mycobacterium tuberculosis infection. *Clin Microbiol Infect* (2019) 25:1468–72. doi: 10.1016/j.cmi.2019.02.015

63. Abebe F. Immunological basis of early clearance of Mycobacterium tuberculosis infection: the role of natural killer cells. *Clin Exp Immunol* (2021) 204:32–40. doi: 10.1111/cei.13565

64. Flynn JL, Chan J. Immune cell interactions in tuberculosis. *Cell* (2022) 185:4682–702. doi: 10.1016/j.cell.2022.10.025

65. Kim H, Shin SJ. Pathological and protective roles of dendritic cells in Mycobacterium tuberculosis infection: Interaction between host immune responses and pathogen evasion. *Front Cell Infect Microbiol* (2022) 12:891878. doi: 10.3389/fcimb.2022.891878

66. Ganchua SKC, White AG, Klein EC, Flynn JL. Lymph nodes—The neglected battlefield in tuberculosis. *PloS Pathog* (2020) 16:e1008632. doi: 10.1371/journal.ppat.1008632

67. Ottenhoff THM, Kaufmann SHE. Vaccines against tuberculosis: where are we and where do we need to go? *PloS Pathog* (2012) 8:e1002607. doi: 10.1371/journal.ppat.1002607

68. Gupta RK, Lawn SD, Bekker LG, Caldwell J, Kaplan R, Wood R. Impact of human immunodeficiency virus and CD4 count on tuberculosis diagnosis: analysis of city-wide data from Cape Town, South Africa. *Int J Tuberc Lung Dis* (2013) 17:1014–22. doi: 10.5588/ijtld.13.0032

69. Lawn SD, Myer L, Edwards D, Bekker LG, Wood R. Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa. *AIDS* (2009) 23:1717–25. doi: 10.1097/QAD.0b013e32832d3b6d

70. Dorhoi A, Kaufmann SHE. Tumor necrosis factor alpha in mycobacterial infection. *Semin Immunol* (2014) 26:203–9. doi: 10.1016/j.smim.2014.04.003

71. Sakai S, Mayer-Barber KD, Barber DL. Defining features of protective CD4 T cell responses to Mycobacterium tuberculosis. *Cur Opin Immunol* (2014) 29:137–42. doi: 10.1016/j.coi.2014.06.003

72. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor α-neutralizing agent. *N Engl J Med* (2001) 345:1098–104. doi: 10.1056/NEJMoa011110

73. Ogongo P, Tezera LB, Ardain A, Nhamoyebonde S, Ramsuran D, Singh A, et al. Tissue-resident-like CD4+ T cells secreting IL-17 control Mycobacterium tuberculosis in the human lung. *J Clin Invest* (2021) 131:e142014. doi: 10.1172/JCI142014

74. Coulter F, Parrish A, Manning D, Kampmann B, Mendy J, Garand M, et al. IL-17 production from T helper 17, mucosal-associated invariant T, and gammadelta cells in tuberculosis infection and disease. *Front Immunol* (2017) 8:1252. doi: 10.3389/fimmu.2017.01252

75. Noschka R, Wondany F, Kizilsavas G, Weil T, Weidinger G, Walther P, et al. Gran1: A Granulysin-Derived Peptide with Potent Activity against Intracellular Mycobacterium tuberculosis. *Int J Mol Sci* (2021) 22:8392. doi: 10.3390/ijms22168392

76. Stenger S, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich CJ, et al. An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science* (1998) 282:121–5. doi: 10.1126/science.282.5386.121

77. Woodworth JS, Wu Y, Behar SM. Mycobacterium tuberculosis-specific CD8+ T cells require perforin to kill target cells and provide protection *in vivo*1. *J Immunol* (2008) 181:8595–603. doi: 10.4049/jimmunol.181.12.8595

78. Napier RJ, Adams EJ, Gold MC, Lewinsohn DM. The role of mucosal associated invariant T cells in antimicrobial immunity. *Front Immunol* (2015) 6:344. doi: 10.3389/fimmu.2015.00344

79. Kaufmann SH. Future vaccination strategies against tuberculosis: thinking outside the box. *Immunity* (2010) 33:567–77. doi: 10.1016/j.immuni.2010.09.015

80. Kaufmann SHE. γ/δ and other unconventional T lymphocytes: What do they see and what do they do? *Proc Natl Acad Sci USA* (1996) 93:2272–9. doi: 10.1073/pnas.93.6.2272

81. La Manna MP, Orlando V, Tamburini B, Badami GD, Dieli F, Caccamo N. Harnessing unconventional T cells for immunotherapy of tuberculosis. *Front Immunol* (2020) 11:2107. doi: 10.3389/fimmu.2020.02107

82. Lockhart E, Green AM, Flynn JL. IL-17 Production Is Dominated by γδ T Cells rather than CD4 T Cells during Mycobacterium tuberculosis Infection1. *J Immunol* (2006) 177:4662–9. doi: 10.4049/jimmunol.177.7.4662

83. Ogongo P, Steyn AJC, Karim F, Dullabh KJ, Awala I, Madansein R, et al. Differential skewing of donor-unrestricted and γδ T cell repertoires in tuberculosis-infected human lungs. *J Clin Invest* (2020) 130:214–30. doi: 10.1172/JCI130711

84. Van Rhijn I, Kasmar A, de Jong A, Gras S, Bhati M, Doorenspleet ME, et al. A conserved human T cell population targets mycobacterial antigens presented by CD1b. *Nat Immunol* (2013) 14:706–13. doi: 10.1038/ni.2630

85. Gold MC, Napier RJ, Lewinsohn DM. MR1-restricted mucosal associated invariant T (MAIT) cells in the immune response to Mycobacterium tuberculosis. *Immunol Rev* (2015) 264:154–66. doi: 10.1111/imr.12271

86. Sakai S, Kauffman KD, Oh S, Nelson CE, Barry CE, Barber DL. MAIT cell-directed therapy of Mycobacterium tuberculosis infection. *Mucosal Immunol* (2021) 14:199–208. doi: 10.1038/s41385-020-0332-4

87. Ruibal P, Voogd L, Joosten SA, Ottenhoff THM. The role of donor-unrestricted T-cells, innate lymphoid cells, and NK cells in anti-mycobacterial immunity. *Immunol Rev* (2021) 301:30–47. doi: 10.1111/imr.12948

88. Korchagina AA, Koroleva E, Tumanov AV. Innate lymphoid cells in response to intracellular pathogens: protection versus immunopathology. *Front Cell Infect Microbiol* (2021) 11:775554. doi: 10.3389/fcimb.2021.775554

89. Seo GY, Giles DA, Kronenberg M. The role of innate lymphoid cells in response to microbes at mucosal surfaces. *Mucosal Immunol* (2020) 13:399–412. doi: 10.1038/s41385-020-0265-y

90. Fillatreau S. B cells and their cytokine activities implications in human diseases. *Clin Immunol* (2018) 186:26–31. doi: 10.1016/j.clim.2017.07.020

91. Maglione PJ, Xu J, Chan J. B Cells Moderate Inflammatory Progression and Enhance Bacterial Containment upon Pulmonary Challenge with Mycobacterium tuberculosis1. *J Immunol* (2007) 178:7222–34. doi: 10.4049/jimmunol.178.11.7222

92. Chan J, Mehta S, Bharrhan S, Chen Y, Achkar JM, Casadevall A, et al. The role of B cells and humoral immunity in Mycobacterium tuberculosis infection. *Semin Immunol* (2014) 26:588–600. doi: 10.1016/j.smim.2014.10.005

93. Lu LL, Chung AW, Rosebrock TR, Ghebremichael M, Yu WH, Grace PS, et al. A functional role for antibodies in tuberculosis. *Cell* (2016) 167:433–443.e14. doi: 10.1016/j.cell.2016.08.072

94. Maglione PJ, Xu J, Casadevall A, Chan J. Fcγ Receptors Regulate Immune Activation and Susceptibility during Mycobacterium tuberculosis Infection1. *J Immunol* (2008) 180:3329–38. doi: 10.4049/jimmunol.180.5.3329

95. Wang TT, Ravetch JV. Functional diversification of IgGs through Fc glycosylation. *J Clin Invest* (2019) 129:3492–8. doi: 10.1172/JCI130029

96. Carpenter SM, Lu LL. Leveraging antibody, B cell and fc receptor interactions to understand heterogeneous immune responses in tuberculosis. *Front Immunol* (2022) 13:830482. doi: 10.3389/fimmu.2022.830482

97. Armstrong JA, Hart PD. Response of cultured macrophages to Mycobacterium tuberculosis, with observations on fusion of lysosomes with phagosomes. *J Exp Med* (1971) 134:713–40. doi: 10.1084/jem.134.3.713

98. Armstrong JA, Hart PD. Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival. *J Exp Med* (1975) 142:1–16. doi: 10.1084/jem.142.1.1

99. Gideon HP, Phuah J, Myers AJ, Bryson BD, Rodgers MA, Coleman MT, et al. Variability in tuberculosis granuloma T cell responses exists, but a balance of pro- and anti-inflammatory cytokines is associated with sterilization. *PloS Pathog* (2015) 11:e1004603. doi: 10.1371/journal.ppat.1004603

100. Kaufmann SH, Dorhoi A. Inflammation in tuberculosis: interactions, imbalances and interventions. *Curr Opin Immunol* (2013) 25:441–9. doi: 10.1016/j.coi.2013.05.005

101. Simmons JD, Stein CM, Seshadri C, Campo M, Alter G, Fortune S, et al. Immunological mechanisms of human resistance to persistent Mycobacterium tuberculosis infection. *Nat Rev Immunol* (2018) 18:575–89. doi: 10.1038/s41577-018-0025-3

102. Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, et al. Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. *Clin Microbiol Rev* (2018) 31:e00021–18. doi: 10.1128/cmr.00021-18

103. Dorhoi A, Reece ST, Kaufmann SH. For better or for worse: the immune response against *Mycobacterium tuberculosis* balances pathology and protection. *Immunol Rev* (2011) 240:235–51. doi: 10.1111/j.1600-065X.2010.00994.x

104. Reece ST, Kaufmann SH. Floating between the poles of pathology and protection: can we pin down the granuloma in tuberculosis? Curr. *Opin Microbiol* (2012) 15:63–70. doi: 10.1016/j.mib.2011.10.006

105. Kaushal D, Foreman TW, Gautam US, Alvarez X, Adekambi T, Rangel-Moreno J, et al. Mucosal vaccination with attenuated Mycobacterium tuberculosis induces strong central memory responses and protects against tuberculosis. *Nat Commun* (2015) 6:8533. doi: 10.1038/ncomms9533

106. Kok L, Masopust D, Schumacher TN. The precursors of CD8+ tissue resident memory T cells: from lymphoid organs to infected tissues. *Nat Rev Immunol* (2022) 22:283–93. doi: 10.1038/s41577-021-00590-3

107. Yenyuwadee S, Sanchez-Trincado Lopez JL, Shah R, Rosato PC, Boussiotis VA. The evolving role of tissue-resident memory T cells in infections and cancer. *Sci Adv* (2022) 8:eabo5871. doi: 10.1126/sciadv.abo5871

108. Perdomo C, Zedler U, Kühl AA, Lozza L, Saikali P, Sander LE, et al. Mucosal BCG vaccination induces protective lung-resident memory T cell populations against tuberculosis. *mBio* (2016) 7:e01686–16. doi: 10.1128/mBio.01686-16

109. Swanson RV, Gupta A, Foreman TW, Lu L, Choreno-Parra JA, Mbandi SK, et al. Antigen-specific B cells direct T follicular-like helper cells into lymphoid follicles to mediate Mycobacterium tuberculosis control. *Nat Immunol* (2023) 24:855–68. doi: 10.1038/s41590-023-01476-3

110. Ulrichs T, Kosmiadi GA, Trusov V, Jörg S, Pradl L, Titukhina M, et al. Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. *J Pathol* (2004) 204:217–28. doi: 10.1002/path.1628

111. Knaul JK, Jorg S, Oberbeck-Mueller D, Heinemann E, Scheuermann L, Brinkmann V, et al. Lung-residing myeloid-derived suppressors display dual functionality in murine pulmonary tuberculosis. *Am J Respir Crit Care Med* (2014) 190:1053–66. doi: 10.1164/rccm.201405-0828OC

112. Dorhoi A, Kotzé LA, Berzofsky JA, Sui Y, Gabrilovich DI, Garg A, et al. Therapies for tuberculosis and AIDS: myeloid-derived suppressor cells in focus. *J Clin Invest* (2020) 130:2789–99. doi: 10.1172/JCI136288

113. Ahmed A, Vyakarnam A. Emerging patterns of regulatory T cell function in tuberculosis. *Clin Exp Immunol* (2020) 202:273–87. doi: 10.1111/cei.13488

114. Barber DL, Sakai S, KudChadkar RR, Fling SP, Day TA, Vergara JA, et al. Tuberculosis following PD-1 blockade for cancer immunotherapy. *Sci Transl Med* (2019) 11:eaat2702. doi: 10.1126/scitranslmed.aat2702

115. Jayaraman P, Jacques MK, Zhu C, Steblenko KM, Stowell BL, Madi A, et al. TIM3 Mediates T Cell Exhaustion during Mycobacterium tuberculosis Infection. *PloS Pathog* (2016) 12:e1005490. doi: 10.1371/journal.ppat.1005490

116. Tezera LB, Bielecka MK, Ogongo P, Walker NF, Ellis M, Garay-Baquero DJ, et al. Anti-PD-1 immunotherapy leads to tuberculosis reactivation *via* dysregulation of TNF-α. *eLife* (2020) 9:e52668. doi: 10.7554/eLife.52668

117. Gengenbacher M, Kaufmann SHE. *Mycobacterium tuberculosis*: success through dormancy. *FEMS Microbiol Rev* (2012) 36:514–32. doi: 10.1111/j.1574-6976.2012.00331.x

118. Lewis K. Persister cells. *Annu Rev Microbiol* (2010) 64:357–72. doi: 10.1146/annurev.micro.112408.134306

119. Kaufmann SHE. The TB vaccine development pipeline: present and future priorities and challenges for research and innovation. In: Migliori GB, Raviglione ME, editors. *Essential tuberculosis*. Switzerland: Springer Nature (2021). p. 395–405.

120. Hanthamrongwit J, Aruvornlop P, Saelee C, Wanta N, Poneksawat P, Soe PT, et al. Peptide microarray-based identification of dormancy-associated Mycobacterium

tuberculosis antigens inducing immune responses among latent tuberculosis infection individuals in Thailand. *Sci Rep* (2023) 13:6978. doi: 10.1038/s41598-023-34307-4

121. Behr MA, Kaufmann E, Duffin J, Edelstein PH, Ramakrishnan L. Latent tuberculosis: two centuries of confusion. *Am J Respir Crit Care Med* (2021) 204:142–8. doi: 10.1164/rccm.202011-4239PP

122. Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon LM, et al. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. *Lancet* (2016) 387:2312–22. doi: 10.1016/S0140-6736(15)01316-1

123. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* (2010) 466:973–7. doi: 10.1038/nature09247

124. Penn-Nicholson A, Mbandi SK, Thompson E, Mendelsohn SC, Suliman S, Chegou NN, et al. RISK6, a 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment response. *Sci Rep* (2020) 10:8629. doi: 10.1038/s41598-020-65043-8

125. Suliman S. BCG: From veins to correlates. *Cell Host Microbe* (2023) 31:921–3. doi: 10.1016/j.chom.2023.05.021

126. Weiner J, Maertzdorf J, Sutherland JS, Duffy FJ, Thompson E, Suliman S, et al. Metabolite changes in blood predict the onset of tuberculosis. *Nat Commun* (2018) 9:5208. doi: 10.1038/s41467-018-07635-7

127. Diel R, Goletti D, Ferrara G, Bothamley G, Cirillo D, Kampmann B, et al. Interferon-gamma release assays for the diagnosis of latent Mycobacterium tuberculosis infection: a systematic review and meta-analysis. *Eur Respir J* (2011) 37:88–99. doi: 10.1183/09031936.00115110

128. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* (2008) 149:177–84. doi: 10.7326/0003-4819-149-3-200808050-00241

129. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* (1974) 99:131–8. doi: 10.1093/oxfordjournals.aje.a121593

130. Eisenhut M, Paranjothy S, Abubakar I, Bracebridge S, Lilley M, Mulla R, et al. BCG vaccination reduces risk of infection with Mycobacterium tuberculosis as detected by gamma interferon release assay. *Vaccine* (2009) 27:6116–20. doi: 10.1016/j.vaccine.2009.08.031

131. Kaipilyawar V, Salgame P. Infection resisters: targets of new research for uncovering natural protective immunity against Mycobacterium tuberculosis. *F1000Research* (2019) 8:1698. doi: 10.12688/f1000research.19805.1

132. Stein CM, Nsereko M, Malone LL, Okware B, Kisingo H, Nalukwago S, et al. Long-term stability of resistance to latent mycobacterium tuberculosis infection in highly exposed tuberculosis household contacts in Kampala, Uganda. *Clin Infect Dis* (2018) 68:1705–12. doi: 10.1093/cid/ciy751

133. Medawar L, Tukiman HM, Mbayo G, Donkor S, Owolabi O, Sutherland JS. Analysis of cellular and soluble profiles in QuantiFERON nonconverters, converters, and reverters in the Gambia. *Immun Inflamm Dis* (2019) 7:260–70. doi: 10.1002/iid3.269

134. Garcia-Basteiro AL, White RG, Tait D, Schmidt AC, Rangaka MX, Quaife M, et al. End-point definition and trial design to advance tuberculosis vaccine development. *Eur Respir Rev* (2022) 31:220044. doi: 10.1183/16000617.0044-2022

135. Mpande CAM, Steigler P, Lloyd T, Rozot V, Mosito B, Schreuder C, et al. Mycobacterium tuberculosis-specific T cell functional, memory, and activation profiles in quantiFERON-reverters are consistent with controlled infection. *Front Immunol* (2021) 12:712480. doi: 10.3389/fimmu.2021.712480

136. Dinkele R, Gessner S, McKerry A, Leonard B, Seldon R, Koch AS, et al. Capture and visualization of live Mycobacterium tuberculosis bacilli from tuberculosis patient bioaerosols. *PloS Pathog* (2021) 17:e1009262. doi: 10.1371/journal.ppat.1009262

137. Nguyen HV, Tiemersma E, Nguyen NV, Nguyen HB, Cobelens F. Disease transmission by patients with subclinical tuberculosis. *Clin Infect Dis* (2023) 76:2000–6. doi: 10.1093/cid/ciad027

138. Patterson B, Bryden W, Call C, McKerry A, Leonard B, Seldon R, et al. Cough-independent production of viable Mycobacterium tuberculosis in bioaerosol. *Tuberculosis* (2021) 126:102038. doi: 10.1016/j.tube.2020.102038

139. Ryckman TS, Dowdy DW, Kendall EA. Infectious and clinical tuberculosis trajectories: Bayesian modeling with case finding implications. *Proc Natl Acad Sci U.S.A.* (2022) 119:e2211045119. doi: 10.1073/pnas.2211045119

140. Bouzeyen R, Javid B. Therapeutic vaccines for tuberculosis: an overview. *Front Immunol* (2022) 13:878471. doi: 10.3389/fimmu.2022.878471

141. Nemes E, Fiore-Gartland A, Boggiano C, Coccia M, D'Souza P, Gilbert P, et al. The quest for vaccine-induced immune correlates of protection against tuberculosis. *Vaccine Insights* (2022) 1:165–81. doi: 10.18609/vac/2022.027

142. Sadoff JC, Wittes J. Correlates, surrogates, and vaccines. *J Infect Dis* (2007) 196:1279–81. doi: 10.1086/522432

143. World Health Organization. *Correlates of vaccine-induced protection: methods and implications* (2013). Available at: https://apps.who.int/iris/handle/10665/84288.

144. Britto C, Alter G. The next frontier in vaccine design: blending immune correlates of protection into rational vaccine design. *Curr Opin Immunol* (2022) 78:102234. doi: 10.1016/j.coi.2022.102234

145. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol* (2010) 17:1055–65. doi: 10.1128/cvi.00131-10

146. Plotkin SA, Gilbert PB. Nomenclature for immune correlates of protection after vaccination. *Clin Infect Dis* (2012) 54:1615–7. doi: 10.1093/cid/cis238

147. Bitencourt J, Peralta-Álvarez MP, Wilkie M, Jacobs A, Wright D, Salman Almujri S, et al. Induction of functional specific antibodies, igG-secreting plasmablasts and memory B cells following BCG vaccination. *Front Immunol* (2021) 12:798207. doi: 10.3389/fimmu.2021.798207

148. Kawahara JY, Irvine EB, Alter G. A case for antibodies as mechanistic correlates of immunity in tuberculosis. *Front Immunol* (2019) 10:996. doi: 10.3389/fimmu.2019.00996

149. Nziza N, Cizmeci D, Davies L, Irvine EB, Jung W, Fenderson BA, et al. Defining discriminatory antibody fingerprints in active and latent tuberculosis. *Front Immunol* (2022) 13:856906. doi: 10.3389/fimmu.2022.856906

150. Musvosvi M, Huang H, Wang C, Xia Q, Rozot V, Krishnan A, et al. T cell receptor repertoires associated with control and disease progression following Mycobacterium tuberculosis infection. *Nat Med* (2023) 29:258–69. doi: 10.1038/s41591-022-02110-9

151. Kaufmann SH, Evans TG, Hanekom WA. Tuberculosis vaccines: time for a global strategy. *Sci Transl Med* (2015) 7:276fs8. doi: 10.1126/scitranslmed.aaa4730

152. Kaufmann SHE. Vaccination against tuberculosis: revamping BCG by molecular genetics guided by immunology. *Front Immunol* (2020) 11:316. doi: 10.3389/fimmu.2020.00316

153. Calmette A, Guérin C, Boquet A, Négre L. *La vaccination préventive contre la tuberculose par le "BCG"*. Masson, Paris: Masson et Cie (1927). p. 250.

154. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet* (2006) 367:1173–80. doi: 10.1016/s0140-6736(06)68507-3

155. Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, Bilek N, et al. Prevention of M. tuberculosis infection with H4:IC31 vaccine or BCG revaccination. *N Engl J Med* (2018) 379:138–49. doi: 10.1056/NEJMoa1714021

156. Darrah PA, Zeppa JJ, Maiello P, Hackney JA, Wadsworth MH, Hughes TK, et al. Prevention of tuberculosis in macaques after intravenous BCG immunization. *Nature* (2020) 577:95–102. doi: 10.1038/s41586-019-1817-8

157. Darrah PA, Zeppa JJ, Wang C, Irvine EB, Bucsan AN, Rodgers MA, et al. Airway T cells are a correlate of i.v. Bacille Calmette-Guerin-mediated protection against tuberculosis in rhesus macaques. *Cell Host Microbe* (2023) 31:962–977.e8. doi: 10.1016/j.chom.2023.05.006

158. Liu YE, Darrah PA, Zeppa JJ, Kamath M, Laboune F, Douek DC, et al. Blood transcriptional correlates of BCG-induced protection against tuberculosis in rhesus macaques. *Cell Rep Med* (2023) 4:101096. doi: 10.1016/j.xcrm.2023.101096

159. Jenum S, Tonby K, Rueegg CS, Rühwald M, Kristiansen MP, Bang P, et al. A Phase I/II randomized trial of H56:IC31 vaccination and adjunctive cyclooxygenase-2-inhibitor treatment in tuberculosis patients. *Nat Commun* (2021) 12:6774. doi: 10.1038/s41467-021-27029-6

160. Sagawa ZK, Goman C, Frevol A, Blazevic A, Tennant J, Fisher B, et al. Safety and immunogenicity of a thermostable ID93 + GLA-SE tuberculosis vaccine candidate in healthy adults. *Nat Commun* (2023) 14:1138. doi: 10.1038/s41467-023-36789-2

161. Tkachuk AP, Bykonia EN, Popova LI, Kleymenov DA, Semashko MA, Chulanov VP, et al. Safety and immunogenicity of the gamTBvac, the recombinant subunit tuberculosis vaccine candidate: A phase II, multi-center, double-blind, randomized, placebo-controlled study. *Vaccines* (2020) 8:652. doi: 10.3390/vaccines8040652

162. Tait DR, Hatherill M, van der Meeren O, Ginsberg AM, Van Brakel E, Salaun B, et al. Final analysis of a trial of M72/AS01E vaccine to prevent tuberculosis. *N Engl J Med* (2019) 381:2429–39. doi: 10.1056/NEJMoa1909953

163. Van Der Meeren O, Hatherill M, Nduba V, Wilkinson RJ, Muyoyeta M, Van Brakel E, et al. Phase 2b controlled trial of M72/AS01E vaccine to prevent tuberculosis. *N Engl J Med* (2018) 379:1621–34. doi: 10.1056/NEJMoa1803484

164. Jeyanathan M, Fritz DK, Afkhami S, Aguirre E, Howie KJ, Zganiacz A, et al. but not intramuscular injection, of adenovirus-vectored tuberculosis vaccine induces respiratory-mucosal immunity in humans. *JCI Insight* (2022) 7:e155655. doi: 10.1172/jci.insight.155655

165. Wilkie M, Satti I, Minhinnick A, Harris S, Riste M, Ramon RL, et al. A phase I trial evaluating the safety and immunogenicity of a candidate tuberculosis vaccination regimen, ChAdOx1 85A prime – MVA85A boost in healthy UK adults. *Vaccine* (2020) 38:779–89. doi: 10.1016/j.vaccine.2019.10.102

166. McShane H, Pathan AA, Sander CR, Keating SM, Gilbert SC, Huygen K, et al. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. *Nat Med* (2004) 10:1240–4. doi: 10.1038/nm1128

167. Ndiaye BP, Thienemann F, Ota M, Landry BS, Camara M, Dieye S, et al. Safety, immunogenicity, and efficacy of the candidate tuberculosis vaccine MVA85A in healthy adults infected with HIV-1: a randomised, placebo-controlled, phase 2 trial. *Lancet Respir Med* (2015) 3:190–200. doi: 10.1016/S2213-2600(15)00037-5

168. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet* (2013) 381:1021–8. doi: 10.1016/S0140-6736(13)60177-4

169. Cardona PJ. RUTI: a new chance to shorten the treatment of latent tuberculosis infection. *Tuberculosis* (2006) 86:273–89. doi: 10.1016/j.tube.2006.01.024

170. Munseri P, Said J, Amour M, Magohe A, Matee M, Rees CA, et al. DAR-901 vaccine for the prevention of infection with Mycobacterium tuberculosis among BCG-

immunized adolescents in Tanzania: A randomized controlled, double-blind phase 2b trial. *Vaccine* (2020) 38:7239–45. doi: 10.1016/j.vaccine.2020.09.055

171. Sharma SK, Katoch K, Sarin R, Balambal R, Kumar Jain N, Patel N, et al. Efficacy and Safety of Mycobacterium indicus pranii as an adjunct therapy in Category II pulmonary tuberculosis in a randomized trial. *Sci Rep* (2017) 7:3354. doi: 10.1038/s41598-017-03514-1

172. Martín C, Marinova D, Aguiló N, Gonzalo-Asensio J. MTBVAC, a live TB vaccine poised to initiate efficacy trials 100 years after BCG. *Vaccine* (2021) 39:7277–85. doi: 10.1016/j.vaccine.2021.06.049

173. Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Nasser Eddine A, et al. Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette-Guérin mutants that secrete listeriolysin. *J Clin Invest* (2005) 115:2472–9. doi: 10.1172/JCI24617

174. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature* (1998) 393:537–44. doi: 10.1038/31159

175. Treatment Action Group (TAG). *Stop TB partnership. Tuberculosis research funding trends, 2005-2020* (2021). Available at: https://www.treatmentactiongroup.org/wp-content/uploads/2021/12/tb_funding_2021.pdf.

176. World Health Organization. *An investment case for new tuberculosis vaccines* (2022). Available at: https://apps.who.int/iris/rest/bitstreams/1485059/retrieve.

177. Boseley S. *'Gamechanging' TB vaccine within reach after $500m pledge to run final trials*. United Kingdom: The Guardian (2023). Available at: https://www.theguardian.com/global-development/2023/jun/28/bill-gates-wellcome-tb-vaccine-within-reach-after-500m-pledge-to-run-final-trials.

178. ITCR/ICMR. *Need for effective TB vaccines*. Available at: https://itrc.icmr.org.in/our-work/thematic-areas/vaccines.

179. Kaufmann SHE. Highly affordable vaccines are critical for our continued efforts to reduce global childhood mortality. *Hum Vaccin Immunother* (2019) 15:2660–5. doi: 10.1080/21645515.2019.1605817

180. GAVI The Vaccine Alliance. *COVAX*. Available at: https://www.gavi.org/covax-facility.

181. World Health Organization. *COVAX*. Available at: https://www.who.int/initiatives/act-accelerator/covax.

182. Portnoy A, Clark RA, Quaife M, Weerasuriya CK, Mukandavire C, Bakker R, et al. The cost and cost-effectiveness of novel tuberculosis vaccines in low- and middle-income countries: A modeling study. *PloS Med* (2023) 20:e1004155. doi: 10.1371/journal.pmed.1004155

183. United Nations. *WHO announces first technology recipients of mRNA vaccine hub with strong support from African and European partners* (2022). Available at: https://www.un.org/africarenewal/magazine/who-announces-first-technology-recipients-mrna-vaccine-hub-strong-support-african-and.

184. Larson HJ, Gakidou E, Murray CJL. The vaccine-hesitant moment. *N Engl J Med* (2022) 387:58–65. doi: 10.1056/NEJMra2106441

185. Sorell T, Butler J. The politics of covid vaccine hesitancy and opposition. . *Polit Q* (2022) 93:347–51. doi: 10.1111/1467-923x.13134

186. Singh P, Dhalaria P, Kashyap S, Soni GK, Nandi P, Ghosh S, et al. Strategies to overcome vaccine hesitancy: a systematic review. *Syst Rev* (2022) 11:78. doi: 10.1186/s13643-022-01941-4

187. Tuckerman J, Kaufman J, Danchin M. Effective approaches to combat vaccine hesitancy. *Pediatr Infect Dis J* (2022) 41:e243–5. doi: 10.1097/inf.0000000000003499

188. World Health Organization. *End TB by 2030. Framework for implementing the "End TB strategy" in the african region 2016 - 2020* (2017). Available at: https://apps.who.int/iris/bitstream/handle/10665/259636/TBstrat-eng.pdf?sequence=1.

189. Stop TB Partnership. *The Global Plan to end TB 2023-2030* (2022). Available at: https://www.stoptb.org/file/15583/download.

190. World Health Organization. *The end TB strategy report* (2015). Available at: https://www.who.int/publications/i/item/WHO-HTM-TB-2015.19.

191. World Health Organization. *UN General Assembly high-level meeting on tuberculosis* (2018). Available at: https://www.who.int/teams/global-tuberculosis-programme/unga-high-level-meeting-on-the-fight-against-tb/unga-high-level-meeting-on-ending-tb.

192. Stop TB Partnership. *TB vaccine clinical pipeline* (2023). Available at: https://newtbvaccines.org/tb-vaccine-pipeline/.

193. Stop TB Partnership. *United nations high-level meeting on TB 2023*. Available at: https://www.stoptb.org/advocate-to-endtb/united-nations-high-level-meeting-tb.

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