Innate Immune Signaling and Proteolytic Pathways in the Resolution or Exacerbation of SARS-CoV-2 in Covid-19: Key Therapeutic Targets?

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COVID-19 is caused by the Severe Acute Respiratory Syndrome (SARS) coronavirus (Cov)-2, an enveloped virus with a positive-polarity, single-stranded RNA genome. The initial outbreak of the pandemic began in December 2019, and it is affecting the human health of the global community. In common with previous pandemics (Influenza H1N1 and SARS-CoV) and the epidemics of Middle east respiratory syndrome (MERS)-CoV, CoVs target bronchial and alveolar epithelial cells. Virus protein ligands (e.g., haemagglutinin or trimeric spike glycoprotein for Influenza and CoV, respectively) interact with cellular receptors, such as (depending on the virus) either sialic acids, Dipeptidyl peptidase 4 (DPP4), or angiotensin-converting enzyme 2 (ACE2). Host proteases, e.g., cathepsins, furin, or members of the type II transmembrane serine proteases (TTSP) family, such as Transmembrane protease serine 2 (TMPRSS2), are involved in virus entry by proteolytically activating virus ligands. Also involved are Toll Like Receptor (TLR) family members, which upregulate anti-viral and pro-inflammatory mediators [interleukin (IL)-6 and IL-8 and type I and type III Interferons among others], through the activation of Nuclear Factor (NF)-kB. When these events (virus cellular entry and innate immune responses) are uncontrolled, a deleterious systemic response is sometimes encountered in infected patients, leading to the well-described "cytokine storm" and an ensuing multiple organ failure promoted by a downregulation of dendritic cell, macrophage, and T-cell function. We aim to describe how the lung and systemic host innate immune responses affect survival either positively, through downregulating initial viral load, or negatively, by triggering uncontrolled inflammation. An emphasis will be put on host cellular signaling pathways and proteases involved with a view on tackling these therapeutically.

Keywords: COVID-19, SARS-CoV-2, coronavirus, protease, lung innate immunity

INTRODUCTION

COVID-19 is a respiratory disease whose aetiologic agent is a novel beta coronavirus (CoV) called Severe Acute Respiratory Syndrome (SARS)-CoV-2/2019-nCov. The initial outbreak of the pandemic began in December 2019, and it is currently affecting the health and safety of the global community. Indeed, on May 12, 2020, 4.5 million worldwide cases were confirmed (probably

a significant under-estimation given the number of untested asymptomatic subjects), with a death toll exceeding 286,000. Before the SARS-CoV-2 outbreak, two related highly pathogenic CoVs viruses, Middle east respiratory syndrome (MERS)-CoV (1) and SARS-CoV (2), provoked catastrophic epidemics and pandemics, respectively. Unfortunately, no drugs nor vaccines have currently been approved to prevent or treat these viral episodes. The first anatomical/histological reports from the lungs of severely SARS-CoV-2-affected patients experiencing acute respiratory disease syndrome (ARDS) revealed excessive inflammatory activation and destruction of the bronchial and alveolar epithelium, features already observed during the first SARS pandemics in 2003 (3, 4). Indeed, in the latter pandemic, lung alveolar epithelial cells were identified as the most likely site of virus replication, and it was suggested that alveolar macrophages may be responsible for the dissemination of viruses within the lungs (3). In accordance, initial histological analyses of lung biopsies from patients positive for SARS-CoV-2 have shown exfoliation of the bronchial epithelium, which may induce altered mucociliary clearance and affect host immune responses (5).

Indeed, there is no doubt that the latter are involved in modulating disease onset and progression. For example, early studies report that, similarly with what was observed with SARS-CoV, lymphopenia [sometimes equivalent or more severe than that observed in human immunodeficiency virus (HIV) infection] is often observed in severely affected patients progressing to ARDS. Despite, or maybe correlated with this, aberrant non-effective innate immune host responses seem associated with severe lung disease during SARS (6–12).

The following sections will give an overview of the molecular and cellular mechanisms underpinning SARS-CoV virus infections and how lung and systemic host innate immune responses affect survival either positively, through downregulating the initial viral load, or negatively, by triggering uncontrolled inflammation. A particular emphasis will be put on the description of the host cellular signaling pathways and proteases involved with a view on tackling these therapeutically.

MECHANISMS OF ENTRY OF CORONAVIRUSES INTO TARGET EPITHELIAL CELLS (SEE FIGURE 1A)

CoVs are enveloped viruses with a positive-polarity, single-stranded RNA genome encoding four structural proteins: the transmembrane trimeric spike glycoprotein (S, composed of two subunits S1 and S2), envelop (E), matrix (M), and nucleocapsid (N) (13).

The entry of CoV viruses into host epithelial cells is mediated by the interaction between the viral envelope S protein homotrimers and the cell surface receptors. Following proteolytic cleavage of the CoV S protein ("priming"), the S1 ecto-domain recognizes a membrane receptor [angiotensin-converting enzyme 2 (ACE2) for SARS-Cov and SARS-Cov-2 as well as Dipeptidyl peptidase 4 (DPP4) for MERS-Cov], whereas the S2 C-terminal domain is involved in cell fusion and viral entry (14–16). This mechanism of action is very similar to that

used by *Influenza*, except that the latter use sialic acids as the cognate receptor for its hemagglutinin (HA) ligand. Importantly, many viruses (*Influenza,* MERS, CoV, and Paramyxoviruses such as Hendra and Nipah viruses) use similar host proteolytic enzymes for cleaving their ligands (HA and S), namely, mostly lysosomal (Cathepsins B, L), furin, or trypsin-like proteases (17, 18). Indeed, it is believed that it is the cellular source of these proteases that may determine the infectivity spectrum of these viruses, with the lung and the gastro-intestinal tract being high producers (19, 20).

Although a variety of these proteases have been studied and shown to be involved to varying degrees in virus activation, including neutrophil elastase (21), proteases of the type II transmembrane serine proteases (TTSP) family [HAT, Transmembrane protease, serine (TMPRSS)2, and TMPRSS4] have recently been demonstrated to be particularly important, albeit probably at different stages of the virus cell cycle (19, 20, 22, 23). In particular, recent research on SARS-Cov-2 has focused on TMPRSS2 and has shown it to be important (although mostly using cell lines infected with pseudotyped virus particles bearing SARS-Cov-2 S protein) for virus entry (24, 25). In that context, it has also been demonstrated that the serine protease inhibitor camostat (see also below section on Therapeutic targets and Conclusion) was protective (24, 25). In contrast, DPP4 which is necessary for the entry of MERS-CoV (26, 27) is not involved in SARS-Cov-2 entry (24). Unlike other SARS-CoVs, the S protein of SARS-CoV-2 has a furin cleavage site at the boundary between the S1 and S2 subunits, which is processed during biogenesis and which may explain CoV-2 high infectivity (28). Although mechanistic studies are obviously still in their infancy, it is very likely that SARS-CoV and SARS-CoV-2 target mainly respiratory epithelial cells with similar mechanisms. Indeed, as indicated above, initial work has shown that ACE2 is the S receptor for both SARS-CoV (29) and SARS-CoV-2 viruses (24, 28, 30), and structural studies using cryo-electron microscopy suggest a binding of two S protein trimer to an ACE2 dimer (28, 30).

Whether this is strictly dependent on ACE2/protease expression is debatable since ACE2 is present in other tissues in humans [such as the intestine, kidney, and testis (31)]. Indeed, "seasonal" low pathogenic CoVs (e.g., CoV-229E, CoV-OC43) infect mostly upper airways, whereas pathogenic CoVs (SARS-CoV/SARS-CoV-2 and MERS) have a tropism for the distal lung and can cause severe pneumonia and ARDS (32), as currently demonstrated again in the present pandemic. Indeed, potentially explaining this is the fact that seasonal coronaviruses do not use ACE2 as a receptor. *In vitro*, primary nasal and tracheobronchial
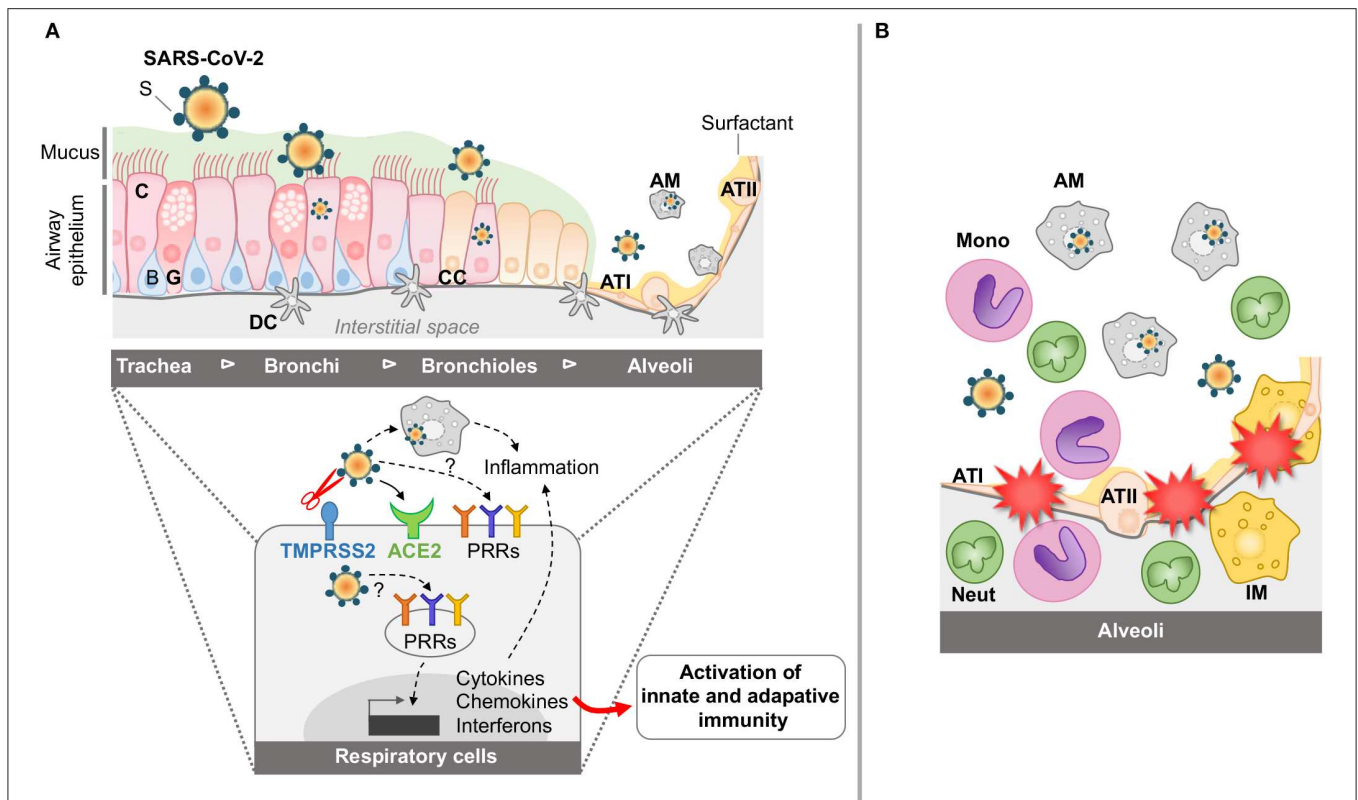
FIGURE 1 | Schematic representation of airway and lung infection by SARS-CoV-2 at early time points **(A)** and during SARS **(B)**. **(A)** The airway epithelium is composed of various cell types including ciliated (C), basal (B), glandular (G), and club cells (CC). It is covered by mucus (M) involved in the mucociliary clearance. Distal to the lung, the alveoli include alveolar type I (ATI) and type II (ATII) cells coated with surfactant (S). The airways are also protected by the resident alveolar macrophages (AM) and dendritic cells (DC). Through the action of host proteases, including the TTSP Transmembrane protease, serine 2 (TMPRSS2), and the interaction of SARS-CoV-2 with its cellular receptor angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 can enter and replicate into airway epithelial cells and AM. Also, interaction of the SARS-CoV-2 with the PRRs is instrumental in inducing cytokine, chemokine, and interferon responses for the establishment of innate and adaptive immune responses. **(B)** During SARS, innate immune responses are exacerbated in the alveolar space, with accumulation of activated monocytes (mono), activated macrophages (AM), interstitial macrophages (IM), and neutrophils (neut), leading to dysregulated inflammation, disruption of the alveolar-capillary membrane and tissue damage.

epithelial cells as well as the Calu-3 bronchial cell line were shown to express ACE2 (the latter not colocalizing with cilia), and their infection with SARS-CoV was shown to be highly cytotoxic (33, 34). In the distal lung, as hinted above, primary alveolar type II epithelial (ATII) cells are also permissive to SARS-CoV infection (35, 36). SARS-CoV-2 has also been shown to infect various respiratory epithelial cell lines including A549 (alveolar origin), BEAS2-B (bronchial origin), Calu-3 cells, as well as primary human bronchial epithelial cells (24). Besides the lung, ACE2 is also highly expressed in the intestine (37), and gastrointestinal symptoms have been recorded with COVID-19 (38). It was shown that SARS-COV2 is able to infect enterocytes as well as intestinal organoids and induces a viral response characterized by the expression of mediators related to type I and III IFN (39).

Even if SARS-CoV2 is thought to originate from bats, the intermediate host between bats and humans is still unknown. SARS-CoV was previously shown to infect various wild and domestic animals, including cats, ferrets and pigs (40–42).

Similarly, recent work reveals that domestic animals, including ferrets and cats, are permissive to SARS-CoV-2 infection. In contrast, the virus replicates poorly in pigs, ducks, chickens, and dogs (43).

Given the described importance of host proteases in mediating infectivity of a number of viruses, it is no surprise that, upon virus infection, murine knock-out (KO) for some of these molecules has shown some protection. For example, TMPRSS2-KO mice were protected from pulmonary disease and death following H1N1 and H7N9 *Influenza* infection, but not from that of the influenza H3N2 subtype, demonstrating some specificity and showing also that other TTSP proteases [such as DESC1 (TMPRSS11E) and MSPL (TMPRSS13)] or other factors may be important (44–47).

Similarly, TMPRSS2 KO mice showed reduced body weight and viral loads compared to WT mice in animals infected with SARS-CoV (48).

Also, it was demonstrated that over-expression of the human DPP4 in mice promoted MERS-CoV infection, causing lethal

FIGURE 2 | Prophylactic/therapeutic approaches to COVID-19. **(A)** Potential therapeutic anti-viral approaches during the early phase of infection. **(B)** Potential anti-inflammatory strategies targeting the alveolar space during the SARS period of COVID disease. **(C)** Immune intervention (vaccination of the population prior to epidemic episodes) or use of convalescent plasma/hyper-immune globulins on infected patients. LYM: lymphocytes; other acronyms are as described in **Figure 1**.

disease (49), and that TMPRSS2 was instrumental in that context (48).

ACTIVATION/MODULATION OF HOST SIGNALING PATHWAYS (SEE FIGURE 1A)

Epithelial Cells

The control of viral infection requires an optimal and innate coordinated host antiviral immunity. This response is activated by various sensors, including pattern recognition receptors (PRR), which recognize pathogen-associated molecular patterns (PAMPs). Although for many viruses, viral RNA is a PAMP classically detected by different sensors, including Toll-Like Receptors (TLR)3 (which senses double stranded (ds)RNA), TLR7 and TLR8 [which sense single stranded (ss)RNA], RIG-I (which senses short dsRNA and ssRNA specific motifs), and MDA-5 (which senses long dsRNA) (50), the sensors potentially recognizing SARS-CoV genomic material are still elusive. In addition, although, as mentioned above, distal peripheral lung alveolar epithelial cells seem to harbor SARS-CoV infection *in vivo*, and although respiratory epithelial cells are known to express TLR3, TLR7, and TLR8 (51, 52) and initiate innate immunity in the lung (53), the study of these cells in anti-CoV responses has been hampered by their general poor permissibility to the virus *in vitro* (except for intestinal Caco-2 and HEK293 kidney epithelial cells) (54). In that respect, although the specific PRR involved was not identified, the M protein of SARS-CoV was indeed shown to induce interferon (IFN)-β in a TLR-related-TRAF3-independent mechanism in HEK293 cells (55). Regarding the lung, the differentiated Calu-3 cell line [when cultured at the air-liquid interface (ALI)] is the model of choice: in that set-up, SARS-CoV infection triggered an inflammatory response characterized by increased production of interleukin (IL)-6, IL-8, gamma interferon (IFN-γ), inducible protein 10 (IP-10), and activation of the transcription factor NF-κB (56). However, the kinetics of this response was extremely slow, and importantly, type I IFN, an important mediator of anti-viral responses, was undetected.

Also, another study involving A549 cells demonstrated that the trimeric spike S glyprotein and virus-like particles were able to modestly upregulate CCL2, an important monocytic chemokine (57).

In addition to lung epithelial cells cultured at ALI, precision-cut lung slices could also be an interesting tool to study SARS-CoV2-cells interactions (58), as demonstrated in Influenza infections with human (59) or animal-derived material (60).

As mentioned above, TTSPs can activate virus-ligands (HA and S protein), but they are also able to modulate cell signaling pathways. For example, recombinant HAT is able to activate mucin gene expression in NCI-H292 lung epithelial cells (61). Relatedly, we have shown both *in vitro* in epithelial cells and in a murine model that *Influenza H3N2* is able to upregulate mucin expression and that this is dependent on human (or mouse) HAT upregulation and TACE activity (62). Interestingly, Haga et al. have shown that inhibiting TACE prevents SARS-CoV cellular entry (63). Strengthening the signaling potential of the receptors, Iwata-Yoshikawa et al. demonstrated *in vivo* that poly IC (TLR3 ligand) induces the expression of a variety of pro-inflammatory mediators (CCL2, KC, and IL-1) through the expression of TMPRSS2 (48).

In addition, although unclear as whether it is beneficial or detrimental to the host cell, SARS-CoV have been shown to activate host stress response, apoptosis, and autophagy (13). These are also various pathways that may also need to be evaluated therapeutically in the context of the current pandemic. Relatedly, we have shown that chloroquine, which also inhibits the autophagic cellular flux by decreasing autophagosome-lysosome fusion, can inhibit Influenza-mediated CCL5 production (64).

Importantly, after having established a foothold in the epithelial compartment, SARS-CoV can disrupt the epithelial polarity, thereby getting access to the parenchyma tissue: for

example, it has been shown that the virus membrane protein E binds to PALS1 (Protein Associated With Lin Seven 1), a junction protein involved in epithelial polarity, and modifies its cellular distribution at the surface of HEK-293 cells (65).

Myeloid Cells and Myeloid-Epithelial Cells Interaction

Myeloid cells, e.g., alveolar and interstitial macrophages or dendritic cells (DCs), elicit different immune responses toward influenza viruses, according to their subtypes (66). It is thus predictable that specificities may also exist with respect to SARS-COV-2 infections. Indeed, although studies are scant, these cells have generally been shown to be poorly permissive to SARS-CoV replication (54, 67, 68).

However, a few studies have shown that myeloid cells can respond to SARS-Cov infection. Indeed, Dosch et al. showed that the S protein could, through TLR2, trigger NF-κB activation and inflammatory responses in peripheral blood mononuclear cells (PBMC) (69). Also, in common with epithelial cells, it was shown that PBMCs and DCs infected with SARS-CoV produced cytokines and chemokines such as and C-C Motif Chemokine Ligand (CCL)-2 and/or C-X-C Motif Chemokine Ligand (CXCL)-10/RANTES/Tumor Necrosis Factor (TNF)/IL-8/IL-6, but, importantly, not IFN-β (67, 68). By contrast, a study performed mostly on THP-1 macrophages suggest that MERS S protein suppresses macrophages pro-inflammatory responses through DPP4-induction of IRAK-M and PPARγ (70).

Furthermore, in an interesting "2-way" system involving differentiated SARS-permissive lung Calu-3 cells and monocyte-derived Macs and DCs, it was shown that mediators produced by Calu-3 cells activate cytokine production by macrophages (IL-1β, G-CSF, MIP-1, and TNF-α) and DCs (IL-12p40, MIP-1, IFN-γ, IL-6, IL-8, and MCP-1) but that some of these Calu-3 derived mediators (in particular IL-6 and IL-8) compromised the ability of DCs and Macs to activate naïve T cells and phagocytosis (4, 56). This echoes data obtained from patients suggesting that SARS may in fact be partly caused by a "paralysis" of the adaptive immune system, characterized by a diminished number of immune cell types including T lymphocytes, DCs and Macs (4).

From Murine Models to Human Genetics

Demonstrating that SARS-CoV can induce TLR-dependent host responses *in vivo*, *Tlr4*, *Tlr3,* and *Tram* KO mice were shown to be more susceptible to mouse-adapted SARS-CoV, albeit without exhibiting extra mortality (71). In comparison, mice deficient for the signaling molecule *Trif* were highly susceptible to CoV infections, exhibited diminished lung function, aberrant inflammatory responses, and importantly, higher mortality (71).

In addition, a mouse genetic study revealed that the TLR adaptor protein *Ticam2* was a susceptibility gene to SARS-CoV (72); mice KO for *Ticam2* (72), but also *MyD88* (73), another TLR adaptor protein, were highly susceptible to a mouse-adapted SARS-CoV lung infection. Since polymorphisms of TLRs and MyD88 have been associated in humans with heightened sensitivity to a variety of pathogens (74), these studies, in addition to demonstrating the role of TLR pathways in the

SARS-CoV infection, suggested a human genetic predisposition to SARS-CoV, and this could explain the variability of severity in patients with COVID-19 disease. Forthcoming human genetic studies from international collaborative efforts (https://www.covid19hg.org) could reveal genetic variants associated with SARS-CoV2 susceptibility, as in the gene encoding ACE2 as recently suggested (75). Indeed, *ACE2* genetic variants may be associated with a modulated ACE2 protein expression, the SARS-Cov-2 receptor, which may explain in part patients' susceptibility to infection. Genes associated with TLR pathways also represent good candidates, as demonstrated in other respiratory viral infection (e.g., influenza) where *TLR3* variants (76) were shown to modulate its virulence.

Maladaptive Activation of Innate Immune Responses (see Figure 1B)

As already mentioned above, aberrant maladaptive innate immune host responses, including "cytokine storm" events, have been associated with severe lung disease and the development of ARDS during SARS and the COVID-19 current episode. Mechanistically, these events usually occur at a late stage of the disease, and several mechanisms have been proposed. In particular, a murine study has shown that a prolonged (albeit delayed, as demonstrated also *in vitro*, see above) type I IFN signaling was instrumental in triggering over-exuberant innate inflammatory monocytes–macrophages immune responses and an impaired virus-specific T-cell response (77).

In complement to the mechanism proposed above, increased lung inflammatory protease (neutrophil elastase and metalloprotease) activity has been demonstrated in ARDS (78, 79), with a concomitant imbalance between protease and protease inhibitors activity (80). In addition, although not yet measured, to our knowledge, in SARS murine models, we and others have shown increased protease-mediated lung damage in mice infected with *Influenza* (81–83). Additionally, in a MERS-CoV murine model, it was shown that excessive complement activation was partly responsible for exacerbated lung inflammation (84).

Lastly, "cytokines storm" may also results from SOCS (suppressors of cytokine signaling) inhibition (85). Indeed, upon Influenza infection, SOCS1 and SOCS3 were shown to reduce type I IFN antiviral responses in human bronchial epithelial cells (86). Also, SOCS4-deficient mice exhibited heightened sensitivity to Influenza infection (87). Studies about SOCS involvement during coronavirus infections are currently lacking and should therefore bring new interesting information.

POTENTIAL THERAPEUTIC TARGETS AND CONCLUSION (SEE FIGURE 2)

On May 12, 2020, using the term "COVID," an unbiased search of already registered trials on https://clinicaltrials.gov/ retrieved 1,409 hits, and, when refined with "double blind/placebo," 119 hits were found. Although the number of trials that are ongoing or "under recruitment" is expectedly very high, the range of molecules tested is relatively narrow and aimed at

targeting mainly antivirals. These include remdesivir (21 hits), lopinavir/ritonavir (also used in AIDS), as well as interferons (46 hits). Also falling in that category are trials testing molecules aiming to block viral entry at the cellular surface by targeting ACE-inhibitors (32 hits) or the membrane proteases of the TTSP family (see above) using camostat mesilate (5 hits). Repurposing of non-antiviral drugs may offer new promising options, such as with Ivermectin—an FDA-approved anti-parasitic drug widely available and recently shown to inhibit SARS-CoV-2 *in vitro* (88).

Because the virus load is not necessarily correlated with symptoms deterioration in SARS (the latter being often caused by worsening of inflammation at day 7–10 post onset of clinical signs), it follows that anti-inflammatory drugs could/should be prescribed during that stage of the disease (8).

In that context, "classical" anti-inflammatory drugs are indeed currently being tested against COVID-19 [e.g., methylprednisolone, budesonide, hydrocortisone, azithromycin, and non-steroïdal anti-inflammatory drugs (NSAIDs)]. In addition, more specific agents are also being investigated, targeting either IL-β (anakinra, 13 hits), IL-6 signaling (Siltuximab/3 hits, Tocilizumab/42 hits, Sarilumab/13 hits), or CD24 (CD24Fc) with the main objective to modulate the "cytokine storm."

However, chloroquine/hydroxychloroquine has, so far, undoubtedly taken the lion's share (178 hits), and it has attracted a lot of media attention. In that respect, the results from an initial pan-European endeavor ("Discovery"), now conducted largely in France because of enrollment difficulties, are eagerly awaited. This drug has a "mixed" mode of action. Indeed, it acts as an anti-viral (presumably through inhibition of lysosomal enzymes requiring an acidic pH and of activation of endolysosomes, see above section "Mechanisms of entry") and as an anti-inflammatory molecule, and it has notably been used in inflammatory rheumatic diseases (89). Despite a relative safe profile, having been administered to millions of people over the years, worries have nevertheless arisen about cardiac issues in many individuals with severe Covid-19, and this will have to be properly assessed (90).

Regardless, the ultimate prize in the fight against COVID-19 (or further SARS-CoV infections) undoubtedly lies with the future generation of effective vaccines and the development of neutralizing antibodies (91, 92).

Unfortunately, coronavirus vaccines in general have attracted less attention compared to the effort dedicated to vaccines against other potential pandemic viruses such as Influenza. For example, from 2012 onwards, few SARS-CoV vaccines reached phase 1 clinical trials for lack of interest from the pharmaceutical industry when it became evident that the virus was not making a "comeback" after its initial appearance. However, although probably too late for affecting the current "first wave" of SARS-CoV-2 pandemic, many pharmaceutical companies and research laboratories are now working on a plethora of vaccine formulations [for a review, see (91) and https://clinicaltrials.gov, the latter reporting so far 83 clinical trials on vaccines].

Indeed, in pre-clinical studies, the determination of cryo-EM structures of the SARS-CoV-2 S ectodomain trimer is providing a blueprint for the design of vaccines and inhibitors of viral entry (28). In this context, promising results show that murine polyclonal antibodies against S protein of SARS-CoV are able to elicit polyclonal antibody responses, preventing SARS-CoV-2 entry into cells, and thus indicating that cross-neutralizing antibodies targeting conserved S epitopes can be elicited upon vaccination (28).

In addition to testing the best SARS-CoV-2 specific epitopes from the most suitable proteins (S, N, etc.) and way of administration (best vectors, etc.), it is important to select the best animal models. Although convincing murine studies are still pending, as indicated above in the section "Mechanisms of entry…", studies in other animals investigated the virus susceptibility of chickens, ducks, dogs, pigs, cats, and ferrets, with the latter two being the most permissive (43). Further up in the phylogenetic scale, a recent study reported that an inactivated vaccine candidate for SARS-CoV-2 was protective in macaques (93).

Finally, large epidemiological studies have demonstrated that Bacille Calmette-Guerin (BCG) can heterologously protect against virus infections [e.g., yellow fever virus (94), probably by tapping on trained immunity mechanisms (95, 96)]. Using such adjuvant-mediated strategies against SARS-CoV viruses may therefore be an exciting avenue worthwhile pursuing (97–99).

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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