MYSTERIES OF TYPE I IFN RESPONSE: BENEFITS VERSUS DETRIMENTS

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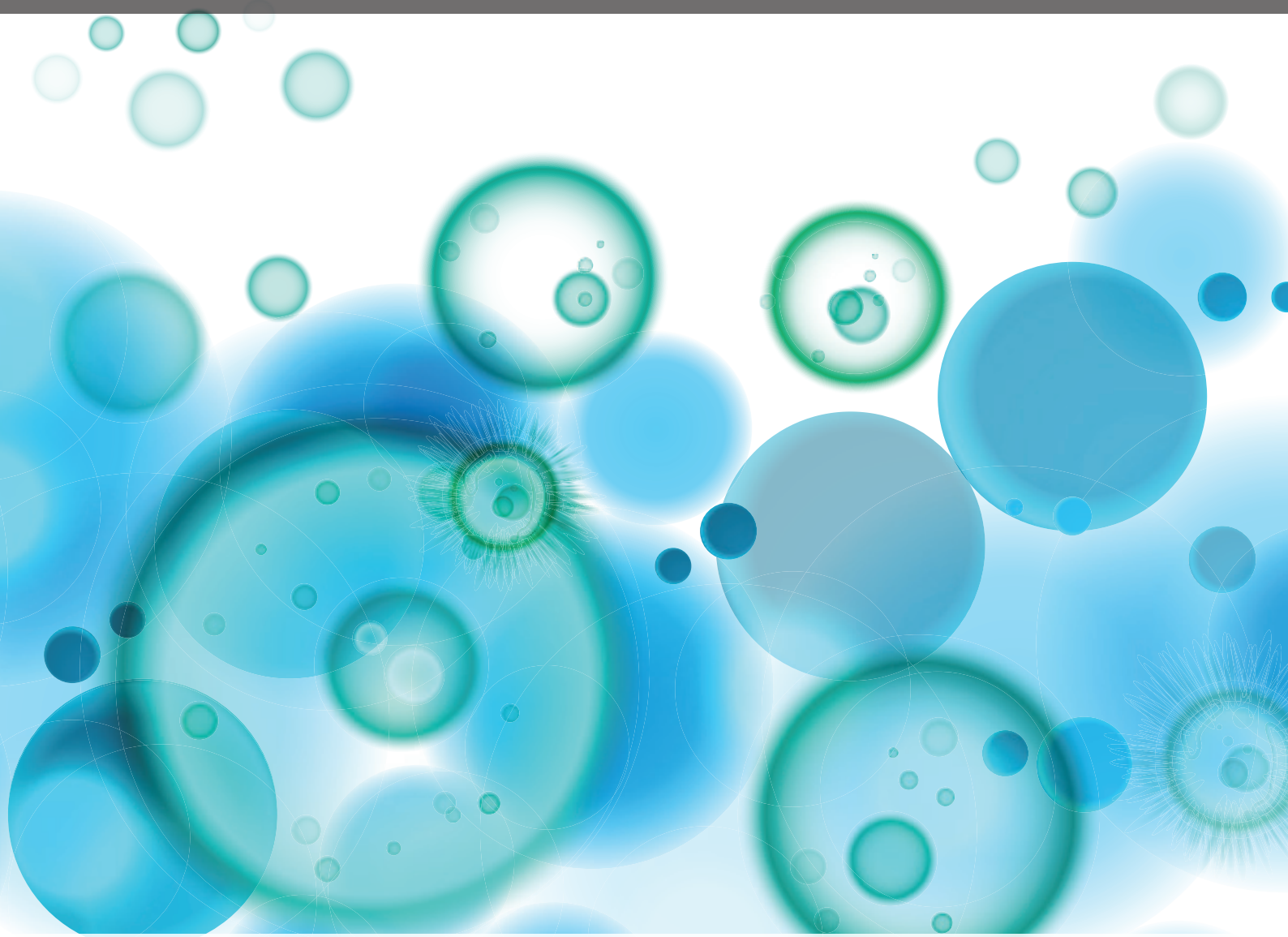

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MYSTERIES OF TYPE I IFN RESPONSE: BENEFITS VERSUS DETRIMENTS

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Successful containment of an infection is dependent on both innate and adaptive immune response. Cytokines are essential effectors of both of these systems. In particular, type I interferons (IFN-I) are important components of early innate immunity against an infection. However, the production of IFN-I could serve as a double edge sword, either containing an infection or enhancing susceptibility. For example, IFN-I, which is essential for early containment of viral infections, has been shown to be detrimental to the host during bacterial infections. In fact, recent significant reports have shown that influenza virus induced IFN-I responses can enhance the host susceptibility to secondary bacterial infections. These recent reports highlight the expanding immunoregulatory role of IFN-I in the host immunity. With these recent findings in mind, the aim of this research topic is to welcome novel data, opinion and literature reviews on the newly identified dual functions of IFN-I. This research topic wills focus on the following areas of IFN-I: 1) a detrimental role of IFN-I during primary bacterial infection; 2) a detrimental role of viral infection induced IFN-I during secondary bacterial infections; 3) evolutionary pressure that drove detrimental IFN-I response during primary bacterial infection; and 4) does benefit of IFN-I responses during primary viral infections outweigh the adverse consequences of IFN-I mediated enhanced susceptibility to secondary bacterial infections.

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Mysteries of type I IFN response: benefits versus detriments

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Keywords: type I interferons, vaccine adjuvant, viral, bacterial

Successful containment of infection is dependent on both innate and adaptive immune responses. Cytokines are essential components of both of these systems. In particular, type I interferons (IFN-I) are important components of early innate immunity against infections. However, the production of IFN-I could serve as a double-edged sword, in that it could help eliminate infections or in certain instances enhance host's susceptibility to infections. For example, IFN-I provide early resistance against acute viral infections, but are detrimental to the host during certain bacterial infections and chronic viral infections. This Research Topic presents seven articles that address the biological roles of IFN-Is in host immunity and various contexts (e.g., autoimmunity, cancer, viral/bacterial infections, IFN-I therapy, etc.) where IFN-Is could either serve to control or exacerbate disease.

The original research performed by Babb et al. investigates the potential adjuvant activity of gamma-irradiated influenza (1). They demonstrate that co-vaccination with gamma-irradiated-influenza virus and poorly immunogenic inactivated Semliki forest virus (SFV) results in enhanced SFV-specific antibody responses without compromising humoral immunity against influenza infections. They have previously shown that gamma-irradiation destroys the ability of SFV but not influenza A virus to elicit strong IFN-I responses (2). Thus, it is likely that the adjuvant activity of gamma-irradiated influenza virus is attributable to its potency of IFN-I induction. These authors speculate that gamma-irradiated influenza virus may therefore be exploited as an adjuvant to improve the efficacy of poorly immunogenic vaccines owing to its ability to stimulate IFN-I production.

The potent immune activating properties of IFN-I can also be detrimental during viral infection, particularly for viruses that establish chronic infection. This certainly is the case for human or simian immunodeficiency virus (HIV/SIV) infection in non-natural hosts. As discussed by Tomasello et al. (3) and Furuya et al. (4), IFN-I induced during HIV infections may play a pathogenic role in driving chronic immune activation associated with $CD4^+$ T cell depletion and loss of T cell function. Indeed, a strong correlation exists between chronic low level productions of IFN-I and disease progression in non-natural hosts. Thus, Furuya et al. hypothesize that regulatory mechanisms must exist in natural hosts that actively suppress IFN-I responses despite viral replication (4). It is speculated that understanding how the host can co-exist with HIV without generating an IFN-I response will be crucial in developing therapeutic interventions that can prevent or dampen progression to an acquired immune deficiency syndrome (AIDS). Perhaps, as pointed out by Tomasello et al., the more important question that needs to be addressed first is whether a causal link exists between low levels of IFN-I signaling and the development of AIDS in non-natural hosts or, rather, the up-regulation of IFN-stimulated genes simply a marker of disease progression.

The pathogenic role of IFN-I is more widely observed in bacterial infections. For example, the facultative intracellular bacterium *Francisella tularensis* is less virulent in mice deficient in IFN-I receptors (5, 6). Furuya et al. propose in an opinion article that the detrimental role of IFN-I during pulmonary tularemia may be linked to its suppressive effects on neutrophil recruitment, a response that may be protective against respiratory *Francisella tularensis* infection (7). The role of neutrophils during pulmonary tularemia has yet to be fully defined, but it is likely that both the magnitude and timing of cellular recruitment to the lung will determine whether neutrophils promote bacterial clearance or contribute to immunopathology.

In three review articles, the mechanisms that have been proposed to explain the IFN-I-mediated increases in bacterial infection susceptibility are discussed. The review article by Eshleman et al. focuses on the suppressive effects of IFN-I on myeloid cells during intracellular bacterial infections (8). These authors also emphasize that IFN-I can exert a positive anti-inflammatory effect in a number of autoimmune diseases and that this is mediated by the suppression of myeloid cell inflammatory responses. The review article from Dhariwala et al. describes multiple pathways of IFN-I-dependent cell deaths that may aid bacteria escape phagocytosis and thereby contributes to bacterial pathogenesis (9). Indeed, as the author pointed out, bacterial pathogens that benefits from IFN-I signaling are often facultative intracellular bacteria. The final review by Wijesundara et al. highlights pathological contexts in which IFN-I could be exploited in therapy and vaccine design with a particular emphasis on IFN-epsilon (10). IFN-epsilon appears to have both overlapping and distinct functions compared to IFN-alpha and -beta, and therefore, the author urge the need of evaluating contribution of the different members of IFN-I to fully exploit its beneficial effects.

Despite IFN-I being perhaps the most studied cytokine, their immunoregulatory roles are not fully understood. As is evident from the articles presented in this Research Topic, the role played by IFN-I is highly context-dependent and can be both beneficial

and detrimental. Further research is clearly required in order for us to selectively harness the protective role of IFN-I while suppressing its damaging effects.

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Gamma-irradiated influenza A virus provides adjuvant activity to a co-administered poorly immunogenic SFV vaccine in mice

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Many currently available inactivated vaccines require "adjuvants" to maximize the protective immune responses generated against the antigens of interest. Recent studies in mice with gamma-irradiated influenza A virus (γ-FLU) have shown its superior efficacy compared to other forms of inactivated FLU vaccines and its ability to induce both potent interferon type-I (IFN-I) responses and the IFN-I-associated partial lymphocyte activation. Commonly, IFN-I responses induced by adjuvants, combined in vaccine preparations, have been shown to effectively enhance the immunogenicity of the antigens of interest. Therefore, we investigated the potential adjuvant activity of γ-FLU and the possible effect on antibody responses against co-administrated antigens, using gamma-irradiated Semliki Forest virus (γ-SFV) as the experimental vaccine in mice. Our data show that co-vaccination with γ-FLU and γ-SFV resulted in enhanced SFV-specific antibody responses in terms of increased titers by six-fold and greater neutralization efficacy, when compared to vaccination with γ-SFV alone. This study provides promising evidence related to the possible use of γ-FLU as an adjuvant to poorly immunogenic vaccines without compromising the vaccine efficacy of γ-FLU.

Keywords: influenza vaccines, combined vaccine, gamma-irradiation, adjuvants, antibodies

INTRODUCTION

Vaccines represent a fundamental aspect of current control strategies against infectious diseases. However, despite the remarkable success of many vaccines, there still remains many challenges in the field of vaccinology, such as generating effective vaccination strategies against notoriously difficult pathogens like hepatitis C virus and human immunodeficiency virus (1). Among the various types of currently used vaccines, live attenuated vaccines can mimic natural infections and consequently have been shown to be very effective in generating long-lived immunity. However, due to the high risk of conversion to their highly pathogenic form and the inability to develop live attenuated vaccines for many pathogens, inactivated vaccines such as inactivated whole viruses and purified antigens have typically been used as the main strategy for vaccine design. However, poor immunogenicity of many inactivated vaccines has severely affected their effectiveness. Therefore, inactivated vaccines often require "adjuvants" to enhance their immunogenicity. Importantly, most immunostimulatory adjuvants have been designed to potently stimulate innate signaling pathways through pattern recognition receptors (PRR) such as toll-like receptors (TLR) and cytosolic receptors (2–5). This ultimately leads to the induction of genes encoding for various immune-modulatory molecules including interferon type-I (IFN-I), which stimulate co-stimulatory molecule expression and antigen presentation (6).

Currently in vaccine design, greater attention is being drawn toward designing adjuvants to effectively boost the immune response toward existing vaccine preparations, which fail to induce sufficient immunity. The influence of IFN-I exogenously or through PRR stimulation has been shown to be very effective (7–9). We have previously reported the superiority of gamma-irradiated influenza virus (γ-FLU), compared to other flu vaccine formulations, in terms of inducing cross-protective immunity (10–12). We have also shown that γ-FLU similarly to its live form is capable of inducing potent IFN-I responses and the associated partial lymphocyte activation 24 h post-challenge (13, 14). Importantly, in contrast to gamma-irradiation of influenza, we have shown that gamma-irradiation of Semliki Forest virus (SFV) abrogates its ability to induce IFN-I responses (15). Therefore, we investigated the potential adjuvant activity of γ-FLU on co-administered γ-SFV as an experimental model for poor immunogenic vaccines. Here, we report that co-vaccination with γ-FLU and γ-SFV resulted in enhanced SFV-specific antibody titers "by six-folds" when compared to vaccination with γ-SFV alone. This enhancement in antibody titer was also associated with greater SFV neutralization efficacy and importantly; the vaccine efficacy of γ-FLU was not affected.

MATERIALS AND METHODS

ETHICS STATEMENT

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of The University of Adelaide. The protocol was approved by the

Animal Ethics Committee at The University of Adelaide (Permit Number: S-2011/119).

VIRUSES AND CELLS

Avirulent SFV (A7 strain) was grown *in vitro* by infecting Vero cells using multiplicity of infection (MOI) of 0.1, and infected flasks were incubated for 24 h at 37°C in a humidified atmosphere with 5% CO_2. Culture supernatants were then collected and clarified to remove cellular debris by centrifugation at 1500 rpm for 5 min. Virus titer was determined by plaque assay on Vero cells to be 3×10^8 PFU/ml. For the vaccine preparation, SFV stock was concentrated using Millipore filtering devices with 100 kDa cut-off (Millipore) and centrifugation at 2000 rpm for 1 h at 4°C using Eppendorf bench top centrifuge. Virus titer of the concentrated SFV was determined by plaque assay on Vero cells to be 5×10^8 PFU/ml.

The influenza type A virus, A/PR/8 [(A/Puerto Rico/8/34 (H1N1)], was grown in 10-day-old embryonated chicken eggs (HiChick, SA, Australia). Each egg was injected with 0.1 ml normal saline containing 1 hemagglutination unit (HAU) of virus, incubated for 48 h at 37°C, and then held at 4°C overnight. The amniotic/allantoic fluids were then harvested, pooled, clarified, and stored at −80°C. Gamma-irradiated A/PR8 vaccine preparations were previously prepared by Dr. Furuya at ANU. Briefly, concentrated virus stocks were prepared using chick erythrocytes as previously described (16). Infectious allantoic fluid was incubated with chicken red blood cells (cRBCs) for 45 min at 4°C allowing the hemagglutinin to bind to erythrocytes, and then centrifuged (4°C, 1500 rpm, 10 min) to remove the allantoic fluid supernatant. The pellets were resuspended in normal saline, incubated for 1 h at 37°C to release the RBCs from the virus, and then centrifuged to remove the erythrocytes and the supernatant containing the virus collected. The titer of the concentrated A/PR8 virus stock (9×10^8 $TCID_{50}$/ml) was determined by TCID50 assay (17).

VIRUS INACTIVATION

Concentrated virus stocks were inactivated by exposure to gamma-irradiation from a ^{60}Co source [Australian Nuclear Science and Technology Organization (ANSTO) at Lucas Heights/NSW]. A/PR8 and SFV received a dose of 10 and 50 kGy, respectively, and they were kept frozen on dry ice during gamma-irradiation. Sterility was tested by two independent methods: plaque assay using MDCK (for A/PR8) or Vero cells (for SFV); and by inoculating embryonated eggs (for A/PR8). The detection limit of our plaque assay is 10 PFU/ml and no plaque forming unit was detected for the irradiated samples. These tests confirmed sterility of inactivated stocks. In addition, we have estimated the minimum inoculum required to cause a positive infection in embryonated eggs and found that the minimum egg infectious dose that causes detectable HA titers in the allantoic fluid after 2 days of incubation is 0.1 TCID50/egg. Embryonated eggs were inoculated with 100 ml of inactivated preparations per egg and incubated for 2 days at 37°C and the allantoic fluid of individual eggs was harvested and tested for virus replication using HA assays. HA titers were negative in the allantoic fluid of these eggs, which illustrates a complete loss of virus infectivity in our inactivated preparations.

MICE AND TREATMENTS

Wild-type C57B/6 mice (9–10-week-old) were bred under specific pathogen-free conditions and supplied by the Animal Laboratory Services at the University of Adelaide, SA, Australia.

In general, vaccine preparations were diluted using 10-fold serial dilutions and each mouse was injected in the tail vein with 200 μl of the relevant virus or vaccine preparation. The following doses were used: live SFV (10^7 PFU/mouse), γ-SFV (either 10^6, 10^7, or 10^8 PFU equivalent/mouse), and γ-FLU (10^4, 10^5 TCID50 equivalent/mouse). Refer to text for specific doses used in each experiment. For co-vaccination, the two vaccine preparations were mixed thoroughly in the same tube and administered as a single injection into experimental animals. Vaccination doses are expressed PFU or TCID50 equivalent. In addition, in some experiments Poly(I:C) was injected intravenously at a dose of 150 μg in 200 μl of PBS per animal as previously reported (18).

ANTIBODY ANALYSIS

Semliki Forest virus-specific and FLU-specific antibody responses in serum samples were determined by enzyme-linked immunosorbent assay (ELISA). In brief, Maxisorp plates were coated with concentrated SFV or FLU viral antigen diluted in bicarbonate coating buffer (Na_2CO_3, $NaHCO_3$, water at pH 9.6) and incubated overnight at room temperature. Non-specific protein binding sites were then blocked with PBS containing 2% skim milk powder for 2 h at room temperature. Fifty microliter volumes of serially diluted serum samples were added to the appropriate wells for 2 h at room temperature followed by the addition of horse radish peroxidase conjugated goat anti-mouse IgG (Thermo Scientific) at room temperature for 2 h. Plates were developed using TMB peroxidase substrate in the dark for 30 min and the reaction was stopped with 2 mol H_2SO_4. Absorbance was measured at 450 nm using a Microplate ELISA reader (Bio-Tek Instruments).

NEUTRALIZATION ASSAYS

Plaque reduction assay modified from (19) was used to analyze SFV neutralization. Twenty-four well tissue culture plates were seeded with 1.5×10^5 Vero cells/well and incubated overnight at 37°C in a humidified atmosphere with 5% CO_2. Aliquots of serum samples from control and vaccinated animals were incubated at 56°C for 30 min to inactivate complements and serially diluted using EMEM media without FCS. Diluted samples were mixed with equivalent amount of DMEM media containing 100 PFU of SFV. Mixtures (sera and virus) were incubated for 1.5 h at 37°C and then used to infect confluent Vero cell monolayer's (in triplicate) prepared earlier. Initially, culturing media was removed from each well prior to addition of virus/serum mixture. Plates were then incubated for 2 h at 37°C to allow infection of monolayers. Following incubation, the infecting mixture was removed and an agar overlay containing 50% of 1.8% Bacto-Agar, 40% DMEM media, 10% FCS, and 0.002% Fungizone was added to each well and plates were incubated for 3 days at 37°C, 5% CO_2. Following incubation, cells were fixed with 5% formalin for 1 h at room temperature. The overlay was then carefully removed and cell monolayers were stained with 0.2% crystal violet. Plaques were enumerated to determine the effect of the serum on virus infectivity.

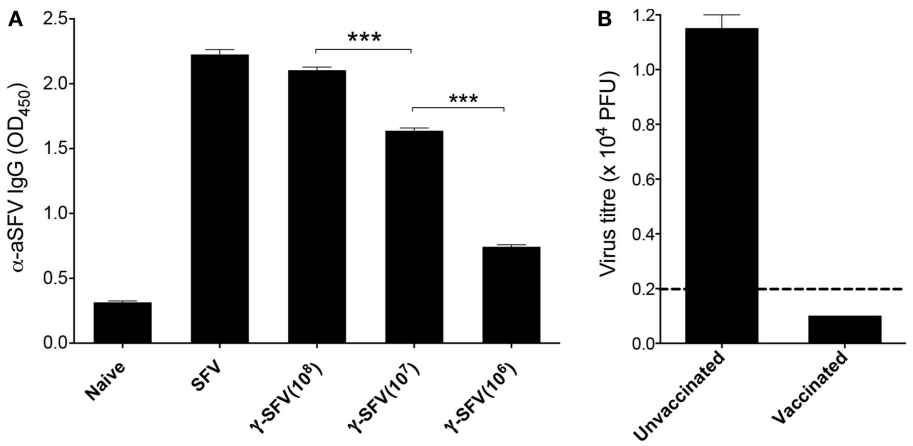

FIGURE 1 | γ-SFV vaccine induces protective antibody responses. **(A)** Mice were injected i.v. with SFV (10^7 PFU) or variable doses of γ-SFV (10^6, 10^7, or 10^8 PFU equivalent/mouse). Twenty days post-injection, serum SFV-specific IgG levels were measured by direct ELISA using a serum dilution of 1/200. Serum from naive mice served as the negative control. **(B)** Mice were vaccinated i.v. with γ-SFV (10^7 PFU equivalent/mouse) and challenged 14 days post-vaccination with SFV (10^7 PFU). Twenty four hours post-challenge, serum SFV titers were determined by plaque assay. Un-vaccinated naive mice infected with SFV served as control. Results are presented as mean ± SEM ($n = 3$) and dashed line represents our assay detection limit of 100 PFU, $^{***}p < 0.001$.

In addition, a hemagglutination inhibition assay (HAI) was used to test the influenza-specific antibody responses, as previously described (17). Aliquots of sera were incubated at 56°C to inactivate complements for 30 min, then diluted in PBS containing 1% RBCs, and left for another 30 min incubation at room temperature. RBCs within samples were then pelleted by centrifugation at 1400 rpm using a microcentrifuge and supernatants were collected. Twofold serial dilutions were performed using a 96-round well-bottom plate. Fifty microliter of diluted virus (FLU) at a concentration of 80 HAU/ml was then added to each dilution of sera and incubated for 30 min at room temperature. PBS containing 1% RBCs was then added to each well and incubated at 4°C. Results were analyzed 24 h later and neutralizing antibodies in each dilution was determined by the presence of a pellet of RBCs at the bottom of the wells.

STATISTICAL ANALYSIS

Results were expressed as mean ± SEM. Statistical significance among samples was calculated using an unpaired Student's *t*-test. *P* values <0.05 were considered statistically significant.

RESULTS

ANTIBODY RESPONSES INDUCED BY γ-SFV

Avirulent SFV causes asymptomatic infection characterized by high-titer viremia in adult mice (20, 21), and effective viral clearance has been attributed to rapid antibody responses generated by the host (22). Interestingly, primary cytotoxic T cell responses have been shown to be associated with MHC-I haplotype and restricted to H-2^k haplotype expressing mice (23). Particularly, C57/B6 mice (H-2^b haplotype) have been classified as cytotoxic T cell non-responders and therefore were used in the study. To confirm that the γ-SFV vaccination strategies used in this study can generate effective antibody responses, serum SFV-specific IgG levels were measured 20 days post-infection with live SFV (10^7 PFU/mouse) or vaccination with variable doses of γ-SFV (10^6, 10^7, or 10^8 PFU equivalent/mouse). Our data illustrate that vaccination with γ-SFV induces high levels of SFV-specific IgG in the serum of vaccinated mice in a dose-dependent manner (**Figure 1A**).

To determine if the detected antibody responses are protective, mice were vaccinated with γ-SFV (10^7 equivalent PFU/mouse) and challenged 21 days later with live SFV (10^7 PFU/mouse). Twenty-four hours post-challenge, serum samples were tested for virus infectivity by plaque assay. No viral infectivity was detected in all serum samples from previously vaccinated mice in contrast to the high viremia observed in un-vaccinated control mice following a challenge with live SFV (**Figure 1B**).

It has been previously shown that live SFV infection induces IFN-I and as a consequence promotes Th1 antibody isotype switching (20). Considering the inability of γ-SFV to induce detectable levels of IFN-I (13, 15), the level of IgG isotypes induced by live SFV vs. γ-SFV was investigated. Mice were infected with SFV (10^7 PFU/mouse) or vaccinated with γ-SFV (10^7 equivalent PFU/mouse) and serum SFV-specific IgG1 and IgG2c levels were measured over a time course. Both IgG1 and IgG2c levels appeared to be lower following vaccination with γ-SFV in comparison to SFV (**Figures 2A,B**).

THE EFFECT OF CO-ADMINISTRATION OF γ-FLU AND γ-SFV ON SFV-SPECIFIC ANTIBODY RESPONSES

Considering the potent IFN-I responses induced by γ-FLU (13, 14), we investigated the effect of γ-FLU and γ-SFV co-administration on SFV-specific antibody responses. Mice were co-injected with γ-SFV (10^7 PFU equivalent/mouse) and γ-FLU (10^4 or 10^5 equivalent TCID50/mouse) and serum SFV-specific IgG levels were measured at day 20 post-vaccination. Our data illustrate the significant enhancement (~six-folds) of SFV-specific IgG levels in mice co-injected with both γ-SFV and γ-FLU compared to mice vaccinated with γ-SFV alone ($p < 0.05$) (**Figures 3A,B**).

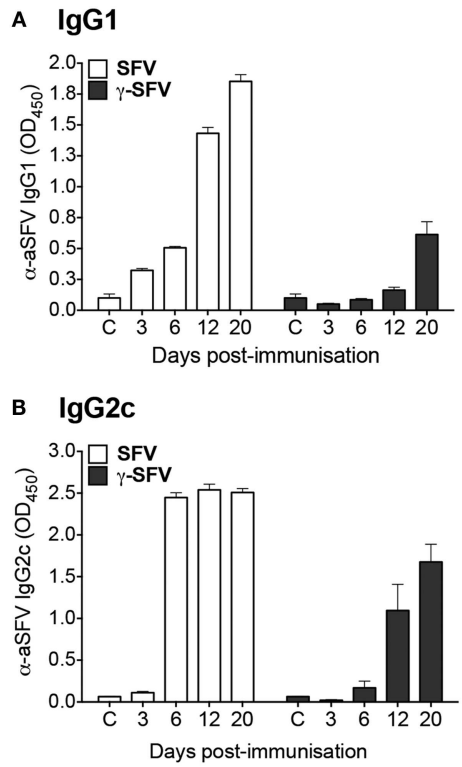

FIGURE 2 | Immunization with γ-SFV promotes lower antibody levels compared to SFV. Mice were injected i.v. with SFV (10^7 PFU) or γ-SFV (10^7 PFU equivalent/mouse). Serum SFV-specific IgG1 **(A)** and IgG2c **(B)** levels were measured at 3, 6, 12, and 20 days post-immunization by direct ELISA at a serum dilution of 1/200. Sera from naive mice served as negative controls (c). Results are presented as mean ± SEM ($n = 3$), $^{**}p < 0.01$.

In addition, we investigated the kinetics of SFV-specific antibody responses post co-administration of γ-SFV and γ-FLU. Similarly, our data illustrate the consistent enhancement of SFV-specific antibody responses at all tested time points ($p < 0.05$) (**Figure 3C**).

To determine whether enhancement of SFV-specific antibody responses mediated by co-administration of γ-FLU coincides with enhanced SFV neutralization, serial dilutions of the immune sera were tested for their ability to neutralize 100 PFU of live SFV *in vitro* using a Vero cells based plaque-inhibition assay. Our results clearly illustrate that while immune sera from γ-SFV alone vaccinated mice have high neutralization activity at 1/100 dilution, this neutralization activity decreased remarkably with every serial dilution to reach ~10% activity at 1/800 serial dilution. In contrast, immune sera from γ-SFV and γ-FLU co-vaccinated mice show significantly higher neutralization values for all tested sera dilutions when compared to sera from γ-SFV alone vaccinated mice (**Figure 3D**).

Next, we investigated the effect of γ-FLU and γ-SFV co-administration on SFV-specific IgG2c and IgG1 levels and whether co-vaccination may promote a particular IgG isotype. Our data illustrates that co-administration of γ-FLU and γ-SFV resulted in significantly enhanced IgG2c levels compared to vaccination with γ-SFV alone ($p < 0.01$). However, co-vaccination did not lead to significant enhancement in IgG1 responses as the detected SFV-specific IgG1 levels appeared similar in both vaccinated groups (**Figures 4A,B**).

THE EFFECT OF CO-VACCINATION ON FLU-SPECIFIC ANTIBODY RESPONSES

Vaccination with γ-FLU has been shown to elicit homotypic neutralizing antibody responses (10, 11). To determine whether the co-administration of γ-FLU and γ-SFV affects the host's ability to generate effective FLU-specific humoral responses, we investigated FLU-specific IgG responses at day 20 post-vaccination. Our data indicates that co-vaccination did not suppress the induction of FLU-specific IgG responses induced by γ-FLU (**Figure 5A**). We have also tested the neutralizing efficacy of FLU-specific antibodies using HA inhibition assay and our data illustrated that the hemagglutinating activity of 80 HAU of A/PR8 was inhibited at similar levels by immune sera from mice vaccinated with γ-FLU alone or mice co-vaccinated with γ-FLU and γ-SFV (**Figure 5B**).

THE EFFECT OF AN IFN-I INDUCING ADJUVANT ON THE IMMUNOGENICITY OF γ-SFV

In line with the common approaches used for adjuvant design, the adjuvant activity of γ-FLU is expected to be related to its ability to induce potent IFN-I responses and the associated IFN-I-mediated partial lymphocyte activation (13). To illustrate the effect of an IFN-I inducing adjuvant on the immunogenicity of γ-SFV, we evaluated the effect of poly(I:C) and γ-SFV co-administration on SFV-specific antibody responses. The ability of poly(I:C) to induce IFN-I and its potential use as an adjuvant to enhance humoral responses toward poorly immunogenic proteins has been well-documented (4, 7, 24). Therefore, mice were vaccinated with γ-SFV with or without co-injection of poly(I:C) and total SFV-specific IgG levels in the immune sera were analyzed at days 3, 6, 12, and 20 post-vaccination. Our data illustrate that co-injection of poly(I:C) and γ-SFV resulted in a significant enhancement in the level of SFV-specific IgG titers in the serum at all time points compared to the injection of γ-SFV alone (**Figure 6**).

DISCUSSION

There has always been an increased demand for safe and effective vaccines to reduce the morbidity and mortality associated with particular viral infections. Non-living antigens are often employed in vaccine strategies and many are poor immunogens (2). We have reported previously the efficacy of γ-FLU to generate protective immunity upon homotypic and heterosubtypic influenza A virus challenges (10, 25). In addition, we have demonstrated the ability of the γ-FLU vaccine to induce potent IFN-I responses and the associated partial systemic lymphocyte activation (13, 14). It has been illustrated previously that IFN-I plays a very influential role in the development of B lymphocytes and consequently antibody production (26, 27). In addition, we have reported that γ-SFV, in contrast to live SFV, does not induce detectable levels of IFN-I (13, 15). Therefore, we used γ-SFV vaccine as an experimental model to test the adjuvant activity of γ-FLU.

In general, adjuvants are often used to achieve qualitative/quantitative differences in the immune responses that may include increasing the speed of an immunological response, which

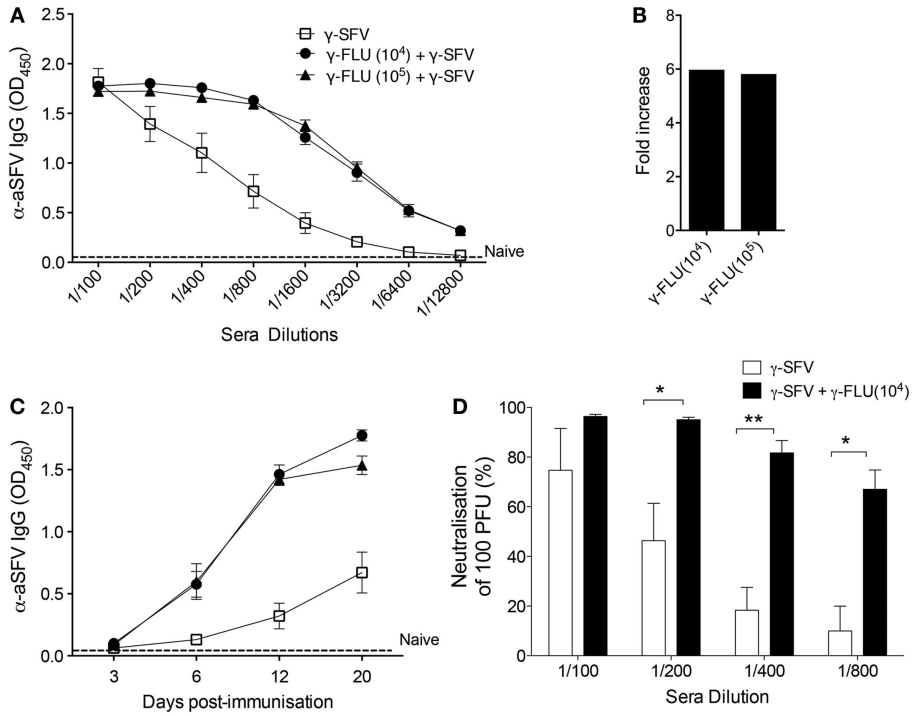

FIGURE 3 | Co-administration of γ-FLU and γ-SFV enhances SFV-specific antibody responses. Mice were injected i.v. with a single dose of γ-SFV (10^7 PFU equivalent/mouse) or co-injected with various doses of γ-FLU (10^4, 10^5 TCID50 equivalent/mouse). **(A)** Serum SFV-specific IgG concentrations 20 days post-vaccination were analyzed at twofold sera dilutions by direct ELISA and serum from naive mice served as the negative control (naïve). **(B)** Folds increase in SFV-specific IgG antibody titers based on the absorbent value of 0.5 for the tested serum dilutions **(A)**. **(C)** Serum SFV-specific IgG levels were analyzed by direct ELISA using a serum dilution of 1/800 at 3, 6, 12, and 20 days post-vaccination. **(D)** Neutralization of SFV by the immune sera collected at day 20 post-vaccination as determined by plaque reduction assay. Data represent mean ± SEM ($n = 3$), $*p < 0.05$, $**p < 0.01$.

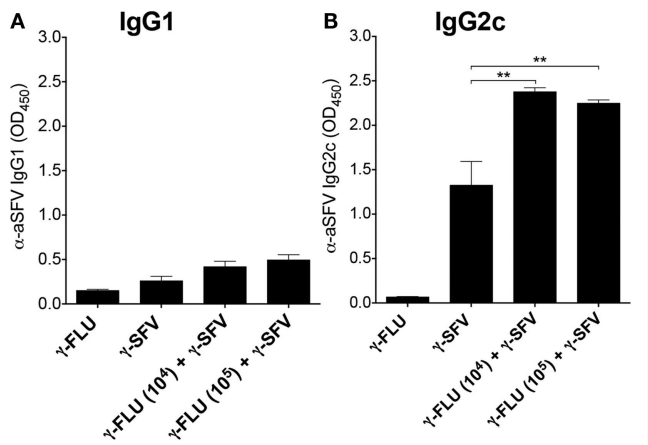

FIGURE 4 | Co-administration of γ-FLU and γ-SFV enhances SFV-specific IgG2c responses. Mice were injected i.v. with a single dose of γ-SFV (10^7 PFU equivalent/mouse) or co-injected with various doses of γ-FLU (10^4, 10^5 TCID50 equivalent/mouse). SFV-specific IgG1 **(A)** and IgG2c **(B)** levels in the serum at day 20 post-vaccination were analyzed by direct ELISA using a serum dilution of 1/200. Sera from γ-FLU vaccinated mice served as negative controls. Data represent mean ± SEM ($n = 3$) $**p < 0.01$.

is important especially during pandemic outbreaks (28, 29). Adjuvants have also been employed to promote specific types of immunity, which may not be efficiently generated by the non-adjuvanted antigens, i.e., Th1 vs. Th2 cells, $CD8^+$ vs. $CD4^+$ cells, and specific types of antibody isotypes (2). Our data clearly show that co-administration of γ-SFV with γ-FLU amplified SFV-specific IgG levels with an overall enhancement of ~six-folds. The enhanced titers observed at day 6 post co-vaccination were equal to the titers detected at day 20 following vaccination with γ-SFV alone. Thus, confirming an earlier induction and amplification of SFV humoral responses. We have also shown this enhancement to be associated with increased efficiency in virus neutralization. Furthermore, our data illustrate that γ-FLU promotes enhancement of type-1 antibody response to co-administered γ-SFV, as illustrated by the significant increase in IgG2c but not IgG1. This outcome is commonly desired within vaccine development due to the competent functions of IgG2a during antigen clearance relative to IgG1 (30–32).

In line with the many approaches used for adjuvant design, IFN-I responses induced by γ-FLU may have played an important role in the observed adjuvant activity of γ-FLU. To evaluate the role of IFN-I, the adjuvant activity of Poly IC on antibody responses to γ-SFV was investigated. The enhancement of

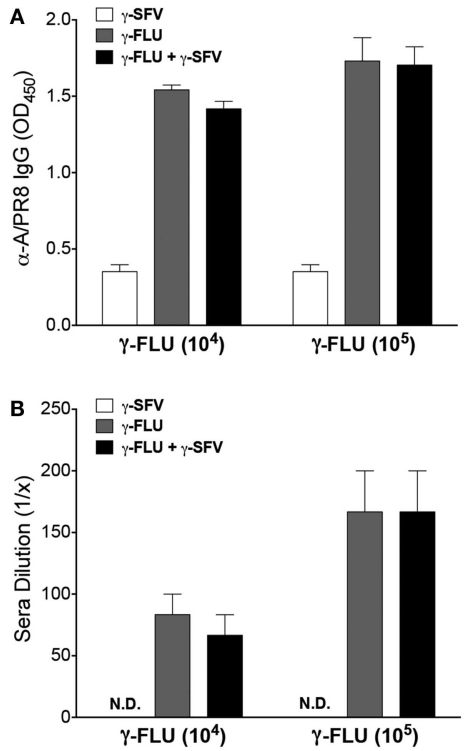

FIGURE 5 | The effect of co-vaccination on FLU-specific antibody responses. **(A)** Mice were injected i.v. with γ-FLU (10^4 or 10^5 TCID50 equivalent/mouse) or co-injected with γ-SFV(10^7 PFU equivalent/mouse) and serum FLU-specific IgG concentrations were analyzed at day 20 post-vaccination by direct ELISA using a serum dilution of 1/200. Sera from γ-SFV injected mice served as negative controls. **(B)** HA inhibition assay showing dilution of the immune sera capable of inhibiting the hemagglutinating activity of 80 HAU of A/PR8. Results are presented as mean ± SEM ($n = 3$).

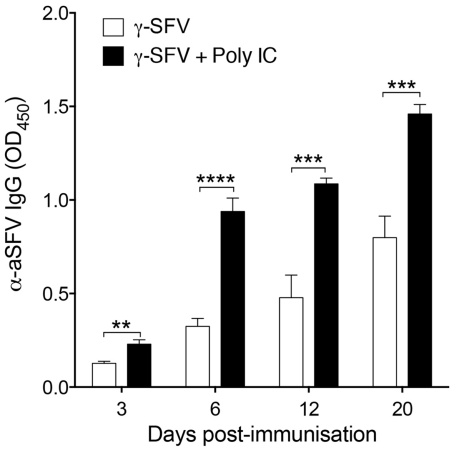

FIGURE 6 | Co-administration of Poly(I:C) and γ-SFV enhances SFV-specific IgG levels. Mice were vaccinated i.v. with γ-SFV (10^7 PFU equivalent/mouse) or co-injected with poly (I:C) (150 μg). Total SFV-specific IgG levels in the serum were analyzed at day 3, 6, 12, and 20 post-vaccination using direct ELISA using a serum dilution of 1/200. Sera from naive mice served as negative controls. Results are presented as mean ± SEM ($n = 3$) (*) denotes statistical significance, $^{**}p < 0.01$, $^{***}p < 0.001$.

humoral responses against poorly immunogenic proteins using Poly I:C is well-documented (4, 7, 24). Poly IC, is a synthetic analog of dsRNA, which is commonly used to stimulate TLR3 and MDA-5 to induce IFN-I and other Th1 priming cytokines such as IL-12 (8, 33, 34). Consistent with previous reports, our data show that co-immunization with Poly IC and γ-SFV resulted in significantly enhanced SFV-specific IgG titers relative to titers observed following vaccination with γ-SFV alone. While this suggest a possible role of IFN-I, more work will be conducted to analyze the underline mechanisms for the adjuvant activity of γ-FLU.

The ultimate goal of using γ-FLU as an adjuvant is to exploit the efficacy of the γ-FLU-mediated immune response toward combined vaccines, in addition to conferring protection against the influenza virus. Our results show that co-administration of γ-FLU and γ-SFV did not affect the titers of FLU-specific IgG and did not affect the neutralizing activity of FLU-specific antibodies. Therefore, humoral responses generated against FLU antigens were not hindered when a second vaccine is present in the environment. Overall, this study is a proof-of-concept illustrating that γ-FLU, a whole virus killed influenza vaccine, can potentially be employed as an adjuvant to increase the quality and magnitude of immune responses toward co-administered less immunogenic vaccines. Future studies will investigate the clinical relevance of the γ-FLU-based combined vaccination strategy, particularly in relation to intranasal and intramuscular routes of administration. Future studies will also examine the effect of co-administered vaccines on the ability of γ-FLU to induce cross-protective immunity.

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Does type I interferon limit protective neutrophil responses during pulmonary *Francisella tularensis* infection?

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FRANCISELLA TULARENSIS

Host–microorganism co-existence has enabled many pathogens to develop mechanisms to evade the immune system. One prime example is *Francisella tularensis*. This Gram-negative bacterium infects various wild animals such as rodents and rabbits, but also exists in water and soil. In rare cases, humans acquire *F. tularensis* infections through inhalation of particles from infected animals, drinking of contaminated water, ingestion of undercooked infected meat, or a bite from an infected tick or mosquito. Respiratory infection is the most deadly form of disease with a mortality rate as high as 50% if untreated. Due to its extreme virulence and ease of aerosol dissemination, it is classified as a Tier 1 bioterrorism agent by the Centers for Disease Control and Prevention. Given that inhalation of aerosolized *F. tularensis* would be the most likely route of transmission during an act of bioterrorism, recent research has shifted from intravenous/intradermal infection models to respiratory models of tularemia. Upon pulmonary infection, many cell types such as alveolar macrophages, neutrophils, and dendritic cells have been shown to harbor live *F. tularensis* (1, 2). Thus, *F. tularensis* is considered as an intracellular bacterium despite its ability to grow in a culture medium. Currently, whether phagocytes contribute to host defense or promote bacterial replication is an intense focus of biodefense research.

TYPE I INTERFERON (IFN-I)-MEDIATED SUPPRESSION OF NEUTROPHIL RECRUITMENT

Francisella tularensis possesses a remarkable ability to evade the host innate immune response. The first line of defense against most respiratory pathogens is the alveolar macrophage, the predominant cell type found in the airways of naïve hosts. A major role for alveolar macrophages is to efficiently eliminate invading pathogens through phagocytosis. However, *F. tularensis* has evolved a mechanism to escape macrophage killing and to replicate within phagocytes. This, together with the relatively inert properties of the *F. tularensis* LPS, renders alveolar macrophages unable to produce the cytokines and chemokines that are necessary to initiate effective immune responses during the early phases of *F. tularensis* infection (3). However, macrophage infection eventually does trigger a host immune response through bacterial recognition by cytosolic receptors, which in turn, results in type I IFN (IFN-I) production (4, 5). IFN-I signaling stimulates expression of "absent in melanoma 2" (AIM-2), a component of the inflammasome that is required for resistance against *F. tularensis* infection (5, 6). Indeed, mice deficient in AIM-2 are highly susceptible to intradermal or subcutaneous *F. tularensis* challenge (6, 7). However, a contradictory finding is that mice deficient in IFN-I receptors (IFN-IR$^{-/-}$) exhibit substantially increased resistance to intranasal and intradermal *Francisella* infection (8, 9). This resistance suggests that the detrimental effects of IFN-I outweigh the benefits of IFN-I-mediated AIM-2 expression that is required for the inflammasome response. The detrimental effects of IFN-I were ascribed to its negative influence on expression of γδ T cells that produce IL-17 (8). Increased survival of IFN-IR$^{-/-}$ mice following subcutaneous tularemia was closely associated with an increased IL-17 response, reduced bacterial burden, and increased influx of neutrophils into the spleen (8). Suppression of IL-17 responses and neutrophil recruitment by IFN-I has similarly been reported in other infectious disease models (10). These various findings have led to the hypothesis that IL-17-mediated neutrophil recruitment is protective against *F. tularensis* infection. Consistent with this report, IL-17A deficient mice were found to be more susceptible to intranasal *F. tularensis* infection (11–13). However, although a role for IL-17 in facilitating neutrophil responses is well established (14), during pneumonic tularemia, recruitment of CD11b^{+}Ly6G/C^{+} cells was not affected by the absence of IL-17A (13). This suggests that IFN-I may limit neutrophil infiltration during pulmonary *F. tularensis* infection independently from its effects on IL-17 expression. It would be of considerable interest to assess production of neutrophil chemoattractants, such as CXCL1 and CXCL2 in the lungs of IFN-IR$^{-/-}$ mice following intranasal *F. tularensis* infection, to elucidate the mechanisms responsible for IFN-I-mediated suppression of neutrophil recruitment.

An alternative or complementary mechanism that may be responsible for decreased neutrophil recruitment to the site of infection by IFN-I involves host cell death. Immune cells such as lymphocytes, macrophages, and neutrophils can be sensitized by IFN-I toward cell death in a mouse model of *Francisella* and *Listeria monocytogenes* infection (5, 15–17). Consistent with the findings in mice, human neutrophils with upregulated interferon-stimulated gene expression exhibit enhanced cell death following *in vitro* exposure to *Staphylococcus aureus*

(15). This phenomenon, however, seems to be pathogen-specific since enhanced cell death was not observed after exposure to *Pseudomonas aeruginosa* (15). Indeed, spontaneous death of neutrophils in the absence of pathogen can be delayed by *in vitro* IFN-I treatment (1). Thus, the direct effect of IFN-I signaling on neutrophils could be context-dependent. In the case of *Francisella infection*, IFN-I signaling may promote neutrophil death and contribute to the overall decrease in neutrophil numbers at the site of infection.

CONTROVERSIAL ROLE OF NEUTROPHILS DURING PULMONARY TULAREMIA

Neutrophils make up the first wave of phagocytic cell migration into the lungs during most pulmonary bacterial infections, including respiratory tularemia (2). However, previous studies utilizing the neutrophil depleting antibody, anti-Gr-1, have failed to show an important role for neutrophils during pulmonary *F. tularensis* infection, as assessed by bacterial burden (18). This is in striking contrast to the known importance of neutrophils in host defense against systemic tularemia (18–20). Recently, it has been proposed that neutrophils may even promote immunopathology during pulmonary tularemia. Mice deficient in metalloproteinase-9 (MMP-9), a mediator of leukocyte migration, have reduced neutrophil recruitment into the lungs following intranasal *F. tularensis* infection (21). Surprisingly, these mice exhibit better survival compared to wild-type mice following pulmonary tularemia. The interpretation from this study was that the excessive neutrophil infiltration observed in wild-type mice was detrimental to the host, but the limited recruitment of neutrophils seen in MMP-9$^{-/-}$ mice was beneficial (21). We have recently found that near complete depletion of neutrophils using a neutrophil-specific mAb, anti-Ly6G, significantly reduced survival of intranasal *Francisella*-infected mice, an observation that is consistent with a protective role for low to moderate recruitment of neutrophils (unpublished observations). Overall, the accumulating evidence suggests that neutrophils do play a role during pneumonic tularemia, but it remains uncertain whether neutrophils exert protective or harmful effects during pneumonic tularemia. It is important to note that some of the discrepancies in results may be due to the differences in experimental conditions such as the type of *in vivo* depleting antibody, timing of antibody administration, and/or mouse strains.

IFN-IR$^{-/-}$ MICE AS A MODEL TO STUDY PROTECTIVE ROLE OF NEUTROPHILS DURING PULMONARY TULAREMIA

We propose that IFN-IR$^{-/-}$ mice can serve as a valuable tool to better understand the role of IFN-I and neutrophils in defense of mucosal tissues against pulmonary tularemia. To the best of our knowledge, only a few studies have been performed using IFN-IR$^{-/-}$ mice in the pulmonary tularemia model. It would be of considerable interest to determine whether the absence of IFN-I signaling alters the kinetics of pulmonary neutrophil recruitment during respiratory tularemia. If so, an important question to address is whether *in vivo* depletion of neutrophils during the early versus late phases of pulmonary tularemia in IFN-IR$^{-/-}$ mice results in a differential survival outcome. Perhaps in the absence of IFN-I signaling, there is an early influx of neutrophils into the lungs, which promotes early bacterial clearance, but later recruitment is not affected or reduced. This scenario would be consistent with the concept that neutrophils are detrimental if pulmonary infiltration is excessive (21), but their complete absence results in reduced survival. A fine balance may exist between the protective role of neutrophils during the early phase of bacterial infection versus excessive neutrophil recruitment during the recovery phase, which impedes resolution of pulmonary inflammation (depicted in **Figure 1**). An

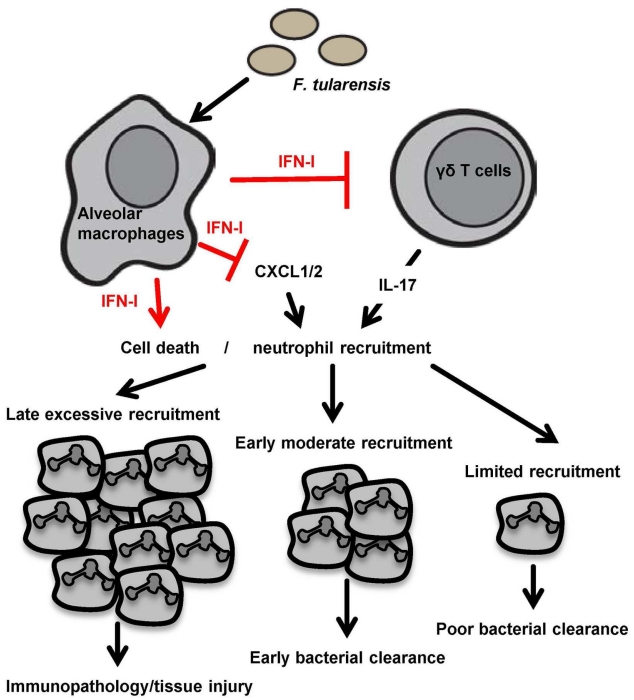

FIGURE 1 | A model depicting the proposed roles of IFN-I and neutrophils during *F. tularensis* infection is shown. Pulmonary infection with *F. tularensis* triggers IFN-I production from innate immune cells such as alveolar macrophages. IFN-I may sensitize *F. tularensis*-infected neutrophils for cell death and contribute to an overall reduction in neutrophil numbers at the site of infection. IFN-I may also directly limit neutrophil recruitment via inhibition of IL-17 producing γδT cells or suppression of other neutrophil chemoattractants. Suppression of neutrophil recruitment may prevent tissue damage associated with excessive infiltration of neutrophils as well as lead to inadequate bacterial clearance, resulting in an unfavorable outcome. An optimal neutrophil response may require moderate recruitment at an early time point to aid in prompt clearance of bacteria without causing significant inflammation later during pulmonary tularemia.

increased understanding of neutrophil-mediated mucosal immunity and the role IFN-I signaling in regulating recruitment of these cells to the lung may ultimately facilitate the development of novel therapeutics for protection against tularemia.

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Unraveling the convoluted biological roles of type I interferons in infection and immunity: a way forward for therapeutics and vaccine design

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It has been well-established that type I interferons (IFN-Is) have pleiotropic effects and play an early central role in the control of many acute viral infections. However, their pleiotropic effects are not always beneficial to the host and in fact several reports suggest that the induction of IFN-Is exacerbate disease outcomes against some bacterial and chronic viral infections. In this brief review, we probe into this mystery and try to develop answers based on past and recent studies evaluating the roles of IFN-Is in infection and immunity as this is vital for developing effective IFN-Is based therapeutics and vaccines. We also discuss the biological roles of an emerging IFN-I, namely IFN-ε, and discuss its potential use as a mucosal therapeutic and/or vaccine adjuvant. Overall, we anticipate the discussions generated in this review will provide new insights for better exploiting the biological functions of IFN-Is in developing efficacious therapeutics and vaccines in the future.

Keywords: type I interferons, human immunodeficiency virus, IFN-ε, vaccine adjuvants, interferon immunity

INTRODUCTION

Since the initial discovery of type I interferons (IFN-Is) as antiviral agents (1), these cytokines have been extensively studied for their anti-microbial and immune regulatory properties. IFN-I family comprises 13 IFN-α subunits, IFN-β, IFN-ω, IFN-ε, IFN-κ, IFN-τ, and IFN-δ (in mice only) (2–8). All IFN-Is signal through the IFN-α receptor (IFN-AR) complex to induce synthesis and secretion of IFN-inducible genes or effector proteins with antiviral, pro-apoptotic, and ubiquitination-modifying properties (9–11). The signaling pathways that IFN-Is utilize to exert various biological effects have been comprehensively reviewed elsewhere and will not be reviewed here [see Ref. (12)]. Numerous cell types produce IFN-Is (e.g., macrophages, myeloid dendritic cells (DCs), fibroblasts, and epithelial cells), but plasmacytoid DCs (pDCs) appear to be the most prolific producers of IFN-Is (13, 14). The production of these cytokines tends to be beneficial to the host particularly against acute viral infections, but there are considerable evidences to suggest that IFN-Is play detrimental roles in autoimmune diseases (15), bacterial and persistent viral infections. Herein, we review how IFN-Is could play beneficial or detrimental roles in pathogen control predominantly with respect to viral infections and discuss how they could be used as therapeutics and vaccine adjuvants. Furthermore, the importance of considering the emerging IFN-ε in immunity and vaccine development will be discussed.

THE BENEFITS AND DETRIMENTS OF IFN-Is IN THE CONTROL OF PATHOGENS

The importance of IFN-Is in protecting hosts against pathogens has been demonstrated in several contexts. Firstly, IFN-AR deficient mice tend to be more susceptible to infection with viruses (particularly acute viral infections) compared to wild-type mice. Some examples include Henipavirus (16), acute Friend virus (17), encephalitic flavivirus (18), lymphocytic choriomeningitis virus (LCMV) Armstrong (19), Hazara virus (20), Dengue virus (21), Respiratory Syncytial Virus (22), and numerous other viral infections (23). Secondly, systemic exhaustion of IFN-Is following a primary viral infection has been shown to increase the host susceptibility to secondary unrelated viral infections in mice (24). Thirdly, therapeutic administration of IFN-Is can reduce viral loads in individuals infected with chronic viruses and promote cancer regression (see below Section "The Use of IFN-Is as Therapeutics and Adjuvants"). Finally, pathogens can attenuate IFN-I responses to promote immune evasion. For instance, human immunodeficiency virus (HIV)-1 can reduce the capacity of IFN producing cells to produce IFN-Is (25–27), induce cytopathic effects on these cells (28–32), and/or block IFN-I mediated intracellular signaling events (33) to help establish a chronic phase infection. Similarly, cancer immune evasion and development could also involve attenuation of IFN-I responses. In agreement with this, Critchley-Thorne et al. (34) have shown that various cancer patients have significantly attenuated expression of interferon stimulate genes in lymphocytes compared to healthy controls.

The benefits of IFN-Is in conferring protection against microbes have been mostly demonstrated using acute viral infection models, but several studies suggest that IFN-Is can also assist in the control of bacterial infections. This was first demonstrated *in vitro* where De la Maza and colleagues (35) showed that IFN-I inhibit *Chlamydia trachomatis* infectivity of human and mouse cell lines. Several subsequent studies have shown that

IFN-I could indeed play important roles for inhibiting various stages of bacterial infections. Some examples include replication of *Chlamydophila pneumoniae* (36), recruitment of *Myobacterium tuberculosis* target cells into the lung during early infection (37), and invasion and transmigration of *Streptococcus pneumoniae* in the lungs (38). However, IFN-Is do not always appear to render beneficial outcomes in anti-bacterial immunity. Several studies have reported that IFN-AR deficient mice are better protected than WT controls following bacterial infections such as *Ehrlichia muris* (39), *Chlamydia muridarum* (40), *Listeria monocytogenes* (41, 42), *Myobacterium* species (43, 44), and *Francisella tularensis* (45). Furthermore, induction of IFN-Is following virus infections could make hosts more susceptible to secondary bacterial infections (46–48). The mechanisms as to how IFN-Is exacerbate or make hosts more susceptible to bacterial disease may vary depending on the infection. For instance, IFN-I mediated disease exacerbation has been linked to reduction of interleukin (IL)-17 expressing γδ T cells, increased expression of IL-10 or reduction in cell-mediate immune responses following *F. tularensis*, *M. Leprae*, or *L. monocytogenes*, respectively (42, 44, 45).

Several reports suggest that the detrimental effects of IFN-Is could also support the establishment of persistent viral infections depending on the quantities and duration of IFN-I induction. IFN-Is have been shown to play significant roles in inhibiting various stages (e.g., replication, virus assembly, protein trafficking, and transcription) of HIV-1 life cycle (49–53). However, sustained unlike transient production of IFN-Is resulting from chronic stimulation of pDCs has been proposed to facilitate HIV-1 persistence (54). Similarly following clone 13 LCMV infection transient (within 24 h) hyper-induction of IFN-α and -β has been reported to exacerbate virus pathogenesis and promote viral persistence (19). However, in the same study IFN-Is were crucial for the control of acute Armstrong LCMV infection, which was likely due to lower IFN-I induction following Armstrong compared to clone 13 LCMV infection. In chronic simian immunodeficiency virus (SIV) infection studies, disease free phenotypes of sooty mangabeys have been associated with the abolishment of interferon stimulated gene expression during chronic, but not in acute phase infection (55). Overall, it can be speculated that early, transient yet non-excessive induction of IFN-Is (at least α and β species) are important in the control of acute viral infections. On the contrary, chronic and/or hyper-induction of IFN-Is could provide an environment for enhanced persistence and/or pathogenesis of chronic viral infections.

IFN-Is AND REGULATION OF ADAPTIVE IMMUNITY

Apart from their most celebrated role as direct anti-viral agents, IFN-Is have also been increasingly recognized as potent regulators of cellular immune responses. Of particular interest to vaccine development has been the ability of these cytokines to regulate adaptive immune responses and this aspect is discussed here.

Dendritic cells are often crucial for initiating adaptive immune responses and serve as important targets for IFN-Is to regulate adaptive immunity. Exposure of IFN-Is facilitates maturation of DCs via increasing the expression of DC-associated chemokine receptors, co-stimulatory molecules, and major histocompatibility complex class I and class II antigen presentation (56–60). Consequently, DCs that mature following IFN-I exposure can effectively prime protective T cell responses (61). A caveat here is that IFN-I responses could operate in a threshold dependent manner where excessive responsiveness is inhibitory to the ability of DCs to prime T cell responses. For instance, following LCMV infection higher induction of IFN-Is has been associated with heightened expression of programed death-ligand 1 (PD-L1) on DCs and PD-L1 interaction with programed death 1 (PD-1) on T cells can inhibit T cell activation (19, 62).

IFN-Is could also act directly on lymphocytes to alter adaptive immune outcomes. Naïve B cells up-regulate the expression of activation markers CD69, CD86, and CD25 following IFN-I exposure *in vitro* (63), but *in vivo* IFN-Is only up-regulate CD69 and CD86 expression on naïve B and T cells (64). The consequences of up-regulating these activation markers are not clear, but *in vitro* studies suggest it could serve to reduce the activation thresholds of naïve B cells unlike T cells (63, 65). Alternatively, CD69 expression resulting from IFN-I exposure can down-regulate sphigosine-1 phosphate receptor-1 on naïve lymphocytes to retain these cells in secondary lymphoid organs (66). This retention mechanism could facilitate a more durable interaction between naïve lymphocytes and DCs for efficient lymphocyte activation to occur. IFN-Is have been reported to represent a distinct third signal for naïve T cell activation to occur and prevent the expansion of regulatory T cells that can inhibit T cell activation (67–69). Furthermore, IFN-Is regulate the functions of lymphocytes even after naïve lymphocyte activation or effector/memory differentiation. Some examples of this include IFN-I mediated enhancement in cell division (63, 70), survival (71, 72), interferon-γ secretion (73), cytotoxicity (74), germinal center formation, and antibody isotype switching (75).

Despite the many studies demonstrating that IFN-Is are capable of boosting adaptive immunity; there have also been several studies in bacterial and chronic viral infection settings suggesting that IFN-I signaling leads to IL-10 production (19, 44, 76, 77). IL-10 is thought to be detrimental to the clearance of these pathogens as has been demonstrated with HIV-1 (78). It is likely that IFN-Is up-regulate PD-1 expression (e.g., on regulatory T cells) and PD-L1 (e.g., on DCs) on cells resulting in a milieu where PD-1/PD-L1 interactions occur; this could facilitate IL-10 production and exhaustion of T cell function during chronic viral infections (19, 76–80). A caveat here is that IFN-Is in some instances can also inhibit IL-10 production and IL-10 production can occur independently of IFN-I signaling (76, 81). Furthermore, IFN-Is up-regulate pro-apoptotic molecules such as Bak on T cells to induce apoptosis independently of T cell exhaustion (82).

Overall, IFN-Is play pivotal roles in boosting adaptive immunity, but the switch from becoming a booster to an inhibitor of adaptive immunity may reflect on how much apoptosis, PD-1/PD-L1 interactions and IL-10 signaling are induced on immune cells due to IFN-Is.

THE USE OF IFN-Is AS THERAPEUTICS AND ADJUVANTS

The development of efficient methods to purify IFN-I and subsequent high yield purification of IFN-α2 during the late 1970s paved way for the first IFN-I based human clinical trial in 1986 where IFN-α2 was used for treating hairy cell leukemia (83, 84). Since then the therapeutic use of IFN-Is have shown promising

outcomes for treatment of several cancers and viral infections. Therapeutic administration of pegylated IFN-α2 have rendered potent anti-viral and immune enhancing effects against hepatitis B virus infection (85, 86). A recent clinical trial has shown that similar outcomes could be achieved even when pegylated IFN-α2 is administered to HIV-infected patients (87). Systemic administration of IFN-α and/or IFN-β has also been reported to reduce viral growth and clinical manifestations of herpes zoster, herpes simplex virus, and cytomegalovirus (CMV) infections (88–91). Furthermore, systemic or intralesional administration of IFN-α and/or IFN-β has been shown to induce a regression of skin-associated wart infections following papilloma virus infections (92–98). IFN-Is have also been used in synergic regimens where administration of IFN-α2 or -β2 and anti-viral drugs (e.g., ribavirin and faldaprevir) could effectively reduce viral loads of certain hepatitis C virus (HCV) genotypes and is currently the best treatment for HCV-infected patients (99–102). A caveat here is that these regimens have also been reported to cause adverse side-effects (103). Apart from treatment of pathogen infections, IFN-Is especially IFN-α2, have also been used for treatment and regression of various cancers (e.g., leukemia, prostrate cancer, and cervical intraepithelial neoplasia) (104–106).

Studies in pre-clinical models suggest that IFN-Is could also be potent vaccine adjuvants for inducing adaptive immune responses. Some examples include when an influenza vaccine adjuvanted with IFN-α/β administered mucosally induced significantly higher IgG2a and IgA antibody responses and protection compared to non-adjuvanted vaccines (107, 108). Interestingly, the species of IFN-Is used as immune adjuvants could have different immune outcomes in terms of enhancing adaptive immunity. Studies in our laboratory suggest that recombinant pox viral vectors encoding IFN-β compared to those encoding IFN-α4 or IFN-ε significantly enhanced systemic T cell immunity against co-encoded antigens in prime-boost vaccination settings (109). However, Xi et al. (110) using similar prime-boost vaccination settings demonstrated that the use of IFN-ε was much more efficient in inducing T cell immunity in mucosal compartments (e.g., lung and gut) compared to IFN-α4 and IFN-β when used as vaccine adjuvants. Another important consideration here is that the vaccine vectors (i.e., pox viruses) used in our studies are acute attenuated viruses and do not chronically induce IFN-Is as is usually the case with persistent virus infections.

There are several confounding factors that could dictate the use of IFN-I in therapy and as vaccine adjuvants. Firstly, unique biological effects have been reported with different members of the IFN-I family and subtypes of IFN-α. Thus, the choice of IFN-I species (e.g., IFN-α2 or IFN-β) could dictate the success of IFN-I treatment or IFN-I based vaccine formulations. Secondly, members of the IFN-I family have different binding affinities and kinetics to the IFN-AR subunits with current comparative studies suggesting that IFN-β has the highest affinity to IFN-AR and anti-viral capacity (111–113). A caveat with these studies is that not all members of the IFN-I family were compared. Thirdly, IFN-Is can cause numerous adverse side-effects and induce autoimmunity (e.g., lupus, thyroiditis, diabetes, dermatitis, Sjogren's syndrome, and arthritis) especially in patients with a history of autoimmune manifestations (114). The autoimmune outcomes in these settings are thought to be a combination of tolerogenic immune function failures and IFN-I mediated maturation of DCs that present autoantigens to activate autoreactive T cells and B cells that make autoantibodies (115).

Collectively, IFN-Is have shown considerable promise for the treatment of cancers and pathogen infections (e.g., chronic viruses) in some clinical settings. IFN-Is are also promising for use as vaccine adjuvants, but the species of IFN-Is used for this purpose could have a significant bearing on adaptive immunity generated at certain immune compartments. For instance, IFN-β could be used to effectively enhance systemic T cell immune responses, whereas IFN-ε is more promising as an adjuvant to enhance mucosal T cell immunity in the lung and the gut mucosae.

IMPORTANCE OF IFN-ε IN IMMUNITY AND VACCINE DEVELOPMENT

Most studies investigating the roles of IFN-Is have done so mainly analyzing the roles of IFN-α and -β. However, investigating the roles of other IFN-I family members is beneficial for effective therapeutic and vaccine development strategies especially given that higher induction of IFN-α and -β could be detrimental to the host as discussed previously. For this purpose, it is indeed intriguing to evaluate the roles of IFN-ε, which unlike other IFN-Is is constitutively expressed and plays various protective roles in reproductive tissues, gut, lung, and the brain (**Table 1**). Since our initial studies characterizing the roles of IFN-ε in inducing anti-viral states on cells (109), we have found that this cytokine also possesses potent immune regulatory capacity. Our recent studies indicated that, intranasal immunization of mice with vaccinia virus (VV) encoding murine IFN-ε (VV-HIV-IFN-ε) unlike IFN-α (VV-HIV-IFN-α4) or IFN-β (VV-HIV-IFN-β) could induce rapid clearance of VV in the lung (110). Viral clearance in this instance correlated with several immune outcomes: (i) elevated lung VV-specific $CD8^+CD107a^+IFN\text{-}\gamma^+$ cell population expressing activation markers CD69/CD103, (ii) enhanced lymphocyte recruitment to lung alveoli with reduced inflammation, and (iii) highly functional $CD8^+CD4^+$ double positive T cell subset [$CD3_{high}$C–C chemokine receptor (CCR)$7_{high}CD62L_{low}$] in lung lymph nodes (110). Next when IFN-ε was used in an intranasal/intramuscular

Table 1 | Site-specific effects of IFN-ε.

Site	Function	Reference
Brain	Maintenance of the structure and function	(116)
Lung	Promote clearance of viral infections Recruitment of unique yet highly anti-viral $CD4^+CD8^+$ T cells	(110)
Gut	Enhance expression of CCR9 and α4β7 on anti-viral T cells to promote homing to the gut (i.e., Peyer's patches)	(110)
Reproductive tissues	Regulation of embryonic development Protect male and female reproductive tissues against infections (e.g., herpes and *Chlamydia*)	(117, 118)

heterologous HIV-1 prime-boost vaccination regimen, elevated HIV-specific effector, but not memory $CD8^+$ T cells responses were detected in spleen, genito-rectal nodes, and Peyer's patches. Furthermore, homing marker α4β7 and CCR9 analysis showed that unlike other IFN-Is, IFN-ε promoted the migration of antigen-specific $CD8^+$ T cells to the gut mucosae (110). These results for the first time established that unlike other IFN-Is, IFN-ε played a unique role at the mucosae. Another recent study has also further substantiated our findings demonstrating that IFN-ε deficient mice were more susceptible to intra-vaginal herpes simplex virus 2 and *Chlamydia muridarum* infections compared to wild-type mice (117). This suggests that IFN-ε could also be beneficial for the control of certain bacterial infections. A caveat here is that it is unknown whether IFN-ε could cause adverse side-effects in humans as it has not yet been used for treatment or vaccination purposes in humans.

Overall, IFN-ε has great potential to be used as a topical microbicide or a therapeutic to control local lung/gut infections or modulate tissue-specific immunity at sites where pathogens are initially encountered (i.e., mucosal surfaces). Specifically, IFN-ε's ability to enhance $CD8^+$ T cell homing to the gut [gut is the primary site of HIV virus replication and $CD4^+$ T-cell depletion (119)] and also its ability to control infections at the lung mucosae suggest that administration of pegylated forms of IFN-ε or vaccines encoding IFN-ε could be effective for controlling mucosal pathogens such as HIV-1.

CONCLUDING REMARKS

The dual roles of IFN-Is in providing beneficial and detrimental effects to the host in pathogen control is intriguing for developing IFN-I based vaccines and therapies. Lessons learned from acute viral infection models and studies comparing acute versus chronic infection states suggest that transient, but not sustained and/or excessive induction of IFN-Is is likely to confer protective outcomes. IFN-Is have also proven to be promising therapeutic agents against various pathogens and cancers and could also be used as vaccine adjuvants. The caveat here is that the vaccine vector used should ideally not chronically stimulate the production of IFN-Is, which is expected to be detrimental for the generation of robust adaptive immune responses. Our laboratory and others have demonstrated that IFN-ε has great potential to provide protective outcomes against not only mucosal viral infections, but also certain mucosal bacterial infections. Keeping this in mind, more studies need to evaluate the contribution of the different species of IFN-Is not just IFN-α and -β in immunity against infections. These studies are expected to pave way for the development of novel and effective IFN-I based vaccines/therapies against chronic pathogens and cancers.

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The curious case of type I IFN and MxA: tipping the immune balance in AIDS

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Human immunodeficiency virus (HIV) remains a significant public health challenge. According to the World Health Organization, there were approximately 35.3 million people living with HIV/AIDS worldwide in 2012, with Sub-Saharan Africa being the most affected region (1). HIV has claimed 27 million lives. It is estimated that two million people die from HIV/AIDS each year.

Highly active antiretroviral therapies (HAART) now allow HIV-infected individuals to live longer and healthier lives. HAART is, however, burdened with side effects and high costs, and threatened by evolving viral resistance. A hallmark of both HIV and simian immunodeficiency virus (SIV) infection is chronic immune activation together with uncontrollable viremia and lymphocyte apoptosis that progresses to acquired immunodeficiency syndrome (AIDS) (2, 3). The deleterious role for immune activation in HIV infection is supported by animal studies. Although the exact mechanism underlying AIDS resistance of natural hosts for SIV, like sooty mangabey (SM) monkeys, is unknown, reports support the idea that disease progression in non-natural hosts such as rhesus macaques (RM) is mainly due to immune system dysfunction triggered by HIV/SIV infections (4, 5). In non-natural hosts, SIV causes progressive impairment of the immune system characterized by high viremia, $CD4^+$ T cell depletion, and loss of T cell function. Continuous $CD4^+$ T cell loss eventually leads to AIDS as the regenerative capacity of the immune system gradually decreases despite the excess of homeostatic cytokines (6). This exhaustion and increased T cell apoptosis, a phenomenon seen in pathogenic infections, may be due to direct and indirect killing by the virus. Indirector bystander-killing, is the loss of uninfected and abortively infected T cells possibly due to generalized immune activation (7, 8). It is widely accepted that type I interferon-producing (IFN I) plasmacytoid dendritic cells (pDC) play a central role in this generalized immune activation (9–11). On the other hand, natural simian hosts preserve T lymphocyte populations despite high viremia and show attenuated immune activation that favors the maintenance of $CD4^+$ T cells; hence, SMs do not develop AIDS (7). Although HIV preferentially infects $CD4^+$ T cells and macrophages, efficient binding and infection of pDCs by HIV have been demonstrated and may contribute to AIDS pathogenesis (12, 13).

Plasmacytoid dendritic cells are specialized cells found in blood and lymphoid tissues. The main function of pDCs is to produce IFN in response to bacterial and viral DNA. Following activation, these produce 1000-fold more IFN I than other IFN producing cells (11). Upon HIV infection, pCDs become activated and express CCR7 and CXCR3, migration markers that induce redistribution to the lymph nodes (LN) (14–16). Further, recent reports suggest that HIV-induced pathogenesis occurs mainly in LNs where the IFN I that pDCs produce elevates serum levels during both acute and chronic infections (13, 17–19).

Type I IFNs are powerful cytokines and adaptive immune system modulators. They are produced upon viral infection, replication, and/or the introduction of double-stranded RNA (20). IFNs trigger antiviral activity and induce the maturation of effector T cells. Therefore, an interesting immunoregulatory role for type I IFN is in lymphocyte activation during viral infection (21). There is, however, evidence that this otherwise beneficial interferon can become detrimental to the host during chronic HIV infection. Persistent levels of IFN I induce apoptosis in both HIV-infected $CD4^+$ T cells and in those that do not become productively infected (bystander-killing) leading to accelerated depletion of $CD4^+$ T cells during pathogenic infection (22). This effect is due to type I IFN triggered apoptosis of uninfected $CD4^+$ T cells via TNF-related apoptosis inducing ligand (TRAIL) (17). Originally, SM, which are natural SIV hosts, were thought to have reduced immune system activation during both acute and chronic SIV infections. Moreover, SM pDCs produce less IFN I *ex vivo* in response to SIV, which leads to less immune activation during chronic infection (23). Other works, however, have shown that natural hosts exhibit an initially strong, but rapidly controlled, IFN I response (24–26). Therefore, both natural and non-natural hosts mount strong type I IFN responses during the acute stage of infection but only natural hosts suppress the response by the chronic stage, 4–16 months after infection. This downregulation occurs despite sustained high levels of viremia. Unlike natural hosts, non-natural hosts maintain high levels of IFN I production at all times. It is important to note that cells from SIV-infected natural hosts can be repeatedly stimulated *in vitro* to produce type I IFN and to upregulate interferon-stimulated genes (ISG) during the chronic stage of SIV infections suggesting that these cells are neither more

refractory nor resistant to re-stimulation (26). Therefore, the downmodulation of IFN I in natural hosts is likely due to negative control mechanisms. The exact mechanism underlying the downregulation of IFN I is, however, not fully understood. The regulatory complexity of the IFN pathway and its overall effects on target cells make pinpointing a resolution mechanism challenging. A role for immunomodulatory proteins, negative regulation of IFN responses, and other mechanisms have been suggested [reviewed in Ref. (2)]. Efforts have been made to discover the IFN I downregulation pathway by examining the regulation of ISGs as well as immunosuppressive genes (24, 26). One common denominator is the myxovirus resistance protein (MxA or Mx1) gene. This finding was supported by a recent clinical study in which Chang et al. found that persistent higher expression of type I IFN and ISGs, including MxA, may explain, at least in part, the increased immune activation and more rapid disease progression in females with chronic HIV infections when compared to males with similar viral loads (27). Harris et al. convincingly showed that natural hosts African green monkeys (AGM) and SM begin to downregulate MxA responses by 28 dpi whereas non-natural hosts (RM) maintained high levels of MxA in LN. This work, however, did not reveal a mechanism of IFN I resolution (24). Further, it is unclear whether IFN is directly downregulated or whether downstream signaling negatively regulates IFN. Understanding the mechanism of Mx regulation in the transition phase, from acute to chronic infection of natural hosts, should reveal new targets for therapies to block the chronic immune system activation associated with disease progression.

MxA is an IFN induced protein expressed in cells like macrophages and hepatocytes. It is best known for its antiviral activity against orthomyxoviruses (28). MxA was first described in 1962 when Lindenmann showed that A2G inbred mice were resistant to doses of mouse-adapted influenza virus that were lethal to other inbred mice. This resistance was dependent on a single dominant locus named Mx1 and was exquisitely specific for orthomyxoviruses (29). Later, it was discovered that Mx1 was the first member of a small gene family and that the spectrum of antiviral activity is in fact much larger than originally thought. Most species have one to three Mx protein isoforms with different antiviral activity depending on their intracellular localization (28). Mouse Mx1 protein is found primarily in the nucleus whereas human MxA is cytoplasmic. Hence, each protein blocks influenza virus at a different stage of the viral replication cycle (30). Of note, Mx2, an interferon-induced protein, has recently been shown to inhibit HIV infection after entry (31, 32). Mx2, however, differs from Mx1 in that Mx2 localizes to the nucleus and its antiviral activity relies on a nuclear localization signal (32).

What are Mx proteins and how do they exert their antiviral activity? Most importantly, how are Mx proteins involved in SIV/HIV infections? Mx proteins belong to the superfamily of GTPases, which includes dynamins, dynamin-like proteins, and mitofusins [described in more detail in Ref. (33)]. These proteins are involved in endocytosis, intracellular vesicle transport, and mitochondria distribution. Mx proteins are mainly characterized by three conserved domains: an N-terminal GTPase domain (GTP binding), a middle domain responsible for interaction with the GTPase effector domain (GED), and the C-terminus GED domain, which recognizes the virus. Two amphipatic α-helices form leucine zippers in the C-terminus. Furthermore, Mx proteins can self-assemble into higher order ring-like structures to form a helical stack (28). The higher order structures may represent a storage form whereas the monomers are likely the active form of MxA (34). Human *MxA* can be induced by IFN or directly by the virus through different pathways. Activation of Mx by IFN involves the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway and the formation of an IFN-stimulated gene factor 3 (ISGF3) multimeric complex, which in turn migrates into the nucleus and binds an IFN-stimulated response element (ISRE) upstream of the Mx gene (35, 36). It is not yet clear how viral infection activates Mx but it seems to be independent of ISGF3, involving STAT1 instead and possibly the IFN-regulatory factors 1 and 3 (IRF1 and IRF3) (37–39). Mx gene activation is fast. Its protein product is detectable within 4 h (40). It is thus likely that Mx gene activation in response to both virus and IFN through two different pathways is evolutionarily advantageous to the host. Indeed HIV can induce MxA transcription and activate ISGs independently of IFN (41). As mentioned previously, in contrast to non-natural hosts, natural SIV hosts downregulate MxA responses during the transition to chronic infection despite high viremia (24). Moreover with every upregulation/activation, there must be a downregulation of the response in order to maintain homeostasis. Indeed overexpression of MxA is a common pathogenic link in Fanconi Anemia (FA), which consists of a group of at least five autosomal recessive disorders. Overexpression of MxA can lead to cancer susceptibility, apoptosis, bone marrow failure, and abnormal instability in cells (42). One potential mechanism for regulation of Mx gene expression relies on the IRF-1 and IRF-2 proteins. The most probable mediator of Mx induction is IRF-1, which is increased in the presence of virus and after IFN treatment (43, 44). This suggests that the functional synergy between the two independent pathways converges in upregulation of Mx. Additionally, the virus may synergize with IFN to increase Mx levels. IRF-1 induces IRF-2 production to repress ISG transcription. This in turn inhibits IRF-1 function. IRF-1 and IRF-2 share homology in their DNA binding domain; however, IRF-2 has higher affinity for binding and a longer half-life (45). Interestingly, IRF-2 also protects quiescent hematopoietic stem cells (HSC) from type I IFN exhaustion (46). In order to avoid cancer susceptibility, increased apoptosis, bone marrow failure, and HSC depletion, natural SIV hosts apparently evolved to control the IFN responses (**Figure 1**); however, the exact mechanism is not yet clear. Studying variations in the Mx genes, simultaneously with standardized screening of their antiviral properties in natural and non-natural SIV hosts, could explain why natural hosts can downregulate MxA responses with the onset of the chronic stage of infection. Additionally, mining for polymorphisms in regulatory genes, such as IRF-2, which correlate with enhanced DNA binding or half-life of the activated protein may offer an avenue for designing new therapeutics for controlling the immune system hyperactivation that is associated with AIDS progression.

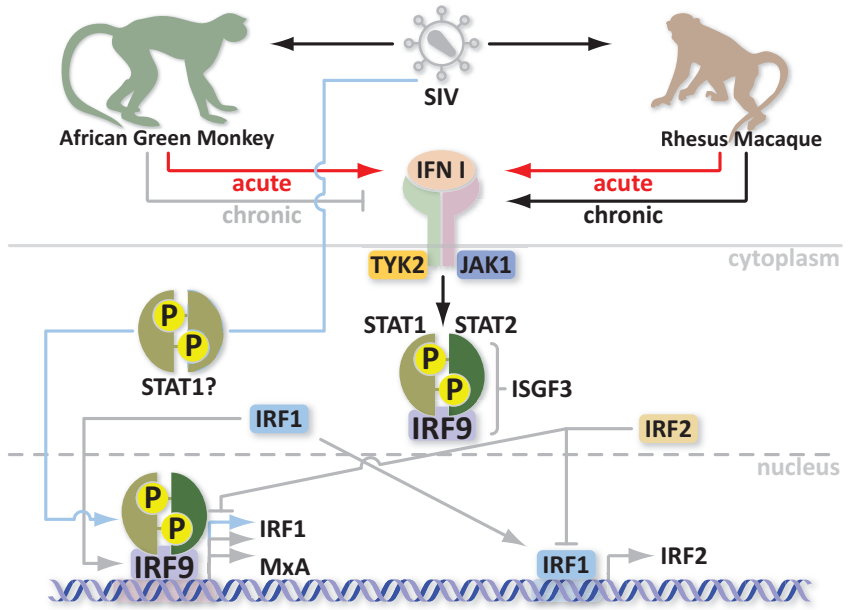

FIGURE 1 | Schematic of IFN and Mx protein regulation in acute and chronic SIV infection models.

CONCLUSION

Studies in non-human primates have revealed the importance of immune activation in HIV/SIV pathogenesis. Chronic immune activation, associated with $CD4^+$ T cell depletion and loss of T cell function, is likely due to increased serum levels of type I IFN. Although type I IFN is necessary to control viral infections, its detrimental effects during the chronic stage of HIV/SIV infection has been well documented. Natural SIV hosts, in sharp contrast to non-natural SIV hosts, control IFN responses with active regulatory mechanisms. Currently, it is not clear how this regulation is achieved on the molecular level. Understanding the basis of active IFN response repression is crucial as these may play an important role in protection against disease progression. Regulation of the type I IFN pathway is very complex making it hard to narrow down the exact factor or factors responsible for repression of this pathway. Evidence supporting a role for MxA genes, warrants studying polymorphisms in MxA genes, which may shed light on the mechanism of active IFN downregulation. Additionally, better understanding of how Mx genes are regulated will further broaden our perspective for understanding the evolution of host–virus interactions.

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Type I interferons in bacterial infections: taming of myeloid cells and possible implications for autoimmunity

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Type I interferons (IFNs) were first described for their ability to protect the host from viral infections and may also have beneficial effects under specific conditions within some bacterial infections. Yet, these pleiotropic cytokines are now known to exacerbate infections by numerous life-threatening bacteria, including the intracellular pathogens *Listeria monocytogenes* and *Mycobacterium tuberculosis*. The evidence that such detrimental effects occur during bacterial infections in both animals and humans argues for selective pressure. In this review, we summarize the evidence demonstrating a pro-bacterial role for type I IFNs and discuss possible mechanisms that have been proposed to explain such effects. The theme emerges that type I IFNs act to suppress myeloid cell immune responses. The evolutionary conservation of such anti-inflammatory effects, particularly in the context of infections, suggests they may be important for limiting chronic inflammation. Given the effectiveness of type I IFNs in treatment of certain autoimmune diseases, their production may also act to raise the threshold for activation of immune responses to self-antigens.

Keywords: interferons, interferon receptors, bacterial pathogens, macrophage activation, immune suppression, autoimmunity

INTRODUCTION

Type I interferons (IFNs) are a class of cytokines that includes numerous IFNα subtypes, IFNβ, IFNδ, IFNε, IFNκ, IFNτ, and IFNω (1, 2). These secreted factors are predominantly produced by innate immune and non-immune cells of humans and other animals in response to recognition of conserved microbial products, rather than specific antigens. The different type I IFNs vary in their sequences but bind and signal using a common, ubiquitously expressed, heteromeric cell surface receptor (IFNAR) comprised of IFNAR1 and IFNAR2 chains. Ligation of IFNAR in diverse cell types activates a canonical JAK/STAT signaling cascade primarily involving JAK1, Tyk2, STAT1, and STAT2 proteins. Activation of these factors leads to induced transcription of numerous type I IFN-stimulated genes (ISGs), the protein products of which largely act to disrupt various stages of viral replication (3, 4). Type I IFNs are thus important for resistance to several viral infections and are used in the clinic for effective antiviral therapy (2). However, type I IFNs also exert a variety of other effects on cellular functions and immune responses. For example, they up or down regulate production of and responsiveness to other cytokines, chemokines, and can stimulate cell growth, cell survival, or apoptosis (2, 5). Consequently, these cytokines are also used for treatment of melanomas, leukemias, and other cancers (6), and as immune modulatory agents to suppress neuroinflammation in patients suffering from relapse-remitting multiple sclerosis (MS) (2). Hence, type I IFNs exert seemingly opposing pro- or anti-inflammatory effects and pro- or anti-apoptotic effects. It is likely that these opposing effects reflect cell type-specific differences in the activation of secondary or "non-canonical" signaling events and/or variations in the dominance of a specific type I IFN species. Indeed, individual type I IFN proteins vary in their ability to elicit specific responses and stimulation of different cell types can cause distinct signaling events (7, 8).

The second class of IFN protein (type II or IFNγ) is more critical for host defense against intracellular bacterial pathogens, such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*. IFNγ signals through its own ubiquitously expressed heteromeric receptor (IFNGR), which utilizes IFNGR1 and IFNGR2 chains to activate a canonical JAK/STAT pathway primarily involving JAK1, JAK2, and STAT1. In contrast to type I IFNs, which are broadly expressed, IFNγ is produced primarily by lymphocytes. Antigen-specific IFNγ production occurs when appropriate T lymphocyte populations respond to specific microbial antigens, while antigen non-specific production of IFNγ is stimulated by cytokines such as interleukin (IL)-12 and IL-18. Studies using *L. monocytogenes* and other bacterial infection models indicate that both T and natural killer (NK) cells are capable of this antigen non-specific IFNγ production (9–11). Myeloid cells such as macrophages and dendritic cells (DCs) are key targets of IFNγ, as shown by the increased susceptibility to *L. monocytogenes* infection in mice selectively defective for functional IFNGR1 in myeloid cells (12, 13). The expression of numerous IFNγ activated genes (GAGs) is induced by the cytokine. Some of these genes are identical to ISGs and have antiviral effects. However, IFNγ is unique in its ability to elicit a potent anti-microbial state of activation in macrophages. This "M1-type" activation is associated with increased expression of GAGs such as nitric oxide synthase 2 (NOS2) and NADPH oxidase subunits. These enzymes generate nitric oxide (NO) and reactive oxygen species (ROS) that alter cell signaling and under appropriate circumstances can mediate direct killing of bacteria

(14, 15). IFNγ-inducible GTPases also promote macrophage resistance to bacterial and parasite infections by increasing the ability of phagosomal compartments to contain and kill engulfed microbes (16–18). IFNγ also upregulates myeloid cell expression of MHC II and other factors important for antigen presentation and T cell activation (19). In addition, IFNγ impacts maintenance and proliferation of hematopoietic stem cells (HSC). Specifically, basal production of IFNγ in the absence of infection drives HSC cycling and the elevated levels occurring during infection can activate HSC proliferation and myelopoiesis to replenish monocytes and other immune cells (20, 21).

Despite the antiviral effects of type I IFNs, and in the context of the antibacterial effects of IFNγ, it is increasingly evident that host responsiveness to type I IFNs correlates with increased host susceptibility to infections by *L. monocytogenes*, *M. tuberculosis*, *Francisella tularensis*, and several other intracellular bacterial pathogens (22–24). Here, relying heavily on the *L. monocytogenes* model, we review the pathways involved in the induction of type I IFNs by intracellular bacteria and various mechanisms proposed to account for the suppressive effects of type I IFN signaling. A theme that emerges from these studies is that type I IFNs have suppressive effects on anti-microbial and antigen-presenting function of myeloid cells. Such effects may contribute to both the observed ability of these cytokines to increase host susceptibility during bacterial infections and to their effectiveness in therapy of neuroinflammatory disease.

BACTERIAL FACTORS CONTRIBUTING TO TYPE I IFN PRODUCTION DURING *L. MONOCYTOGENES* INFECTION

Listeria monocytogenes is a Gram-positive facultative intracellular bacterium that causes the systemic disease Listeriosis. The mortality rate of Listeriosis is quite high even in hospitalized patients; hence, *L. monocytogenes* remains a leading cause of death from foodborne illnesses within the United States. *L. monocytogenes* can infect hematopoietic and non-hematopoietic cell types through phagocytosis or cellular mediated uptake (25, 26). Following systemic infection in the murine model *L. monocytogenes* localizes to the liver and spleen where resident phagocytes, primarily macrophages and DCs, engulf the bacteria. *L. monocytogenes* that escape from phagosomal compartments in these cells can replicate within the cell cytosol and further propagate the infection into neighboring cells. To facilitate vacuolar escape, the bacteria secrete a pore-forming hemolysin (hly) known as listeriolysin O (LLO) (27).

Like many other bacteria, *L. monocytogenes* induces production of pro-inflammatory cytokines such as TNFα and type I IFNs when engulfed by professional phagocytes. Studies of infection in bone marrow derived macrophages (BMM) suggest that there are two waves of the cellular response to *L. monocytogenes* infection (28, 29). An "early phase" gene expression profile is seen at 1–2 h post infection by both virulent wild-type *L. monocytogenes* and avirulent Δhly or heat-killed bacteria that cannot escape from vacuolar compartments into the host cytosol (28, 29). Several of these "early phase" genes, including *il1b*, *tnfa*, and those encoding several chemokines are induced through the activation of Toll-like receptor (TLRs) and the ensuing activation of NFκB (28, 29). A subsequent "late-phase" response is observed at 4–8 h after infection by wild-type, but not killed or Δhly, *L. monocytogenes* strains (28, 29). "Late-phase" genes include IFNβ, multiple subtypes of IFNα, and several ISGs (28, 29). The fact that killed and Δhly *L. monocytogenes* strains fail to induce this late-phase IFN-dominated response supports the interpretation that products from bacteria replicating within the BMM cytosol stimulate cytosolic pathogen recognition receptors (PRR), though TLR stimulation can augment the induction of type I IFNs during *L. monocytogenes* infection (29, 30). There has been considerable interest in identifying the cytosolic PRRs responsible for type I IFN production during *L. monocytogenes* infection.

PATHWAYS LEADING TO THE PRODUCTION OF TYPE I IFNs DURING BACTERIAL INFECTION

Pathways known to be important for induction of type I IFN within *L. monocytogenes*-infected phagocytes are diagrammed in **Figure 1**. Amongst the earliest identified cytosolic PRRs were the nucleotide-binding oligomerization domain (NOD)-containing proteins; members of the nucleotide-binding domain, leucine-rich repeat (LRR) protein family referred to as NLRs. NOD1 and NOD2 proteins sense distinct muropeptide fragments from the cell wall from *L. monocytogenes* and other bacteria (31–33). Recognition of appropriate muropeptides activates a serine/threonine kinase receptor interacting protein (RIP2) to initiate downstream signaling and activation of NFκB (31). With regards to triggering of type I IFN production, NOD1, NOD2, and RIP2 seem to play an ancillary role. They augment the induction of type I IFN and other cytokine expression in response to *L. monocytogenes* (29, 31–33), but mice and BMM deficient for any one of these proteins retain the ability to mount inflammatory responses and synthesize type I IFNs in response to *L. monocytogenes* (31, 34, 35). Thus, the recognition of bacterial cell wall components by the cytosolic NOD1 and NOD2 proteins is not crucial for the induction of type I IFNs during *L. monocytogenes* infection.

Nucleic acids are potent inducers of type I IFN production and it was shown that extracts from *L. monocytogenes* induce IFNβ production in a manner sensitive to DNAse treatment of the extracts (36). *L. monocytogenes* was also reported to actively secrete both RNA and DNA during infection of macrophages (37). Cytosolic RNA is detected by the RNA helicase retinoic acid inducible gene 1 protein (RIG-I), related RIG-I-like (RLR) proteins including melanoma differentiation-associated gene 5 (MDA5), as well as other non-RLR helicases and PRRs (38–40). RNA recognition by RIG-I and MDA5 induces their recruitment to mitochondria, where they encounter an adaptor protein [mitochondrial antiviral signaling (MAVS)] that regulates downstream signaling to induce type I IFNs (41–43). Secreted *L. monocytogenes* DNA could also be detected using these RNA receptor systems if it is transcribed into RNA by host cell RNA polymerase III (37). However, deficiency in RIG-I, MDA5, or MAVS fails to ablate IFNβ production by *L. monocytogenes*-infected BMM (37, 44, 45). Thus, it does not appear that RNA sensing is crucial for recognizing cytosolic *L. monocytogenes* infection in this cell type, although it may play a more important role in sensing *L. monocytogenes* infection of other cell types and for sensing infection by other bacteria, namely *Legionella pneumophila* (46, 47).

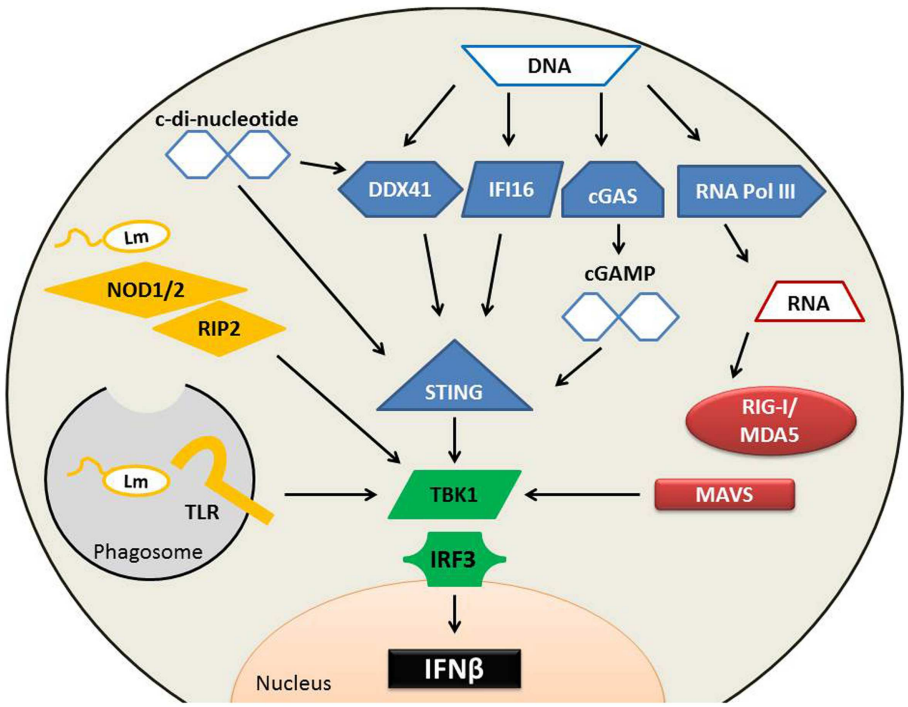

FIGURE 1 | Pathways implicated in type I IFN production following *L. monocytogenes* infection. Several cytosolic receptors are able to recognize *L. monocytogenes* (Lm) microbial components to induce type I IFN production. (Yellow) Endosomal TLRs recognize a variety of Lm bacterial patterns including cell wall fragments, which can also stimulate NOD proteins. NOD proteins require association with RIP2 to activate TBK1. Lm secretes RNA, DNA, and cyclic-di-nucleotides. Secreted RNA (red) binds to RIG-I or MDA5, both of which associate with MAVS. Cytosolic DNA (blue) can alternatively be converted into RNA by RNA polymerase III and induce type I IFNs through the RIG-I pathway. DNA is also directly sensed by DDX41 or IFI16 to induce the production of type I IFNs in a STING-dependent mechanism. cGAS can sense DNA and convert it into cGAMP, which binds with high affinity to STING to stimulate IFNβ production. C-di-nucleotides bind to STING directly and to DDX41, either of which may result in IFNβ synthesis. TBK1 and IRF3 are essential for the induction of type I IFN production during Lm infection, and each of these upstream sensing pathways converges on TBK1 activation.

DNA present in the host cell cytosol can also be detected and trigger the production of type I IFNs (40). Several putative receptors have been identified that might mediate such recognition, including the DNA-dependent activator of IRFs (DAI), IFN-inducible gene (IFI)-16, and LRR flightless-interacting protein (LRRFIP1). Deficiency or knockdown of DAI, IFI16, or LRRFIP1 fails to completely ablate type I IFN production by infected murine BMM (48–50). However, recently IFI16 was shown to have a larger role in the recognition of *L. monocytogenes* DNA in the human macrophage cell line, THP1. Knockdown of IFI16 in these cells drastically reduced the production of IFNβ in response to *L. monocytogenes* DNA (51). The DNA-binding DEAD-box helicase DDX41 has also been shown to bind *L. monocytogenes* DNA. DDX41 elicits type I IFN production through a mechanism requiring the stimulator of interferon genes protein (STING), also called MITA, MPYS, or ERIS (52). STING induces type I IFN production by activating the TNFR-associated NF-κB kinase (TANK)-binding kinase 1 (TBK1), which phosphorylates a C-terminal serine residue on the transcription factor IFN regulatory factor 3 (IRF3) to induce IRF3 dimerization and nuclear translocation (34, 53). IRF3 and other IRF family members bind to the promoters of type I IFN genes and ISGs to regulate and initiate their transcription. TBK1 and IRF3 are thus not surprisingly essential for the production of type I IFNs during *L. monocytogenes* infection (34, 54). Likewise, deficiency or knockdown of STING significantly decreases IRF3 activation and IFNβ production in BMM, DC, or fibroblasts infected with *L. monocytogenes* (55–57).

It appears that STING does not respond to intact DNA, but rather to endogenous or exogenous cyclic-di-nucleotides (40). In the presence of cytosolic dsDNA, the enzyme cGAS synthesizes an endogenous cyclic-di-nucleotide, cGAMP (58). Binding of cGAMP to STING occurs with a very high affinity (~4 nM) and induced conformational changes that presumably initiate the downstream events that culminate in type I IFN production (59). Exogenous cyclic-di-nucleotides can also activate STING (56, 57, 60). Both cyclic-di-GMP (cdGMP) and cyclic-di-AMP (cdAMP) are produced by bacteria and function as second messengers. There is evidence that cdAMP is released from replicating *L. monocytogenes* (61, 62), thus it is conceivable that cdAMP secreted by the bacterium mediates the STING-dependent production of type I IFNs in *L. monocytogenes*-infected macrophages. However, while the affinity of cdAMP for STING is not known, STING binds cdGMP ~300-fold lower affinity (~1 μM) than cGAMP DNA (59). Thus, it is also conceivable that cGAMP produced by cGAS

in response to secreted bacterial DNA contributes to STING-dependent type I IFN production. Regardless, it is important to keep in mind that STING deficient mice showed significantly reduced serum IFNβ only very early (8 h) after *L. monocytogenes* infection (56, 57). Thus, systemic *L. monocytogenes* infection can trigger type I IFN through multiple pathways and the impact of STING on overall type I IFN production in this model is limited.

TYPE I IFN SIGNALING AND INCREASED SUSCEPTIBILITY TO BACTERIAL INFECTION

In certain bacterial infection models, protective effects of type I IFNs have been reported. For example, type I IFN can reduce bacterial burdens in cultured cells infected with *L. pneumophila* or *Chlamydia trachomatis* and survival of mice is increased in sepsis models with group B *Streptococcus* and *E. coli* (63–65). Mice lacking expression of IFNε, which is abundantly expressed within the female reproductive tract, were also reported to be highly susceptible to urogenital infection by *C. muridarum* (66). The precise mechanisms are not clear in these cases, but the observed protective effects appear to reflect unique aspects of the models and/or pathogens studied since there is considerable evidence to indicate that type I IFNs instead play a deleterious role during infections by numerous other bacterial pathogens (22–24). Specifically, studies with mice lacking IFNAR1 report that bacterial burdens are significantly reduced and survival increased following systemic or mucosal infections with intracellular bacteria that infect the cytosol of host cells, such as *L. monocytogenes* (54, 67–70) and *F. tularensis* (71) as well as bacteria like *M. tuberculosis* (72–74) and *C. muridarum* (75, 76) that reside within vacuolar compartments. In addition, heightened type I IFN production correlates with increased host susceptibility to several bacterial infections. In mice, examples of this include the correlation of increased type I IFN production in mice with a mutated ubiquitin specific peptidase (USP18) and sensitivity to *Salmonella typhimurium* (77). Furthermore, isolates of *L. monocytogenes* and *M. tuberculosis* that hyper-induce type I IFN production have heightened pathogenicity in animal models (78, 79). The administration of type I IFNs or agents that induce these cytokines also causes increased susceptibility to *L. monocytogenes* and *M. tuberculosis* in model infections (54, 78, 80). Type I IFN production is also increased during viral infections. In mice, lymphocytic choriomeningitis virus (LCMV) infection potently induces type I IFNs and leads to ~1000-fold increased susceptibility to a secondary *L. monocytogenes* infection as measured by bacterial burdens (81). In humans, a similar situation occurs following infection with influenza virus. Influenza infections are often associated with secondary bacterial infections and secondary bacterial pneumonias are estimated to account for up to 25% of the more than 250,000 annual deaths attributed to influenza (82, 83). Such secondary infections are also thought to have caused most of the deaths from the 1918 influenza pandemic (84). *Streptococcus pneumoniae* is a prevalent bacterial cause of pneumonias and a model of influenza and secondary *S. pneumoniae* infection showed that increased bacterial burdens and mortality was dependent on IFNAR expression (85). Severe bacterial infections have also been noted in patients receiving prolonged IFNα2 therapy for chronic hepatitis C virus infection (86–88). Moreover, in the absence of obvious viral infections, signatures of type I IFN responses correlate with disease progression in human tuberculosis and leprosy patients (89, 90). Thus, despite numerous differences in the receptors and cytokines themselves, the association of type I IFNs with exacerbated bacterial infections appears to have been conserved in murine and human systems. An improved understanding how these cytokine responses are deleterious to their hosts and what has driven their conservation across this evolutionary span are important questions to address.

MECHANISMS PROPOSED TO ACCOUNT FOR THE PRO-BACTERIAL EFFECTS OF TYPE I IFNs

A summary of the proposed mechanisms for the deleterious effects of type I IFN signaling during bacterial infections is outlined in **Figure 2**.

INDUCTION OF HOST CELL DEATH

It has long been known that bacterial infections can induce death of multiple cell types within tissues of murine hosts. In the systemic *L. monocytogenes* infection model, this cell death is exacerbated by type I IFNs. O'Connell et al. observed that expression of pro-apoptotic genes such as TNF-related apoptosis-inducing ligand (TRAIL), promyelocytic leukemia (PML), and death-associated protein 6 (Daxx) were increased in the spleens of *L. monocytogenes*-infected wild-type, but not IFNAR1 deficient, mice (54). Consistent with increased apoptosis in these tissues, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining is also increased in the spleens of infected wild-type mice, when compared to infected IFNAR1$^{-/-}$ or IRF3$^{-/-}$ mice (54, 69). This TUNEL staining was localized to lymphocyte rich follicles within the spleens, suggesting that type I IFN might induce apoptosis of lymphocytes and that such apoptosis could itself be detrimental (54, 69). The possibility that lymphocyte apoptosis is deleterious to the host is also consistent with the observation that mice deficient in lymphocytes or T cells alone are resistant to acute systemic *L. monocytogenes* infection (91–93). Resistance in T cell-deficient hosts is thought to reflect constitutively heightened macrophage activation (94), and correlates with reduced production of the anti-inflammatory cytokine IL-10 (91). It was thus proposed that in mice responsive to type I IFNs, the uptake of apoptotic cells by macrophages triggers their production of IL-10, which in turn inhibits host resistance (91). Myeloid cells are also sensitive to apoptosis in response to type I IFN and IFNAR expression has also been correlated with increased apoptosis of splenic and pulmonary macrophages during *L. monocytogenes* and pulmonary *C. muridarum* infections, respectively (54, 76). As mentioned above, these type I IFNs increase expression of the pro-apoptotic factor TRAIL during *L. monocytogenes* infection (54). Similar to IFNAR1$^{-/-}$ mice, mice lacking TRAIL demonstrate reduced TUNEL staining and increased resistance during *L. monocytogenes* infection (95). These effects were further correlated with increased numbers of splenic lymphocytes and monocytes. Type I IFN production induced by LCMV infection also correlates with granulocyte apoptosis and impaired control of *L. monocytogenes* infection (81). Thus, there is a clear association between type I IFNs, cellular death, and impaired myeloid cell responses during bacterial infections. Nonetheless, it remains

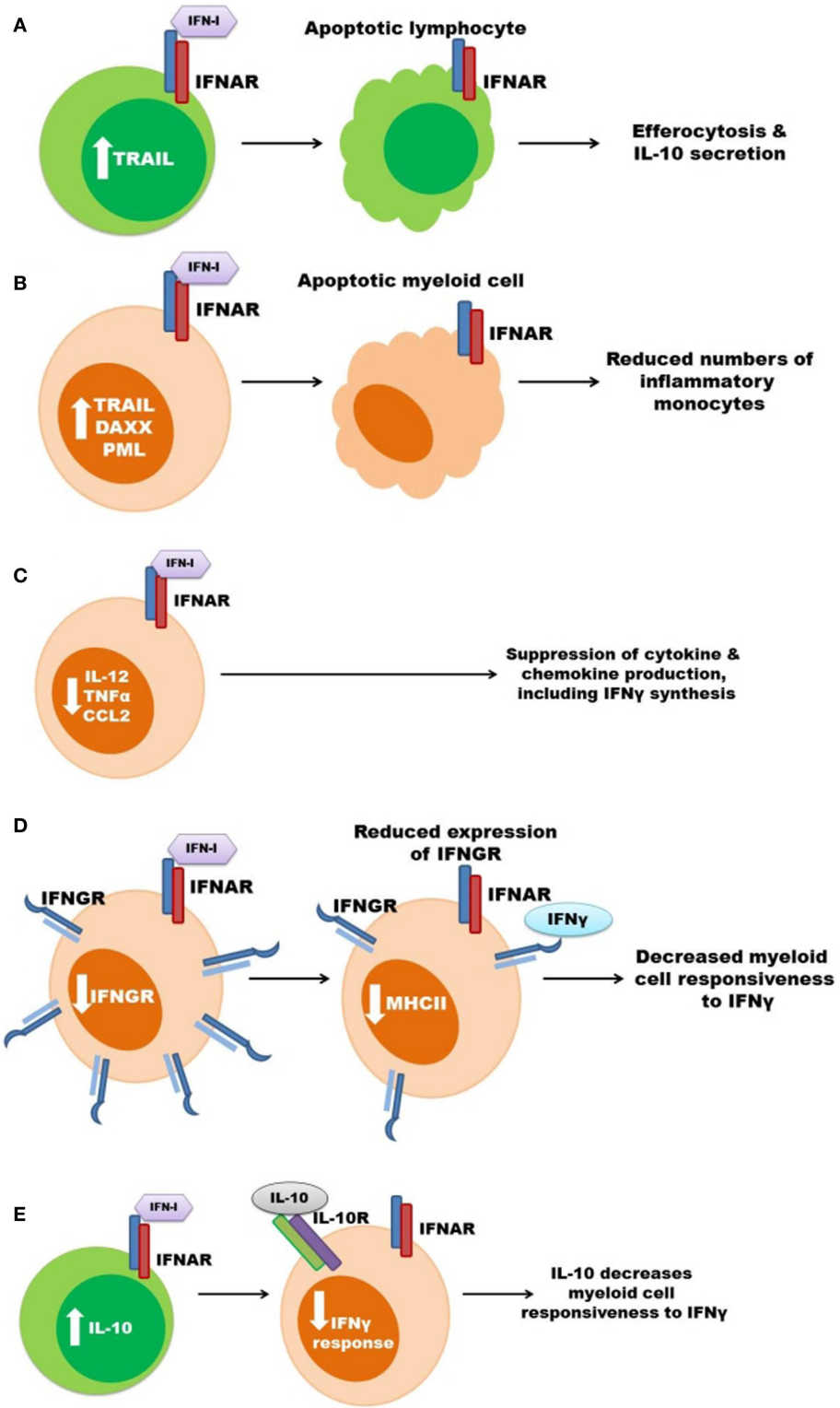

FIGURE 2 | Some mechanisms previously proposed to account for the pro-bacterial effects of type I IFNs. **(A)** Type I IFNs up regulate pro-apoptotic genes resulting in lymphocyte (green) apoptosis. Apoptotic lymphocytes stimulate myeloid cell IL-10 secretion. **(B)** Increased apoptosis of myeloid cells (orange) leads to reduced amounts of inflammatory monocytes during infection. **(C)** Signaling through the IFNAR suppresses myeloid cell secretion of pro-inflammatory cytokines and chemokines, which can result in decreased IFNγ production. **(D)** Expression of IFNGR is suppressed in response to type I IFN signaling in myeloid cells thus decreasing cellular responsiveness to IFNγ. **(E)** Type I IFNs induce the production of IL-10 to inhibit IFNγ responsiveness.

unclear whether apoptosis of T or myeloid cells is a primary cause of the increased host susceptibility.

SUPPRESSION OF PRO-INFLAMMATORY CYTOKINE AND CHEMOKINE PRODUCTION

Type I IFN production during viral infection is known to suppress production of IL-12 and other pro-inflammatory cytokines (96). Similarly, type I IFN production was associated with reduced secretion of IL-12 and TNFα in both *L. monocytogenes* and *M. tuberculosis* infection models (68, 72). Type I IFNs also suppress IL-1β production by inhibiting inflammasome activation (97), and reduced IL-1β secretion correlated with increased host susceptibility in *M. tuberculosis* infection models (98, 99). Expression of chemokines such as CCL2 is also regulated by type I IFNs (100, 101). CCL2 and its chemokine receptor CCR2 are critical for migration of inflammatory monocytes to sites of infection by *L. monocytogenes* and other bacteria (102–104). Spleens of IFNAR1$^{-/-}$ mice have increased accumulation of inflammatory monocytes during *L. monocytogenes* infection (68), however, type I IFNs upregulate CCL2 and recruitment of monocytes into the lung during *M. tuberculosis* infection (80). In the latter study, accumulation of monocytes correlated with more severe infection. By contrast, type I IFNs were reported to impair production of CXCL1, CXCL2, and neutrophil accumulation in lungs and more severe infection in mice infected with *S. pneumoniae* (85). Moderately, impaired neutrophil recruitment was also correlated with reduced IL-17 production and increased disease severity in mice in response to type I IFNs during *F. tularensis* and *L. monocytogenes* infections (71). However, it is debated whether or not neutrophils are protective during infections by *L. monocytogenes* and other intracellular bacteria (105, 106). Moreover, the neutropenia seen in patients treated with type I IFNs fails to correlate with their susceptibility to bacterial infections (86, 87). Thus, type I IFNs can alter production of cytokines and chemokines involved in neutrophil or inflammatory macrophage recruitment.

SUPPRESSION OF MYELOID CELL RESPONSIVENESS TO IFNγ

IFNγ is critical for the pro-inflammatory anti-microbial (M1) type activation of macrophages and transgenic mice lacking responsiveness to IFNγ selectively in myeloid cells are highly susceptible to *L. monocytogenes* and other intracellular pathogens (12, 13, 107). The macrophages activated by IFNγ have increased expression of molecules involved in both MHC class I and MHC class II antigen presentation, as well as enzymes producing reactive oxygen and nitrogen species with potential anti-microbial functions and pro-inflammatory cytokines and chemokines, including IL-12 (19). Expression of some, but not all, of these genes can also be induced when macrophages are stimulated with type I IFNs. In contrast, only IFNγ stimulates macrophages to express or upregulate MHC class II molecules (108). Indeed, stimulation of macrophages with type I IFNs suppresses their induction of MHC II expression in response to IFNγ. As mentioned above, type I IFNs also suppress production of IL-12 and CXCL1 and 2 (85, 96). These data suggest that type I IFNs are able to prevent or dampen classical M1-type anti-microbial macrophage activation in response to IFNγ. Consistent with this interpretation, a recent report revealed an inverse correlation between IFNβ and IFNγ gene expression patterns in lesions of human leprosy patients (90). The IFNβ-driven response also correlated with IL-10 production, and IFNβ production contributed to IL-10 secretion, leading the authors to conclude that the impaired IFNγ responses in *M. leprae* infected macrophages is due to IL-10 production (90). Indeed, IFNβ and IL-10 treatments both impaired the ability of IFNγ to induce expression of the vitamin D receptor, the vitamin D-1a-hydroxylase, and the anti-microbial peptides cathelicidin and DEFB4 in macrophages (90).

Type I IFNs were also associated with the induction of IL-10 secretion and Programed death-ligand 1 (PD-L1) expression by myeloid cells during chronic LCMV infection (109, 110). Experiments using antibody blockade of IFNAR showed reduced expression of these immune suppressive factors and increased clearance of persistent viral infections (109, 110). Interestingly, the blockade of IFNAR also suppressed production of IL-1β and IL-18, arguing against the notion that improved viral clearance was due to increased inflammasome activation. Rather, the improved viral clearance was associated with increased serum IFNγ and blockade of IFNAR failed to improve viral clearance in mice treated with antibody to block IFNγ (109, 110). The therapeutic effects of blocking type I IFN signaling also correlated with an improved ratio of stimulatory versus immune regulatory antigen-presenting cells (APCs) and enhanced antiviral T cell responses. Although the authors of one study further suggested that the suppression of inflammatory and immune responses in these studies reflected chronic type I IFN signaling (109), a second study observed that type I IFNs increased IL-10 secretion and PD-L1 expression by DCs as early as 1 day post infection (110).

Leading up to these recent studies, prior efforts had also demonstrated suppressive effects of type I IFNs on myeloid cell activation during systemic *L. monocytogenes* infection of mice (70). In this model, the suppressive effects of type I IFNs correlate with reductions in myeloid cell surface IFNGR1. Similarly, surface IFNGR1 staining is significantly reduced on myeloid cells from *M. tuberculosis* infected patients compared to healthy control and effective treatment of these patients correlates with restored myeloid surface expression of IFNGR1 (111). IFNAR expression is necessary and recombinant type I IFNs are sufficient to trigger IFNGR1 down regulation in mouse and human myeloid, but not T cells (70, 112), suggesting this mechanism might contribute to a selective inhibition of myeloid cell responsiveness to IFNγ. Indeed, the reduced expression of IFNGR1 correlates with decreased responsiveness to IFNγ as indicated by reduced STAT1 phosphorylation and impaired induction of MHC class II expression in the context of *L. monocytogenes*, *M. tuberculosis*, and *F. novicida* infections (70, 111, 113).

Additional mechanistic studies have revealed that type I IFNs suppress myeloid cell surface IFNGR1 within hours of stimulating the IFNAR and that this effect is due to transcriptional silencing of the otherwise constitutively expressed *ifngr1* gene (70, 111, 112). The rapid reductions in *ifngr1* transcript abundance following IFNβ stimulation are preceded by loss of activated RNA polymerase II at the *ifngr1* transcriptional start site and the accumulation of epigenetic marks on nearby histones that are indicative of condensed chromatin (112). The reduction in *ifngr1* transcription is also associated with recruitment of the early growth response 3 (Egr3) transcription factor shortly after IFNβ treatment (112).

Egr3 is a member of the Egr family of zinc finger transcription factors originally defined for their role in regulation of cell growth and differentiation (114, 115). Egr3 can act as an activator or repressor in response to various stimuli, depending on post-translational modifications and association with various adapter proteins (114–117). One such adaptor protein, the NGFI-A binding protein Nab1 is a known corepressor and is also recruited to the *ifngr1* promoter shortly after Egr3 (112). Knockdown of Nab1, but not Nab2, prevented IFNGR1 down regulation in macrophages treated with IFNβ, suggesting that recruitment of a repressive Egr3/Nab1 complex is responsible for rapid silencing of *ifngr1* transcription (112). Given that the half-life of IFNGR1 protein is estimated at 3–4 h (118), such transcriptional silencing is sufficient to rapidly reduce myeloid cell responsiveness to IFNγ. Nonetheless, type I IFN stimulation does not appear to cause a complete loss of myeloid cell surface IFNGR1, possibly due to the induction of SOCS proteins and other endogenous negative feedback circuits that attenuate cellular responses to IFNAR signaling. These results suggest that down regulation of IFNGR1 expression might be an early step in the cascade of events leading to the suppression of myeloid cell responses that result in increased bacterial burdens and disease severity during acute and chronic bacterial infections, and the establishment or maintenance of chronic viral infections.

COMMON FEATURES OF PROPOSED MECHANISMS

Given the numerous effects of type I IFNs on various cells of the immune system and on non-immune cells, it is plausible that their pro-pathogen effects vary for different pathogens. This seems particularly likely for pathogens infect different tissue or cell types, where the responses to type I IFNs may differ. For instance, a recent study demonstrated that IFNAR expression on non-hematopoietic cells was required to increase host susceptibility to the intracellular bacterial pathogen *Ixodes ovatus Ehrlichia* (119). The effects of these cytokines may also differ depending on the route of infection and the presence or nature of competing commensal microbes. For example, Kerbauer et al. suggested that type I IFNs might not be as detrimental to the host following gastric infection of mice with *L. monocytogenes* (120). Regardless, results from the studies highlighted above clearly implicate myeloid cells/APCs as key targets of type I IFNs in settings where these cytokines are deleterious to the host. Precisely, how these cytokines act to dampen myeloid cell immunity and what selective advantage this confers on the host remains to be discerned.

APPOSING EFFECTS OF TYPE I IFNs IN AUTOIMMUNE DISEASES

The role of type I IFN signaling during autoimmune disease remains controversial, possibly indicating that these cytokines have opposing effects in different disease settings. For example, chronic type I IFN production is a hallmark of systemic lupus erythematosus (SLE) and several groups have reported a subset of ISGs upregulated in SLE patients compared to healthy controls (121–124). ISG signatures were also associated with disease severity and progression in SLE patients (121–123). It has thus been suggested that type I IFNs promote SLE pathology through the activation of effector cells. Type I IFNs can paradoxically promote not just death of lymphocytes, but also T cell survival, proliferation, cytotoxicity, and B cell differentiation and antibody production. Any of these effects might conceivably contribute to increased tissue damage and disease progression in SLE patients.

In contrast to the exacerbation of SLE by type I IFNs, these same cytokines confer therapeutic benefits in certain other autoimmune diseases. The most obvious example of this is the neuroinflammatory disease MS. IFNβ is a common therapy and has been shown to reduce the frequency of clinical exacerbations in patients with relapse-remitting MS (125). The mechanisms for these beneficial effects remain uncertain. However, type I IFNs also suppress disease in the murine experimental autoimmune encephalomyelitis (EAE) model of MS. As for MS, IFNβ is therapeutic in the EAE model and deletion of the *ifnb* gene or IFNAR1 robustly increased EAE pathogenesis in mice (126, 127). Furthermore, using conditional knockouts, it was shown that IFNAR1 expression on myeloid cells was specifically required for the therapeutic effects of IFNβ during EAE (127). Deficiencies in IFNAR1 expression on myeloid cells also severely exacerbated disease and correlated with increased secretion of TNFα and CCL2 as well as increased expression of MHC II (127). These immunosuppressive effects of IFNβ treatment in humans may likewise target myeloid cells.

Other autoimmune diseases where type I IFNs appear to play a protective role include collagen type II induced arthritis in non-human primates (128). Treatment with double-stranded RNA species or recombinant IFNα also lowered the frequency and severity of arthritic symptoms in the murine model of antigen-induced arthritis (129). The pharmacokinetics of IFNβ therapies have shown to be a barrier is translating many of these treatments from animal models to clinical use in humans. However, Mullen et al. engineered a latent form of IFNβ that can only become activated when cleaved by aggrecanase (130). Aggrecanases are highly expressed within the joints and synovial fluid of rheumatoid arthritis and osteoarthritis patients and are responsible for the cleavage of aggrecan, an important component of joint tissue (130). This delivery method allows for temporal and tissue specific release of IFNβ that resulted in a significantly increased half-life of IFNβ as well as reduced pathology and joint swelling from collagen induced arthritis (130).

Humans with autoimmunity often carry a single nucleotide mutation in protein tyrosine phosphatase non-receptor type 22 (PTPN22) (131). PTPN22 is an intracellular protein tyrosine phosphatase that is exclusively found in immune cells (131), and was recently associated with TLR signaling for type I IFN synthesis in myeloid cells (132). Functional PTPN22 was also shown to suppress inflammatory arthritis and promote gut homeostasis (132). Mice deficient in PTPN22 demonstrate increased susceptibility in the dextran sodium sulfate (DSS) mouse model of acute colitis (132). TLR stimulation by microbiota also induced immunosuppressive effects that correlated with type I IFN production and decreased progression of experimental colitis in mice (133). Treatment with recombinant IFNβ phenocopied the decreased colitis achieved through TLR stimulation (133). Moreover, in a randomized placebo controlled study of active ulcerative colitis, a significant clinical response, and in some cases, disease remission, was seen in patients that received IFNβ therapy compared to the patients that received placebo (134, 135). It was noted that the therapeutic effects of IFNβ treatment in this disease

correlated with reduced production of IL-13, an effector cytokine driving intestinal inflammation (134). Mice deficient in type I IFN signaling have been shown to have exacerbated DSS-induced acute colitis (136, 137). Furthermore, mice with IFNAR1 deletion specifically in myeloid cells demonstrated significantly increased weight loss and colitis disease activity score when treated with DSS (136). These data suggest that type I IFN signaling specifically in myeloid cell is protective during DSS-induced acute colitis. Interestingly, the authors further showed that IFNAR1$^{-/-}$ mice recovered from DSS treatment more quickly than wild-type mice, suggesting a deleterious role of type I IFNs during the recovery phase of colitis (136).

CONCLUSION

Interferons are important mediators and regulators of the immune response to viruses, bacteria, and other pathogens. They can suppress inflammatory responses and exacerbate the pathogenesis in certain autoimmune diseases and several intracellular bacterial infections. Indeed, pathogens such as *L. monocytogenes* may actively promote type I IFN production through secretion of nucleic acids or cyclic-di-nucleotides that are recognized by cytosolic pattern recognition receptors to stimulate a type I IFN response. However, type I IFNs appear to be protective in certain other bacterial infections and in many viral infections, and may exacerbate the autoimmune disease SLE. Thus, blindly blocking their production as a therapy for bacterial infections would likely have severe untoward effects in these other disease settings. It thus remains an important challenge to dissect the mechanisms for the divergent pro- and anti-inflammatory effects of type I IFNs, as well as their paradoxical protective and deleterious effects during infectious and other diseases. As we review here, a number of observations have been correlated with the pro-bacterial effects of type I IFNs. However, while these observations have led to the proposal of several differing mechanisms to explain these detrimental effects of type I IFNs during intracellular bacterial infections, a common theme is the suppression of myeloid cell inflammatory responses. Whether such suppression results from the induction of effector cell death and IL-10 production, suppression of T cell cytokine or chemokine production, suppression of inflammasomes, or down modulation of IFNGR expression remains to be seen. However, even in the absence of experimental proof that points to a specific mechanism, it is attractive to speculate that the deleterious effects of type I IFN signaling during bacterial infections are tolerated because their ability to suppress myeloid cell responses also has a beneficial effect in protecting the host from MS and other autoimmune diseases.

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Bacterial programming of host responses: coordination between type I interferon and cell death

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During mammalian infection, bacteria induce cell death from an extracellular or intracellular niche that can protect or hurt the host. Data is accumulating that associate type I interferon (IFN) signaling activated by intracellular bacteria with programmed death of immune effector cells and enhanced virulence. Multiple pathways leading to IFN-dependent host cell death have been described, and in some cases it is becoming clear how these mechanisms contribute to virulence. Yet common mechanisms of IFN-enhanced bacterial pathogenesis are not obvious and no specific interferon stimulated genes have yet been identified that cause sensitivity to pathogen-induced cell death. In this review, we will summarize some bacterial infections caused by facultative intracellular pathogens and what is known about how type I IFN signaling may promote the replication of extracellular bacteria rather than stimulate protection. Each of these pathogens can survive phagocytosis but their intracellular life cycles are very different, they express distinct virulence factors and trigger different pathways of immune activation and crosstalk. These differences likely lead to widely varying amounts of type I IFN expression and a different inflammatory environment, but these may not be important to the pathologic effects on the host. Instead, each pathogen induces programmed cell death of key immune cells that have been sensitized by the activation of the type I IFN response. We will discuss how IFN-dependent host cell death may increase host susceptibility and try to understand common pathways of pathogenesis that lead to IFN-enhanced bacterial virulence.

Keywords: ***Yersinia*, plague, *Francisella, Salmonella, Listeria*, type I interferon, cell death, bacterial infection**

INTRODUCTION

Type I interferon (IFN) is a major component of the mammalian innate immune system, especially important for defense against viral infection (Stark et al., 1998). Nearly all cells in the body express the type I IFN receptor (IFNAR), making this potent anti-viral response capable of protecting every type of cell. Against bacterial infection, type I IFN can activate inflammatory responses that protect the host, but can also lead to hyper-inflammatory responses and programmed cell death which can hurt the host (Decker et al., 2005). In addition, type I IFN induced during viral infection can lead to increased apoptosis of granulocytes which can prevent clearance of a super-infection caused by Gram-positive or Gram-negative bacterial pathogens (Navarini et al., 2006).

Interferon-β is typically induced following detection of pathogen associated molecular patterns (PAMPs) by membrane-bound or cytoplasmic pattern recognition receptors (PRRs; Takeuchi and Akira, 2010). Expression of type I IFN is regulated at the transcriptional level, with binding sites for multiple activators in *Ifn* promoters. Membrane or cytoplasmic PRRs in the host cell signal through adaptor proteins to activate interferon regulatory transcription factors (IRFs), such as IRF-1, 3, 5, or 7. Phosphorylated IRF migrates to the nucleus, and cooperates with NF-κB and other co-activators to form an enhanceosome that binds the *Ifn*β promoter and activates transcription (Panne et al., 2007). Secreted IFN-β binds to IFNAR which results in the activation of the JAK-STAT pathway leading to the formation of the interferon-stimulated gene factor 3 (ISGF3) complex (Ivashkiv and Donlin, 2014). This complex translocates to the nucleus and can initiate the transcription of interferon-stimulated genes (ISGs) via their 5′ enhancer elements known as Interferon Stimulated Response Elements (ISREs). These ISGs encode *Ifn*β, pro- and anti-inflammatory cytokines, activators or inhibitors of programmed cell death, and numerous anti-viral proteins (Sato et al., 1998; de Veer et al., 2001; Schoggins and Rice, 2011).

Nucleic acids, secondary messengers, cell wall, or membrane fragments from bacteria activate expression of IFN-β following its detection by phagosomal or cytoplasmic PRRs (Takeuchi and Akira, 2010; Woodward et al., 2010; Jin et al., 2011a; Parvatiyar et al., 2012). Many pathogenic bacteria survive phagocytosis and may even grow in the intracellular compartment. When intracellular PRRs are activated, a downstream type I IFN response may include increased pro-inflammatory cytokine expression, down-regulation of cytokine receptors, or the sensitizing of key immune cells to undergo programmed cell death. Increasing evidence associates IFN-dependent host cell death during bacterial infection with increased susceptibility to disease. It is clear that the factors that determine the outcome of IFN signaling are complex and influenced by cell-and tissue-specific

host-pathogen interactions. In this review, we will discuss type I IFN-dependent sensitization of immune cells to programmed cell death during bacterial infection, the host-pathogen interactions that might enhance this outcome and how it might contribute to disease.

IFN-DEPENDENT DEPLETION OF IMMUNE EFFECTOR CELLS CRIPPLES HOST DEFENSE

Yersinia pestis is a recently evolved vector borne pathogen that causes the lethal diseases bubonic, septicemic, and pneumonic plague (Pollitzer, 1954). All three forms lead to systemic disease and after the infection eliminates virtually all of the phagocytic cells, extracellular bacterial growth is uncontrolled (Heine et al., 2013). When mammals, including humans, inhale *Y. pestis* aerosols, primary pneumonic plague develops in a short period, resulting in a deadly bronchopneumonia that becomes untreatable shortly after symptoms present (Butler, 2013). Neutrophil recruitment and function is critical for host defense against *Y. pestis* infection as well as antibody-mediated protection (Laws et al., 2010; Eisele et al., 2011).

Evasion of the innate immune system by *Y. pestis* is driven by two dominant virulence mechanisms: tetraacylated LPS and a type 3 secretion system (T3SS). Thermal control of acetylases causes hypoacetylation of lipid A at the mammalian body temperature resulting in predominantly the tetraacylated form during infection (Kawahara et al., 2002; Rebeil et al., 2006). Tetraacylated LPS does not stimulate toll-like receptor 4 (TLR-4) and may have anti-inflammatory properties that limit the activation of immune cells by extracellular bacteria (Montminy et al., 2006; Valdimer et al., 2012).

Upon intimate contact with a host cell, the T3SS spans the inner and outer membranes and a translocation pore is formed in the host cell plasma membrane but detection of this pore by the host inflammasome is blocked by the bacterial protein YopK (Cornelis, 2006; Brodsky et al., 2010). YopK is one of seven effector proteins of the T3SS, collectively referred to as *Yersinia* Outer Proteins (Yops), that are transported into the cytoplasm of the host cell where their combined action disrupts signaling pathways, reduces phagocytosis, halts the expression of pro-inflammatory cytokines, and induces programmed cell death through multiple mechanisms (Raymond et al., 2013). This action stalls the inflammatory response, creating an anti-inflammatory environment that is permissive for bacterial growth (Price et al., 2012). Substantial evidence suggests that extracellular bacteria preferentially target macrophages and neutrophils and cause their depletion as the infection progresses (Marketon et al., 2005; Maldonado-Arocho et al., 2013; Pechous et al., 2013).

YopJ is a T3SS effector protein with deubiquitinase and acetylase activity that prevents activation of NF-κB, MAP kinase kinase, and IRF-3, as well as other proteins in the host causing suppression of pro-inflammatory cytokine expression (Monack et al., 1997; Palmer et al., 1998; Orth et al., 1999; Zhou et al., 2005; Sweet et al., 2007). Suppression of NF-κB and activation of RIP1 by YopJ leads to the initiation of apoptosis and pyroptosis, respectively (Monack et al., 1997; Zheng et al., 2011; Philip et al., 2014). The amount of YopJ injected, host cell type and the action of other Yops such as YopK influence the amount of cell death that is caused by YopJ and, although it appears that evolution is favoring reduced secretion of YopJ, this protein and its proper regulation are important to *Yersinia* virulence (Holmstrom et al., 1995; Lemaitre et al., 2006; Brodsky and Medzhitov, 2008; Zauberman et al., 2009; Brodsky et al., 2010; Peters et al., 2013).

The combined evasion provided by YopJ and the tetraacylated lipid A leads to immune suppression that delays neutrophil recruitment allowing for establishment of bacterial colonies in susceptible tissues. Type I IFN expression can be detected early during infection when other pro-inflammatory cytokines are suppressed (Patel et al., 2012). Nevertheless, the absence of type I IFN signaling does not alter the expression of pro-inflammatory cytokines including those harboring ISREs. Overall the data suggest that the immune suppressive environment established during the early stages of *Y. pestis* infection does not prevent expression of IFN-β but nevertheless, at least some ISGs are suppressed.

If a macrophage succeeds in taking up *Y. pestis* before it is injected by the T3SS, the bacteria can remain viable inside a membrane-enclosed compartment known as the *Yersinia* containing vacuole (YCV) where the T3SS functions poorly (Zhang et al., 2011). Intracellular survival requires bacterial stress response pathways which presumably allow the bacteria to adjust to an adverse, nutrient-limiting environment (Oyston et al., 2000; Grabenstein et al., 2004). Intracellular *Y. pestis* eventually lyse the cell and once extracellular, the bacteria appear to have acquired increased resistance to phagocytosis and killing by neutrophils (Ke et al., 2013). *Y. pestis* mutants that are unable to survive in activated macrophages were less virulent in murine plague models suggesting the intracellular life cycle is a biologically relevant process that contributes to the success of infection (Oyston et al., 2000).

Host pathogen interactions that occur as a result of the intracellular life cycle of *Yersinia* are largely uncharacterized. Although extracellular bacteria effectively suppress the expression of pro-inflammatory cytokines, the host likely detects intracellular *Y. pestis* where an abundance of PRRs can bind nucleic acids as well as surface located PAMPs and, to date, no microbial species have been described that escape detection inside host cells. Recently, expression of the mitochondrial-located adaptor protein MAVS was identified as induced by *Y. pestis* infection of macrophages (Du et al., 2014). Mice lacking MAVS were more resistant to *Y. pestis* infection, suggesting that MAVS could play a role in inducing type I IFN. Together the data support the likelihood that one or more intracellular PRRs are activated by *Yersinia*, causing expression of IFN-β.

Pulmonary infection of mice by *Y. pestis* leads to neutropenia that becomes pronounced as the infection progresses (Patel et al., 2012). Mice lacking *Ifnar* were more resistant to lethal disease and this was associated with an increased population of neutrophils in the bone marrow and spleen without detectable changes to the inflammatory response. In contrast, $Ifnar^{+/+}$ mice had reduced populations of Gr-1^{+} neutrophils in both primary and secondary immune tissues that became more pronounced as the infection progressed. These observations suggest that neutrophil migration is not impacted by type I IFN but more likely it has a direct effect on maturation or viability of this effector

population (**Figure 1**). *In vitro*, T3SS^{+} *Yersinia* caused similar levels of cytotoxicity of WT and *Ifnar*$^{-/-}$ bone marrow derived macrophages after 5.5 h infection. While this does not rule out the possibility that *Yersinia* infection directly causes IFN-dependent cell death through another mechanism, the data are consistent with an IFN-dependent depletion of neutrophils, perhaps by sensitizing them to undergo cell death *in vivo*.

Interferon-dependent sensitization of immune cells to programmed cell death was among the initial observations of pathology conferred by type I interferon during bacterial infections in the well-characterized model system of another facultative intracellular pathogen *Listeria monocytogenes*. We now know the details of a number of IFN-dependent host responses to *L. monocytogenes*, a pathogen with multiple mechanisms for inducing programmed cell death. Detection of *Listeria* by TLR-2 leads to expression of pro-inflammatory cytokines (McCaffrey et al., 2004; Torres et al., 2004). *L. monocytogenes* can escape this response by invading phagocytic and non-phagocytic cells where it escapes from intracellular vacuoles and grows in the cytoplasm. Detection of nucleotide secreted by bacteria in the cytoplasm is signaled through the adaptor protein STING which leads to the phosphorylation of IRF-3 and expression of IFN-β (Burdette and Vance, 2013).

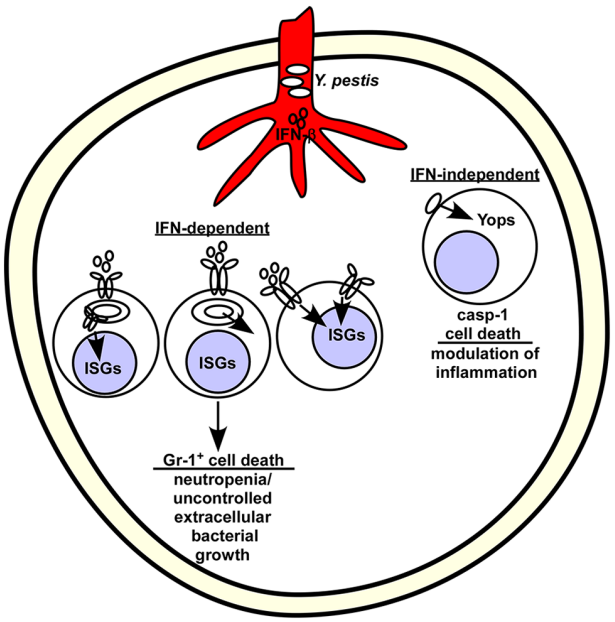

FIGURE 1 | Model for interferon (IFN)-β stimulated depletion of neutrophils in the bone marrow following *Yersinia pestis* infection. *Y. pestis* stimulate IFN-β production in the blood shortly after infection. Bacteria likely disseminate from the infection site through the vasculature, where they reach and colonize the bone marrow. Given their tropism for phagocytic cells, *Yersinia* might preferentially interact with Gr-1^{+} cells, which are typically mature neutrophils and monocytes. Extracellular bacteria use the type III secretion system to inject Yops and stimulate caspase-1-dependent, IFN-independent cell death. Intracellular bacteria may stimulate host pathways that combine with IFN to activate cell death (left) or secrete a protein that activates IFN-dependent cell death (middle). Alternatively, the inflammatory signals received by Gr-1^{+} cells may prevent their activation (not shown) or induce cell death (shown).

Multiple mechanisms are believed to contribute to the increased resistance of *Ifnar*$^{-/-}$ mice to *L. monocytogenes* infection (Auerbach et al., 2004; Carrero et al., 2004; O'Connell et al., 2004). IFN-dependent susceptibility to infection correlated with increased apoptosis of splenic T cells, not necessarily infected by *Listeria*, which resulted in a reduction of IFN-γ and an increase in IL-10 expression, both of which would suppress macrophage activation and bacterial killing. Further, *Il10*$^{-/-}$ mice were also more resistant to *L. monocytogenes* infection, supporting the model whereby IFN enhanced pathogenesis may be affected by changes in IL-10 (Carrero et al., 2006; Biswas et al., 2007). However, the mechanism whereby T cells become sensitized to apoptosis is not yet clear. Paradoxically, IFNAR signaling also causes up-regulation of CD69 in T cells which increased their sensitivity to antigenic stimulation during *Listeria* infection (Feng et al., 2005). Deletion of CD69 blocks protective immunity to *Listeria* even though *Cd69*$^{-/-}$ mice produced increased levels of IFN-β and this induced increased levels of T cell apoptosis (Vega-Ramos et al., 2010).

Listeriolysin O (LLO) is a toxin secreted by *L. monocytogenes* that can cause host cell death and is required for bacteria to escape the phagosome and induce type I IFN (Schnupf and Portnoy, 2007). IFN-β sensitizes macrophages to undergo LLO-mediated necrosis, lowering the amount of toxin required to cause cell death *in vitro* (Zwaferink et al., 2008). As macrophages are important for bacterial clearance, this mechanism likely also contributes to disease progression. Thus at least two key immune cells, T cells and macrophages, are sensitized by IFN-β signaling to induce programmed cell death thereby crippling host defense against *Listeria* infection. In addition, IFN-dependent down-regulation of the IFN-γ receptor also decreases activation of infected macrophages, and since IFN-γ is required for bacterial clearance, this likely also contributes to increased susceptibility (Rayamajhi et al., 2010). Overall multiple IFN-dependent changes, some of which involve programmed cell death, may contribute to increased susceptibility of mice to *Listeria* infection.

IFN-DEPENDENT MODULATION OF INFLAMMASOME ACTIVATION

Francisella tularensis causes tularemia, a disease that begins with a very low infectious dose entering via one of a number of routes including inhalation (Dennis et al., 2002; Foley and Nieto, 2010). Inhalation of aerosolized *F. tularensis* leads to bacterial evasion of inflammatory responses and efficient invasion of alveolar macrophages (Hall et al., 2008). *Francisella* escape the phagosome and replicate in the host cytosol, eventually lysing the macrophage. Extracellular bacteria replicate and disseminate systemically likely through the vasculature. Pneumonic tularemia manifests in humans as an interstitial pneumonia that can cause death due to systemic disease and multi-organ failure.

Evasion of innate immune responses by *Francisella* can be attributed to its invasion of host cells, combined with a non-canonical LPS and absence of flagella (Jones et al., 2011). Thus, even though extracellular bacteria are recognized by TLR-2, intracellular bacteria are only weakly immunostimulatory. Once inside macrophages, *Francisella* escape the phagosome and replicate in the cytoplasm, where they eventually cause host cell death. Type A

Francisella strains, including those that cause disease in humans, carry a duplicated copy of a 30 kb high pathogenicity island that encodes a type 6 secretion system (T6SS) which is required for virulence in the mouse model (Broms et al., 2010; Bröms et al., 2011; Long et al., 2012). Escape from the phagosome, replication in the cytoplasm and host cell death all depend on the T6SS (Lindgren et al., 2013). Like *Listeria*, *Francisella* escape from the phagosome occurs prior to lysosomal fusion and is detected by host PRRs in the cytoplasm which signal through STING to the IRF-3-dependent expression of type I IFN (Jones et al., 2010).

Francisella tularensis mutants that are unable to escape the phagosome or that survive poorly in the cytoplasm induce increased expression of IFN-β and increased cytotoxicity due to activation of pyroptosis in macrophages (Peng et al., 2011). Phagosomal escape of *F. tularensis* subspecies *novicida* (*F. novicida*), a type A strain that is virulent in mice but avirulent in humans, and type I IFN signaling activate the absent in melanoma-2 (AIM-2) inflammasome, which leads to cleavage of pro-caspase-1, secretion of IL-1β and host cell death (Henry et al., 2007). *F. novicida* mutants that fail to escape the phagosome were attenuated in the mouse model, suggesting that intracellular survival is necessary for virulence. Similarly, the absence of caspase-1, AIM-2 or the inflammasome adaptor protein ASC all individually caused increased susceptibility to *F. novicida* suggesting that the inflammasome contributes to host defense (Fernandes-Alnemri et al., 2010; Pierini et al., 2013). Paradoxically, IFNAR is required to activate caspase-1 during *F. novicida* infection *in vitro*, but *Ifnar*$^{-/-}$ mice were more resistant to pulmonary infection by this strain. This could be explained by type II IFN activation of the inflammasome *in vivo* which has been observed as a compensatory mechanism in the absence of type I IFN. Therefore, *in vivo*, *Ifnar*$^{-/-}$ mice are likely not deficient in activating the inflammasome during *F. novicida* infection.

Inflammasome activation by *Francisella* varies depending on cell type as well as bacterial strain which complicate interpretation of the *in vivo* data. Human dendritic cells, for example, induced much less caspase-1 dependent inflammasome activation when infected by *F. tularensis* SchuS4, a type A *Francisella* strain that is fully virulent in humans and mice (Bosio et al., 2007; Ireland et al., 2013). Similar to *Y. pestis* infection, SchuS4 suppresses activation of TLRs and the expression of pro-inflammatory cytokines, but is not able to prevent expression of IFN-β from human dendritic cells (Bauler et al., 2011). In addition, caspase-1 may not play a significant role in host defense against SchuS4 (Dotson et al., 2013). These data suggest that intracellular SchuS4 may escape host cells through a distinct, caspase-1-independent mechanism and the role of type I IFN during infection by this strain is not clear (Lindemann et al., 2011).

Like *Y. pestis*, virulence of *F. novicida* may be enhanced by IFN signaling, as *Ifnar*$^{-/-}$ mice were more resistant to pulmonary infection (Henry et al., 2010). Larger populations of IL-17A-producing γδT-cells were found in the spleens of infected *Ifnar*$^{-/-}$ mice and this correlated with increased neutrophil recruitment and survival. *In vitro*, IFN-β signaling caused a decrease in *F. novicida*-induced IL-17A expression by γδT-cells suggesting a direct effect of IFN-β on these cells. However, this effect may not extend to the virulent *Francisella* strain SchuS4 which is not only resistant to neutrophil-mediated killing but neutrophils may even contribute to disease caused by this strain (Bosio et al., 2007; Schwartz et al., 2012). Although the role of IFN-β during challenge of mice with *F. tularensis* SchuS4 has not yet been described, pulmonary challenge of *Il17Rα*$^{-/-}$ mice by *F. tularensis* SchuS4 did not result in increased survival suggesting IL-17A may not play an important role in this model (Skyberg et al., 2013). It will be interesting to see whether γδT-cells produce IFN-dependent IL-17A during infection by SchuS4 or if this strain induces an alternative response to type I IFN.

IFN-DEPENDENT ESCAPE FROM HOST CELLS

Salmonella enterica is a gastrointestinal pathogen with many serotypes that cause a range of diseases including the lethal typhoid fever (Santos, 2014). *S. enterica* infection begins as an interaction with intestinal M cells and enterocytes which take up bacteria from the small intestine. The bacteria survive in a modified phagosome, also called the *Salmonella* containing vacuole (SCV), and intracellular survival is essential for virulence in the murine and calf models (Libby et al., 1997; García-del Portillo, 2001). Intracellular bacteria eventually cause host cell death, allowing the bacteria access to an extracellular replicative niche where it can grow rapidly. Host cell death appears to be induced by multiple virulence factors that are exported from the SCVs via the type III secretion systems. Occasionally, *Salmonella* gains access to the vasculature and disseminates systemically, resulting in sepsis and multi-organ failure.

Salmonella express multiple PAMPs that are recognized by the host, including LPS and flagellin that strongly stimulate TLR-4 and NLRC4, respectively, and induce the expression of type I

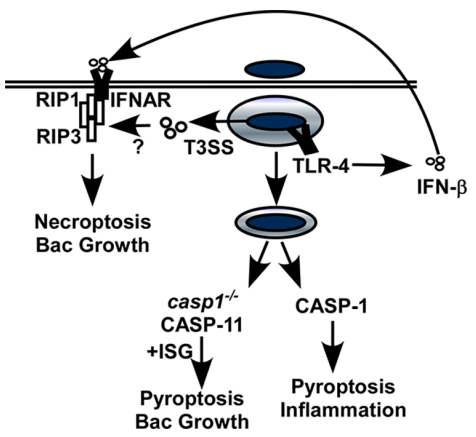

FIGURE 2 | Interferon-dependent and IFN-independent host cell death caused by *Salmonella*. Intracellular *Salmonella* remain in a vacuolar compartment where they undergo little, if any replication. The SPI-2 type III secretion system (T3SS) is required for intracellular survival, replication and host cell death. IFNAR promotes necroptosis by forming a complex with RIP1/RIP3, and may also activate the caspase-11 inflammasome under circumstances where caspase-1 is absent. These two pathways favor bacterial replication, presumably because they provide an escape mechanism for the intracellular bacteria. In contrast, IFNAR-independent activation of caspase-1 leads to pyroptosis and inflammation and contributes to clearance of extracellular bacteria.

Table 1 | Cell death and type I IFN during bacterial infection of macrophages.

Pathogen	PRR[a]/ adaptor	Cell death	Virulence factor[b]	IFNAR[c]	Reference
IFN-β signaling aids the pathogen					
Yersinia pestis					
Extracellular	None	Casp-3	T3SS	No	Zheng et al. (2011), Patel et al. (2012), Weng et al. (2014)
Intracellular	Unknown	RIP1/Casp-8 Casp-1 Necrosis	Unknown	Unknown	
Francisella tularensis					
Extracellular	TLR-2				
Intracellular	STING	Casp-1	T6SS	Yes	Henry et al. (2007), Henry et al. (2010), Jones et al. (2010)
Salmonella typhimurium					
Extracellular	TLR-4				
Intracellular	TLR-5				
	GBP	RIP1-RIP3	T3SS	Yes	Broz et al. (2010, 2012), Robinson et al. (2012)
	NLRP3	Casp-11			
	NLRC4				
Mycobacterium tuberculosis					
Intracellular	NOD2,	Apoptosis	T7SS	Yes	Chen et al. (2006), Pandey et al. (2009), Manzanillo et al. (2012), Repasy et al. (2013), Dorhoi et al. (2014) Koo et al. (2008), Mishra et al. (2010), Shah et al. (2013)
	STING	Necrosis Casp-1			
Staphylococcus aureus					
Extracellular	TLR-2	Necrosis	HlyA	No	Mariathasan et al. (2006), Martin et al. (2009), Parker and Prince (2012), Parker et al. (2014)
Intracellular	TLR-9	Casp-1		Unknown	
	NOD2				
Listeria monocytogenes					
Intracellular	NALP3	Casp-1	LLO	Yes	Auerbach et al. (2004), O'Connell et al. (2004), Mariathasan et al. (2006), Jin et al. (2011b)
	STING				
IFN-β signaling aids the host					
Legionella pneumophila					
	RIG-1	Casp-1		Unknown	Amer et al. (2006), Lightfield et al. (2008), Monroe et al. (2009), Nogueira et al. (2009), Plumlee et al. (2009), Lippmann et al. (2011)
	NLRC4				
GroupB Streptococcus					
	TLR-7	None reported		n/a	Mancuso et al. (2009), Parker and Prince (2012)
Streptococcus pneumoniae					
	STING	Necrosis Apoptosis	Ply	Unknown	Weigent et al. (1986), Colino and Snapper (2003), N'Guessan et al. (2005), Sutterwala et al. (2007), Mancuso et al. (2009), Parker et al. (2011)
Pseudomonas aeruginosa					
	TLR-4	Casp-3	T3SS	Unknown	Faure et al. (2004), Power et al. (2007), Sutterwala et al. (2007), Carrigan et al. (2010), Parker et al. (2012)
	NLRC4	Casp-1	Unknown		

[a] *PRR, pattern recognition receptor.*
[b] *Virulence factor that stimulates cell death.*
[c] *IFNAR = indicates if IFNAR is required for cell death.*

IFN (Wyant et al., 1999; Rosenberger et al., 2000). In addition, bacterial invasion of non-phagocytic cells results in the recognition of bacterial RNA by the cytosolic sensor RIG-I (Schmolke et al., 2014). Bacterial invasion of macrophages and epithelial cells requires the T3SS encoded on *Salmonella* Pathogenicity Island I (SPI-1; Fàbrega and Vilaa, 2013). Intracellular survival and replication require the T3SS encoded on SPI-2, which is also necessary for virulence in some models (Ochman et al., 1996; Libby et al., 1997; Winter et al., 2010). At least two SPI-2 effector proteins (SpvB and SseL) and one SPI-1 effector protein (SipB) are able to induce host cell death (Browne et al., 2002; Rytkonen et al., 2007). The cell death pathway induced by each of these proteins is distinct from one another, and each may provide an escape mechanism from the intracellular compartment.

Mice lacking *Ifnar* were more resistant to *Salmonella* infection with no detectable impact on the expression of pro-inflammatory cytokines (Robinson et al., 2012). Macrophages from *Ifnar*$^{-/-}$ mice were resistant to *Salmonella*-induced cell death compared to macrophages from WT mice suggesting IFN signaling may activate host cell death (**Figure 2**). IFN-β induced an interaction between IFNAR and RIP1 in infected cells which promoted RIP1/RIP3-dependent necroptosis. Like *Ifnar*$^{-/-}$ mice, *Rip3*$^{-/-}$ mice showed improved clearance of *Salmonella* infection suggesting this mechanism contributes to IFN-dependent pathogenesis. While these data provide a direct link between type I IFN, necroptosis and pathogenesis, there are additional mechanisms that are impacted by IFNAR during *Salmonella* infection.

Infection of stationary phase *Salmonella* leads to host cell death *in vitro* through a distinct mechanism involving the SPI-2 T3SS. Through this pathway, *Salmonella* induce the activation of the inflammasome which is enhanced by TLR-4-dependent type I IFN expression (Broz et al., 2012). Yet mice lacking inflammasome caspases 1 and 11 were more sensitive to infection suggesting that inflammasome activation is necessary for host defense against *Salmonella*. This apparent paradox is similar to the observations in the *Francisella* model and therefore may be explained by redundant activation of the inflammasome *in vivo* by type II IFN. Alternatively, specific cells or tissues may mediate susceptibility phenotypes or residual caspase-1 activation in the *Ifnar*$^{-/-}$ mice is sufficient for host defense.

Casp1$^{-/-}$ mice, which express caspase-11 and can activate the inflammasome, were more sensitive to infection than those lacking both caspases that were unable to induce inflammasome activation (Broz et al., 2012). This suggests that caspase-1 and caspase-11 contribute independent rather than redundant functions during infection and that caspase-11-induced cell death may increase disease susceptibility when caspase-1 is absent. Increased resistance of *casp1*$^{+/+}$/*casp11*$^{-/-}$ mice to multiple pathogens has been reported, including *Francisella* and there appears to be a connection with type I IFN signaling and caspase-11-mediated host pathology (Schroder and Tschopp, 2010). Activation of caspase-11 by *Salmonella*-infected macrophages was shown to be dependent on IFNAR, with a role for transcriptional activation of expression pro-caspase-11 as well as an additional function that is not well understood. Overall, these data suggest a second functional link between type I IFN and host cell death through the activation of pyroptosis during *Salmonella* infection. Together, it is clear that even for a single bacterial pathogen, type I IFN sensitizes cells through multiple mechanisms to induce programmed cell death. Escape from the host cell without a protective inflammatory response gives *Salmonella* the opportunity to grow rapidly and disseminate where it can cause severe disease.

CONCLUDING REMARKS

Studies of IFN-dependent host-pathogen interactions that lead to host cell death have been a focus for the last 10 years of research in bacterial pathogenesis, beginning with the initial observations in the *Listeria* model (**Table 1**). Bacterial secretion systems, often encoded within shared high pathogenicity islands, commonly induce type I IFN expression presumably because the secretion pore and/or the effector proteins are detected by cytoplasmic PRRs. The list of bacterial infections that benefit from IFN-β signaling out-numbers those that are protected by it. Strikingly, the pathogens that benefit from IFN-β signaling are all facultative intracellular bacteria.

Current sequencing technologies have revealed nearly 3,500 genes that are responsive to IFN-β signaling, including transcription factors and regulators of programmed cell death (de Weerd et al., 2013). Confounding the ability to define ISGs that confer IFN-enhanced susceptibility to infection is the need to identify critical cells whose IFN-dependent response directly contributes to disease. Type I IFN expression specifically in myeloid cells has recently been shown to be critical to clearance of viral infection (Pinto et al., 2014). With the availability of mouse strains that restrict IFNAR expression to specific cells or tissues, it will be possible to study these issues in the bacterial infection models.

Multiple mechanisms of IFN-induced programmed cell death may contribute to bacterial infection in mouse models. There is beginning to be evidence that IFN-enhanced pathogenesis may also occur in humans. For example, virulence of *Staphylococcus aureus* isolated from human patients appears to correlate with increased production of type I IFN (Parker et al., 2014). Given the growing population of immunocompromised people and the confounding effects of co-infections often present in humans, particularly in hospitals, type I IFN may not always be a safe anti-viral treatment option in spite of its undisputed ability to stimulate clearance of viral infections. As we gain in our understanding of how IFN signaling combines with bacterial virulence factors to enhance disease, it may be possible to stimulate the anti-viral effects of type I IFN without placing the patient at risk for bacterial diseases.

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Harnessing mechanistic knowledge on beneficial versus deleterious IFN-I effects to design innovative immunotherapies targeting cytokine activity to specific cell types

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Type I interferons (IFN-I) were identified over 50 years ago as cytokines critical for host defense against viral infections. IFN-I promote anti-viral defense through two main mechanisms. First, IFN-I directly reinforce or induce *de novo* in potentially all cells the expression of effector molecules of intrinsic anti-viral immunity. Second, IFN-I orchestrate innate and adaptive anti-viral immunity. However, IFN-I responses can be deleterious for the host in a number of circumstances, including secondary bacterial or fungal infections, several autoimmune diseases, and, paradoxically, certain chronic viral infections. We will review the proposed nature of protective versus deleterious IFN-I responses in selected diseases. Emphasis will be put on the potentially deleterious functions of IFN-I in human immunodeficiency virus type 1 (HIV-1) infection, and on the respective roles of IFN-I and IFN-III in promoting resolution of hepatitis C virus (HCV) infection. We will then discuss how the balance between beneficial versus deleterious IFN-I responses is modulated by several key parameters including (i) the subtypes and dose of IFN-I produced, (ii) the cell types affected by IFN-I, and (iii) the source and timing of IFN-I production. Finally, we will speculate how integration of this knowledge combined with advanced biochemical manipulation of the activity of the cytokines should allow designing innovative immunotherapeutic treatments in patients. Specifically, we will discuss how induction or blockade of specific IFN-I responses in targeted cell types could promote the beneficial functions of IFN-I and/or dampen their deleterious effects, in a manner adapted to each disease.

Keywords: type I interferons, dendritic cells, chronic viral infections, immunotherapy, bioengineering

INTRODUCTION

Type I interferons (IFN-I) were the first cytokines discovered, over 50 years ago, based on their potent anti-viral effects (1, 2). IFN-I play a crucial, non-redundant role in vertebrate anti-viral defenses (3–5). IFN-I also mediate protective effects in other physiopathological contexts, including cancer (6) and multiple sclerosis (MS) (7). On the contrary, IFN-I responses can be deleterious in a number of circumstances, including bacterial or fungal infections (8–10), many autoimmune diseases (11), and, paradoxically, certain chronic viral infections (12–14). It is only recently that an integrated picture has emerged of the cellular and molecular mechanisms regulating the production of IFN-I and underlying their functions. Much knowledge was gained recently on another class of potent innate anti-viral interferons, the lambda, or type III IFNs (IFN-III). We will review knowledge on IFN-I/III (IFNs) and discuss how it could be harnessed to develop innovative therapeutic strategies aimed at surgically tuning IFN activity toward protective responses in a manner adapted to each disease. We will focus on IFN-α/β/λ because they are the best characterized IFNs and already used therapeutically. Recent reviews are covering information on other IFN-I subsets including IFN-ε, which is produced at mucosal sites and promotes local anti-viral defenses (15, 16).

Dendritic cells (DCs) are rare heterogeneous mononuclear phagocytes functionally characterized by their unique efficacy for antigen-specific activation of naïve T lymphocytes. DCs are sentinel cells of the immune system, able to sense and integrate a variety of danger input signals for delivery of output signals instructing the activation and functional polarization of effector immune cells. In mammals, five major DC subsets exist, which differ in their expression of innate immune recognition receptors (I_2R_2s) and in their functional specialization: monocyte-derived DCs (MoDCs), Langerhans cells, $CD11b^+$ DCs, $XCR1^+$ DCs, and plasmacytoid DCs (pDCs) (17). A recurrent theme of this review will be the intricate relationships between IFNs and DCs, since these cells can be a major source and/or target of these cytokines under various conditions.

The first section will synthesize current knowledge on IFN production and protective anti-viral functions. The I_2R_2s and downstream signaling pathways responsible for IFN-I production during viral infection will be listed. The roles of different cell types for this function will be discussed. The two main mechanisms through which IFN-I promote anti-viral defense will be reviewed, succinctly for direct anti-viral effects and in greater details for immunoregulatory functions.

The second section will focus on the detrimental functions of IFN-I. Selected diseases will be discussed to illustrate how different, and sometimes opposite, processes underlie deleterious IFN-I responses depending on the physiopathological contexts. IFN-I induction of unbridled inflammatory responses causing lethal tissue damage will be discussed as a major pathological mechanism during bacterial encounters secondary to influenza infection or in some autoimmune diseases. Inappropriate functional polarization of immune responses by IFN-I will be discussed as one potential cause for enhanced susceptibility to bacterial or fungal infections. The complex and disputed role of IFN-I in chronic viral infections will be reviewed, with emphasis on the physiopathology of the infections by human immunodeficiency virus type 1 (HIV-1) and human hepatitis C virus (HCV), with an outlook for the development of novel immunotherapeutic strategies to combine with anti-viral drugs.

The third section will recapitulate how the balance between beneficial versus deleterious IFN-I responses is modulated by several key parameters including (i) the source and timing of IFN-I production, (ii) the cell types affected by IFN-I, and (iii) the signaling pathways activated by IFN-I.

In the last section, we will speculate how integration of all the knowledge discussed before combined with advanced biochemical manipulation of the activity of the cytokines should allow designing innovative immunotherapeutic treatments, based on induction or blockade of specific IFN-I responses in targeted cell types. This "activity-by-targeting" concept is based on the design of novel "immuno-IFNs" consisting in covalent association between a mutated IFN-I with decreased affinity for its receptor and an antibody with high avidity for a molecule specifically expressed on target cell types (18). This design ensures lack of activity of the immuno-IFNs on all cell types but those targeted, contrary to previous strategies using IFNs with close to maximal potency that were still able to mediate strong off-target effects despite their coupling to cell-type specific antibodies and/or their local delivery.

GENERAL CONCEPTS ON IFN PRODUCTION AND FUNCTIONS

HOW IS THE PRODUCTION OF IFN CONTROLLED?

Type I interferons expression is not detectable under steady state conditions *in vivo* using classical methods such as gene expression analysis by RT-PCR or protein titration by ELISA or bioassays. However, mice deficient for the expression of the alpha chain of the IFN-I receptor (IFNAR1) harbor alteration in the ontogeny or functions of various cell types (19–26). Hence, extremely small or localized but functionally relevant quantities of IFN-I must be produced under steady state conditions (27). Indeed, the existence of steady state responses to IFN-I in various organs *in vivo* was demonstrated by using reporter mice expressing the firefly luciferase under the control of the promoter of *Ifnb1* (28) or of *Mx2* (29), a canonical IFN-I-stimulated gene (ISG). Steady state IFN-I responses are promoted by gut commensals (30). Early and transiently after many viral infections, large amounts of IFNs can be detected, in blood and spleen in the case of systemic infections or locally in the case of confined infections. IFN induction during viral infections results from the detection of specific danger signals by specialized I_2R_2s. This includes the detection of pathogen-associated molecular patterns as well as the sensing of stress signals or damage-associated molecular patterns (31, 32). Based on the nature and intracellular location of the danger signals that induce the production of the cytokines, the cellular sources of IFNs during viral infection can be classified in two main groups. Infected cells often contribute to IFN production as a response to their sensing of endogenous viral replication, or consecutive to the metabolic stress induced during massive translation of viral structural proteins, or as a result of plasma membrane perturbations upon viral entry. Specific subsets of uninfected cells can also significantly contribute to IFN production upon engulfment of material containing viral-derived nucleotide sequences and sensing of these molecules in endosomes by specific I_2R_2s. All sensing pathways leading to IFN induction converge on the activation of interferon response factors 3 or 7 (IRF3/7), which are the master transcription factors inducing IFN genes. Most cell types constitutively express IRF3 but not IRF7 or only at low levels. IRF7 expression requires IFN-I stimulation. IFN-β can directly be induced by IRF3. All but one of the IFN-α subtypes require IRF7 for their induction. Hence, IFN-β secretion promotes its own production and that of IFN-α in an autocrine manner (33, 34). This positive feedback loop strongly amplifies IFN production during viral infections, promoting fast and widespread induction of cell-intrinsic anti-viral defenses in uninfected cells to prevent virus dissemination. Other feedback loops tightly regulate IFN-I production positively or negatively. This section reviews different mechanisms controlling IFN production and how they could play different roles in host/virus interactions.

IFN production in infected cells is initiated by sensing of endogenous viral replication

Plasma membrane modifications occur upon virus entry which can induce IFN-I production and ISGs through a STING-dependent signaling. Infected cells can sense abnormal changes in the physical or biochemical properties of their plasma membrane upon virus entry, which can trigger their production of IFN-I (35, 36). This event depends on signaling by the endoplasmic reticulum (ER) – resident transmembrane protein stimulator of interferon genes (STING). Upon virus entry, STING translocates to the cytosol where it is activated by phosphatidylinositol 3-kinase (PI3K) and calcium-dependent pathways to initiate a signaling cascade leading to IRF3-dependent induction of IFN-I and ISGs (**Figure 1**) (31, 37).

Viral nucleotide sequences are sensed by dedicated I_2R_2s in the cytosol of infected cells, which induces IFN-I production. Some I_2R_2s are located in the cytosol and bind viral nucleotide sequences to induce IFN-I production in infected cells. These I_2R_2s are classified as cytosolic RNA or DNA sensors. Their specificity for

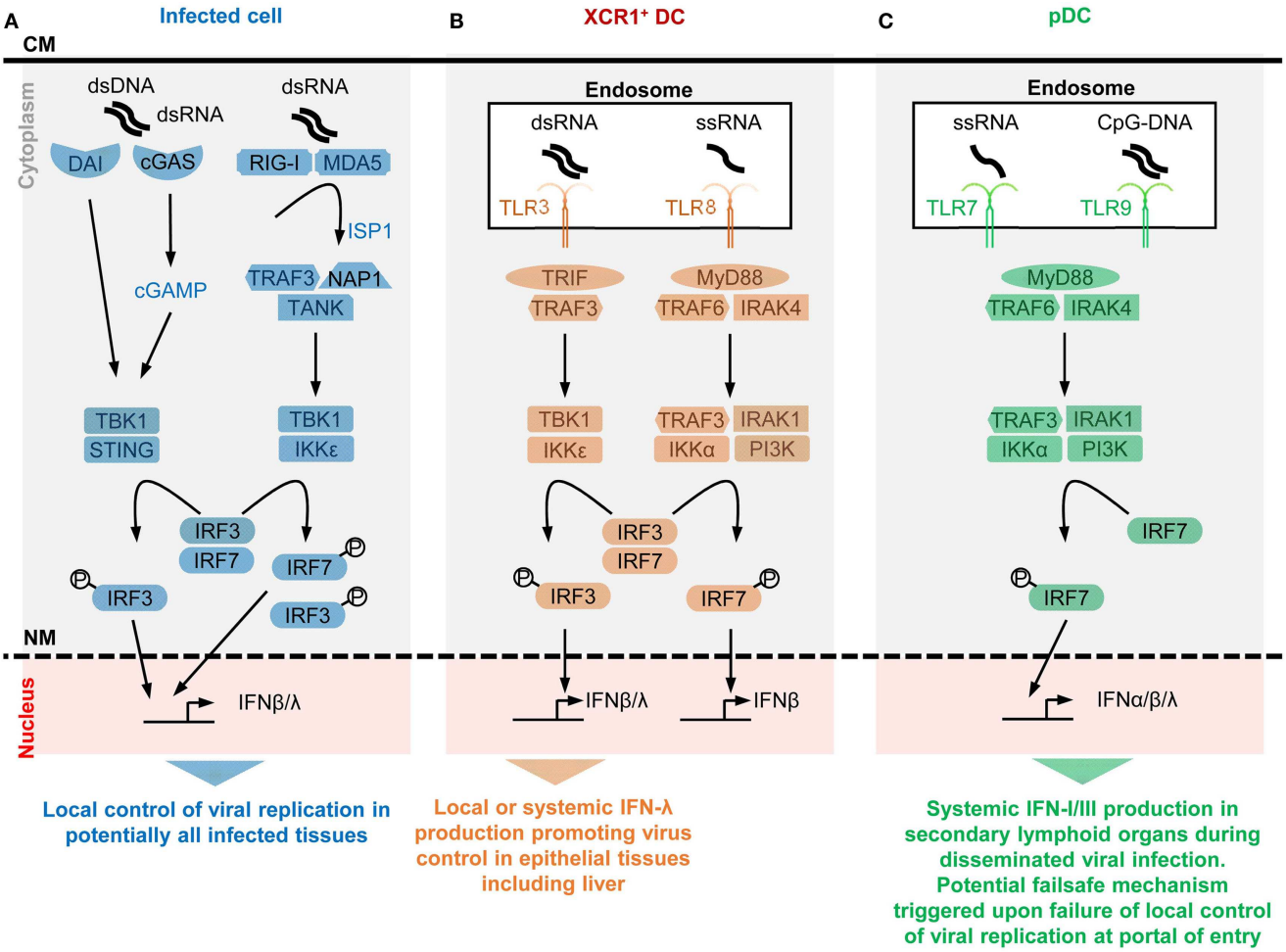

FIGURE 1 | A simplified model of the potential contributions of selective sensors and cell types to IFN production during viral infections. Different innate immune recognition receptors are involved in sensing various types of viral nucleic acids in distinct categories of cells during viral infections, which may promote different types of anti-viral defenses. For each selected sensor shown, the types of viral nucleic acids recognized and the downstream signaling cascade induced are represented in a simplified, schematic manner. The potential specific role of each cell type in anti-viral defenses is also indicated at the bottom of each panel. **(A)** Potentially all types of infected cells can detect endogenous viral replication through cytosolic sensors triggering their local production of IFN-β/λ to control viral replication in an autocrine and paracrine fashion in infected tissues. **(B)** Uninfected XCR1$^+$ DCs selectively produce high levels of IFN-λ and IFN-β upon engulfment of materials containing dsRNA and the consecutive triggering of TLR3 in endosomes. The receptor of IFN-λ is mostly expressed by epithelial cells. Hence, XCR1$^+$ DCs might be involved in inducing local IFN responses in virally infected epithelial tissues. Since XCR1$^+$ DCs are especially efficient at producing IFN-III upon HCV stimulation, they might contribute to local or systemic IFN production during infection with this virus, to promote IFN-λ-mediated protection of hepatocytes. Uninfected XCR1$^+$ DCs and other uninfected cells may produce some IFN-β upon engulfment of materials containing ssRNA and the consecutive triggering of TLR8 in endosomes. The contribution of this pathway to anti-viral defense is not well understood yet, in part because mouse TLR8 is deficient for this function. **(C)** Uninfected pDCs selectively produce high levels of all subsets of IFNs upon engulfment of materials containing ssRNA or CpG DNA and the consecutive triggering of TLR7/9 in endosomes. However, pDCs seem to be activated for this function only in lymphoid tissues. Hence, pDC might contribute to systemic IFN production during blood-borne viral infections or as a failsafe mechanism activated upon abnormal widespread dissemination of a viral infection once it has escaped local confinement at its portal of entry. CM, cell membrane; NM, nuclear membrane.

particular nucleotide sequences or tertiary structures, their signaling pathways and their physiological significance have been recently reviewed (31, 32). Cytosolic RNA sensors encompass DExD/H helicases among which the retinoic-acid-inducible gene (RIG)-I-like receptors (RLRs) have been the most studied, namely RIG-I and melanoma differentiation associated gene 5 (MDA5). RIG-I recognizes RNA with a 5′-PPP or 5′-PP (38) (uncapped) moiety, or double-stranded RNA (dsRNA), both structures being present in viral, but not in cytosolic eukaryotic, RNA molecules. MDA5 might specifically recognize long dsRNA fragments. Both RIG-I and MDA5 contain a DexD/H box-containing RNA helicase domain, and 2 caspase recruitment domains (CARD1/2), which bind to mitochondrial anti-viral signaling protein (MAVS). RNA/RLR/STING molecular complexes initiate a signaling cascade leading to IRF3/7-dependent induction of IFNs (**Figure 1**). Other DExD/H helicases can promote IFN-I production in DCs,

although their physiological roles for *in vivo* immune defenses against viral infections remain to be established (32). Cytosolic DNA sensors able to induce IFN-I (mostly IFN-β) and IFN-III encompass molecules belonging to different protein families, including DExD/H helicases, the inflammasome component IFN-γ-inducible protein 16 (IFI16), the Z-DNA binding protein 1 (ZBP1), and the cyclic GMP-AMP (cGAMP) synthase (cGAS) (31, 32). Most of the cytosolic DNA sensors activate STING and lead to IRF3/7- and NFκB-dependent induction of IFN-β and IFN-III. Many cell types express ZBP1 and are able to produce IFN-I upon triggering of this molecule, including macrophages, DCs, and fibroblasts following an HSV-1 infection (39, 40). Upon DNA binding, cGAS catalyzes the production of cGAMP. cGAS is critical for the detection of lentiviruses including HIV-1/2 (41, 42) and can contribute to sensing of, and protection against, other RNA viruses, including *in vivo* in mice (43). cGAMP also acts as a secreted second messenger signal alerting uninfected cells to directly induce their expression of intrinsic immune anti-viral defenses. The cGAS/STING/IRF3 signaling cascade and the IRF1 transcription factor are "master" inducers of cell-intrinsic immunity able to control the replication of most DNA and some RNA viruses at least in part independently of IFNs (43).

Viral hijacking of the protein synthesis apparatus of the host cell triggers ER overload, a stress, which synergizes with cytosolic sensing to promote IFN-I production. Infected cells become a factory for production of viral particles. Hijacking of the translation apparatus of the host cell for massive production of viral structural proteins leads to an overload of the capacity of the ER for correct folding of newly synthesized proteins. ER overload induces a homeostatic response of the cell, the unfolded protein response (UPR). UPR aims at restoring normal ER functions by inhibiting translation. UPR activation in infected cells contributes to prevent viral replication, including through inhibition of the production of viral proteins, promotion of IFN-I production, and induction of cell suicide (44).

IFN-I production in uninfected cells is initiated by endosomal sensing of viral nucleotide sequences derived from engulfed virions or infected cells

Toll-like receptors (TLRs) are among the first and best characterized I_2R_2s. TLRs are transmembrane proteins with a leucine-rich repeat extracellular domain involved in ligand recognition and an intracellular toll/interleukin-1 receptor domain essential for signaling (45). Among the nine TLRs conserved between mouse and human, TLR3, TLR7, TLR8, and TLR9 are located in endosomes where they can detect the abnormal presence of nucleic acids such as occurs upon endocytosis of virions or of virally infected cell material. TLR3 recognizes dsRNA, TLR7/8 ssRNA, and TLR9 DNA sequences containing unmethylated cytidine-phosphate-guanosine (CpG) motifs. TLR fine specificity and signaling pathways have been reviewed recently (32) and are summarized in **Figure 1**. We will discuss the expression patterns and functions of endosomal TLRs with regards to IFN production in uninfected specialized immune cell types, pDCs and $XCR1^+$ DCs.

Selective expression of TLR7, TLR9, and IRF7 in pDCs endows them with a unique ability to produce very high amounts of all subtypes of IFNs upon virus stimulation irrespective of their own infection. Plasmacytoid DCs uniquely produce very large amounts of IFNs in response to *in vitro* stimulation with many viruses, without being infected (46). IFN-I mRNAs represent up to 40% of all mRNAs in pDCs at the peak of their activation (47). *In vitro*, upon exposure to influenza virus, herpes virus type 1, cytomegaloviruses, or vesicular stomatitis virus, individual pDCs produce 100–1000 times more IFNs than total PBMCs, monocytes, MoDCs, cDCs, neutrophils, and fibroblasts (47–52). However, *in vitro*, high molarity infection of cDCs with certain viruses unable to inhibit IFN-I production in their target cells can also induce massive IFN-β secretion (53). pDCs produce high levels of all subtypes of IFNs, contrary to many other cell types including infected cells, which often preferentially produce IFN-β (46, 47). *In vivo*, pDC depletion during systemic viral infections leads to over 95% decrease of IFN-I production, while the total number of pDCs producing IFN-I (<100,000 in one mouse) is much lower than the total number of infected cells (54–59). This shows that *in vivo* also individual activated pDCs produce much more IFN-I/III than most other cell types, including virus-infected cells. The professional IFN-producing function of pDCs largely results from their high constitutive and selective expression of IRF7, TLR7, and TLR9 (**Figure 1**). These molecules are pre-associated in ready-to-signal complexes located in specialized endosomes specific to pDCs (60, 61). pDCs must also be equipped for efficient sensing and up-take of virions and virus-infected cells. The corresponding cell surface I_2R_2s remain to be identified.

Selective expression of TLR3 in $XCR1^+$ DC endows them with a unique ability to produce very high amounts of IFN-β and IFN-III upon stimulation with dsRNA or HCV irrespective of their own infection. $XCR1^+$ DCs are very potent for antigen-specific activation of $CD8^+$ T cells, in particular through cross-presentation of exogenous antigens that they have captured from other cells and processed for association with class I major complex histocompatibility (MHC-I) molecules (62). In mice, $XCR1^+$ DCs are crucial for the initiation of protective adaptive immune responses against tumors and a variety of viruses (63). Mouse and human $XCR1^+$ DCs constitutively and selectively express high levels of TLR3 (**Figure 1**). They produce large amounts of IFN-III and IFN-β upon stimulation with a synthetic mimetic of dsRNA, Polyinosinic:polycytidylic acid (PolyI:C) (64, 65). Human $XCR1^+$ DCs uniquely respond to stimulation with HCV by producing large amounts of IFN-III in a TLR3-dependent manner (66, 67), irrespective of their own infection.

Positive and negative feedback loops regulating IFN-I production

Positive feedback loops. In addition to IRF7 induction, other positive feedback mechanisms exist to amplify the production of IFNs rapidly after initiation of a viral infection as illustrated by the following selected examples. IFNs induce the expression of many cytosolic RNA/DNA sensors and of TLR7. This broadens the spectrum of host's cell types able to detect endogenous viral replication for IFN induction. Induction of OASL by IFNs in human cells enforces RIG-I signaling, counteracting viral immune

evasion genes interfering with this sensing pathway (68). The IFN-inducible ribonuclease L (RNaseL) generates viral and cellular RNA degradation products, which engage RLRs for amplification of IFN production (69, 70). The IFN-inducible Protein kinase R (PKR) stabilizes IFN-I mRNA (71).

Negative feedback loops. To prevent unbridled responses deleterious for the host, IFN activity must be tightly controlled including during viral infections. Several negative feedback loops exist to terminate IFN production, after anti-viral defenses have been activated. The ISG ubiquitin specific peptidase 18 (USP18) binds to IFNAR2, preventing it from recruiting signal transducer and activator of transcription 1 (STAT1). IFNs induce the expression of TAM receptor tyrosine kinases in DCs, monocytes, and macrophages. TAM receptors associate and signal in part through IFNAR1. They activate the suppressors of cytokine signaling-1/3 (SOCS-1/3). SOCS inhibit TLR and RLR signaling, thereby terminating IFN production (72). TAM receptor ligands, Gas6 and ProS, bind phosphatidylserine on dying cells and are produced by activated DCs and monocytes/macrophages. Thus, IFN induction of TAM inhibitory receptors on uninfected phagocytic immune cells could limit their propensity to produce the cytokines upon engulfment of dying virally infected cells. IFNs induce Tetherin on most cell types. pDCs express a receptor for Tetherin, leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 4 (LILRA4). LILRA4 triggering on pDCs inhibits their production of IFN-I. Hence, through LILRA4 engagement by Tetherin, pDCs can monitor their efficacy at inducing an anti-viral gene expression program in neighboring cells through IFNs, and timely terminate their IFN production.

How positive and negative feedback loops integrate in time and space to promote optimal kinetics and intensity of IFN production in order to efficiently control viral infection without causing severe immunopathology is not completely understood. Positive feedback loops may occur very rapidly after initiation of viral infection to allow rapid secretion of high levels of the cytokines for fast and strong induction of anti-viral cell-intrinsic immunity. Negative feedback loops occur likely later to terminate the response and thus avoid chronicity of cytokine production and its ensuing deleterious effects.

What are the respective roles of infected versus uninfected cells in IFN production during viral infections?

IFN production by infected cells serves as first line of defense to block viral replication at his portal of entry in the body, while IFN production by uninfected pDCs might constitute a failsafe mechanism activated only when viral infection gets systemic.

pDCs do not constitute the major source of IFN production upon local infections by several viruses in the lung or in the female reproductive tract. pDCs are dispensable for resistance against these infections (56, 73, 74). During pulmonary infection by Newcastle disease virus (NDV), IFN-I are produced locally in the lungs mainly by infected alveolar macrophages. Lung pDCs do not express the cytokines (73). Selective depletion of lung alveolar macrophages leads to systemic dissemination of NDV, and to a strong activation of pDCs for IFN-I production specifically in the spleen. Even in the case of systemic viral infections such as caused by intravenous injection of NDV or intraperitoneal injection of mouse cytomegalovirus (MCMV), pDC IFN production is confined to the spleen. It is not detected in other organs even those with high viral replication (59, 73). Hence, splenic pDCs are especially prone to high level IFN production upon systemic acute viral infections. pDCs located in non-lymphoid organs, in particular mucosal barrier tissues, appear to be inhibited for IFN production. Thus, IFN production by infected cells serves as first line of defense to block virus replication at its portal of entry in the body. IFN production by uninfected pDCs might constitute a failsafe mechanism mainly activated in the spleen when viral infection gets systemic (75). Under these conditions, to promote health over disease, the benefits for the host of producing high circulating levels of IFNs in order to induce widespread cell-intrinsic anti-viral defenses might prevail over the deleterious effects that this could cause on certain cell types or tissues. Indeed, pDCs are required for protection against HSV-2 and HSV-1 in mice only in systemic but not local infections (56). This observation is consistent with the crucial role of pDCs for protection of mice against systemic infection by mouse hepatitis virus (MHV), a fast replicating coronavirus (55). Conflicting results have been obtained on the role of pDCs during intranasal influenza infection (74, 76–78). A possible explanation is that pDC IFN production contributes to resistance to highly pathogenic influenza strains that might systemically spread from the lung early after infection, even if at low levels. Another intriguing observation is that IFNs are critical for host resistance to MCMV and that pDCs are the major source of IFNs in this infection model but are dispensable for virus control (54). Studies are ongoing to understand this apparent paradox. Patients bearing genetic mutations disrupting endosomal TLR signaling do not appear to suffer from life-threatening viral infections (79, 80), contrary to patients impaired in IFNAR signaling (4, 81). A notable exception is the specific susceptibility to severe herpes virus encephalitis in individuals' deficient for TLR3 signaling (82, 83). However, contrary to extracellular TLR, endosomal TLR have evolved under strong purifying selection in human beings (84). Hence, while pDCs and endosomal TLR might have been required for protection of our species against viral infections in the past, this appears not to be the case anymore perhaps due to improved social, hygiene, and health care in modern society (75).

IFN production by uninfected pDCs or XCR1$^+$ DCs might promote protection against viruses able to interfere with the signaling pathways inducing cytokine production in infected cells.

Attesting to the importance of IFNs for anti-viral defense in vertebrates, many mammalian viruses encode immune evasion genes specifically inhibiting the production of IFNs in infected cells (39, 85). pDCs or XCR1$^+$ DCs might be essential for IFN-dependent host protection against these viruses, because they are spared from the intracellular functions of viral immune evasion genes (75). To the best of our knowledge, MCMV does not encode for immune evasion genes inhibiting IFN production. However, MCMV manipulates IFN-I responses through specific inhibition of STAT1 functions in infected cells. Thus, pDCs might be dispensable for resistance against systemic MCMV infection due to sufficient levels of IFN production by infected cells locally in all infected tissues. Hepatocyte responses to IFN-III appear to play a

critical role in human resistance to HCV. In infected hepatocytes, HCV induces the expression of cellular microRNAs binding to IFN-III mRNA and leading to its degradation. Uninfected XCR1$^+$ DCs produce high levels of IFN-III *in vitro* upon HCV stimulation (66, 67). Hence, during acute HCV infection *in vivo*, XCR1$^+$ DC may be a strong and early source of IFN-III not subjected to virus immune evasion strategies, therefore, contributing to protect naturally resistant individuals.

Altruistic suicide of subcapsular sinus macrophages in secondary lymphoid organs promotes strong IFN responses to control viral dissemination. In secondary lymphoid organs, a subset of macrophages is critical for the clearance of viruses from the lymph (86). These macrophages are located on viral entry routes, near to subcapsular sinuses where the afferent lymph drained from non-lymphoid tissues flows. Contrary to other subsets of macrophages, subcapsular sinus macrophages are highly susceptible to viral infection, because they constitutively express only low levels of effector molecules of cell-intrinsic anti-viral immunity and because their responses to IFNs are inhibited by their high constitutive expression of USP18. Subcapsular sinus macrophages rapidly become infected by viruses incoming from the lymph and produce large amounts of IFNs. This altruistic suicide prevents virus dissemination to other adjacent cell types and promotes the induction of innate and adaptive anti-viral immunity (87).

HOW ARE IFNs PROMOTING ANTI-VIRAL IMMUNITY?

IFN direct anti-viral effector functions: induction of effector molecules of cell-intrinsic anti-viral immunity

Upon instruction by IFNs, cells express a wide variety of viral restriction factors, whose combined action blocks pathogen invasion by interfering with the different stages of viral life cycle (**Figure 2A**). This has been extensively reviewed recently (88) and will only be described succinctly here. Virus fusion with host cell membrane can be blocked by Cholesterol-25-hydrolase (CH25H) that inhibits sterol biosynthesis. Some viruses enter cells by escaping from endosomes/lysosomes, which can be blocked by interferon inducible transmembrane (IFITM) proteins. Virus uncoating can be blocked by tripartite motif (TRIM) proteins, such as TRIM5α, which bind to HIV-1 capsid thus promoting its degradation, and by Myxoma resistance GTPases, MX1, and MX2, which efficiently trap viral structural proteins at an early stage following virus entry into the cell. MX1 inhibits a number of viruses, including influenza virus through sequestration of its nucleocapsid. MX2 associates with host cyclophilin A and HIV-1 capsid protein. Virion assembly can be blocked at transcriptional, translational, and post-translational levels. The adenosine deaminase acting on RNA 1 (ADAR1) and the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) deaminases induce viral RNA destabilization and hypermutation (89, 90). The sterile alpha motif and histidine-aspartic domain (HD) containing protein 1 (SAMHD1) blocks reverse transcription by hydrolyzing dNTPs (91). ADAR1, APOBEC, and SAMHD1 functions have been mainly studied in infections by HIV-1 and other retroviruses. The 2′,5′-oligoadenylate synthase (OAS) proteins, the IFN-induced proteins with tetratricopeptide repeats (IFIT), and PKR inhibit viral and host protein translation by using complementary mechanisms (88). The major post-translation modification induced by IFNs is the binding of the ubiquitin-like modifier ISG15 to several viral and host proteins, a process called ISGylation. Most of the known ISGylated proteins are targeted for degradation, with few exceptions that are on the contrary stabilized like IRF3 (88). Finally, the egress and budding of virions of many enveloped viruses can be inhibited by Tetherin or by Viperin (88).

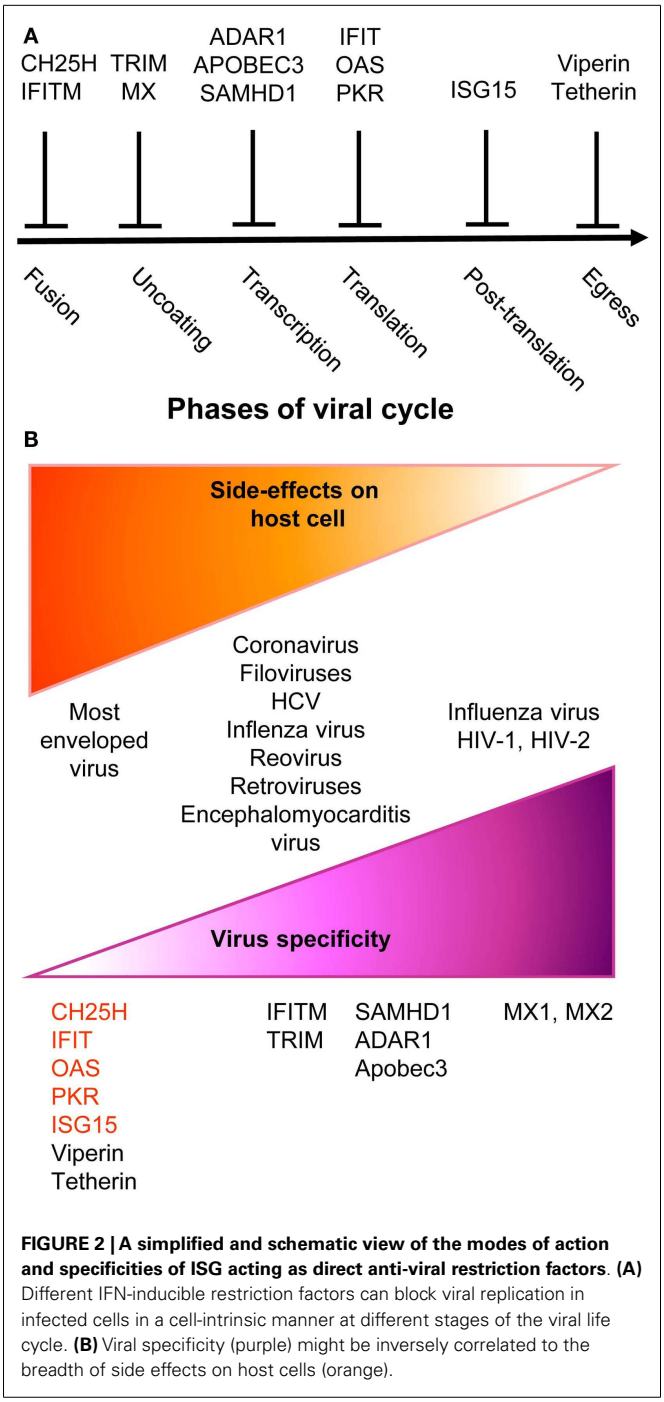

FIGURE 2 | A simplified and schematic view of the modes of action and specificities of ISG acting as direct anti-viral restriction factors. (A) Different IFN-inducible restriction factors can block viral replication in infected cells in a cell-intrinsic manner at different stages of the viral life cycle. **(B)** Viral specificity (purple) might be inversely correlated to the breadth of side effects on host cells (orange).

Many anti-viral ISGs have been functionally characterized only recently, largely thanks to large-scale screening approaches. They display a variable degree of viral specificity (43, 92) that might inversely relate to the extent of their side effects on host cells (**Figure 2B**). Anti-viral effectors acting on a broad spectrum of viruses often target key metabolic pathways that are also crucial for host cell functions. This is the case for the control of cholesterol metabolism by CH25H (93) or of protein translation by PKR, OAS, or IFITs (88). Other anti-viral restriction factors such as MX2 may specifically target one molecule of a very restricted set of viruses with no apparent side effects on host cells. Some anti-viral ISGs target specific functions critical for only a restricted array of viruses and might similarly exert side effects only on a subset of host cell types. For example, SAMHD1 inhibits retrovirus replication through dNTP depletion, which might more specifically affect proliferating host cells. Hence, the infected host must balance the intensity, breadth, and location of ISG induction to circumvent viral replication while preventing life-threatening damages to vital cell types or tissues. One of the mechanisms contributing to this balance is translational control of the expression of ISGs, especially those with pro-apoptotic or anti-proliferative functions (94). While many anti-viral ISGs are transcriptionally activated in most IFN-stimulated cells, their translation can be specifically blocked in uninfected cells by cellular microRNA. This inhibition is relieved upon cell infection through negative regulation of the function of the RNA-induced silencing complex. Hence, IFN stimulation of uninfected cells prepares them for rapid and strong induction of cell-intrinsic anti-viral defenses upon viral infection while avoiding their unnecessary exposure to the toxic effects of certain ISGs.

Further knowledge on the functions and the dynamic regulation of ISGs is essential to develop novel therapeutic strategies against viral infections aiming at modulating IFN responses to promote their protective anti-viral cell-intrinsic functions over their deleterious toxic effects. A better understanding of the immunoregulatory effects of IFNs will also help.

IFN orchestration of anti-viral responses of both innate and adaptive immune cells

Type I interferon can modulate the functions of a broad spectrum of immune cells (**Figure 3A**). We will review this knowledge, focusing on the functions of DCs, NK cells, T cells, and B cells, since they are involved in the control of most viral infections. We will discuss the hypothesis that DCs play a central role in IFN-I orchestration of innate and adaptive immunity for the induction of optimal anti-viral defenses (**Figure 3**).

During viral infections and cancer immunosurveillance, IFN-I constitute one of the most important input signal acting on DCs to promote their delivery of appropriate output signals to T cells, B cells, and NK cells for protective immunity (**Figure 3A**). DCs deliver three types of signals to activate and functionally polarize T cells. Signal 1 is the triggering of the T cell receptor by viral peptide-MHC complexes. Signal 2 is the triggering of activating T cell co-stimulation receptors such as CD28 or CD27 by the CD80/86 and CD70 co-stimulation molecules expressed on DCs. Signal 3 corresponds to cytokines, which can promote T cell proliferation and acquisition of specific effector functions. Under steady state conditions, most DCs are in an immature state characterized by low level expression of MHC-II (signal 1) and co-stimulation molecules (signal 2) and by the lack of production of T cell-activating

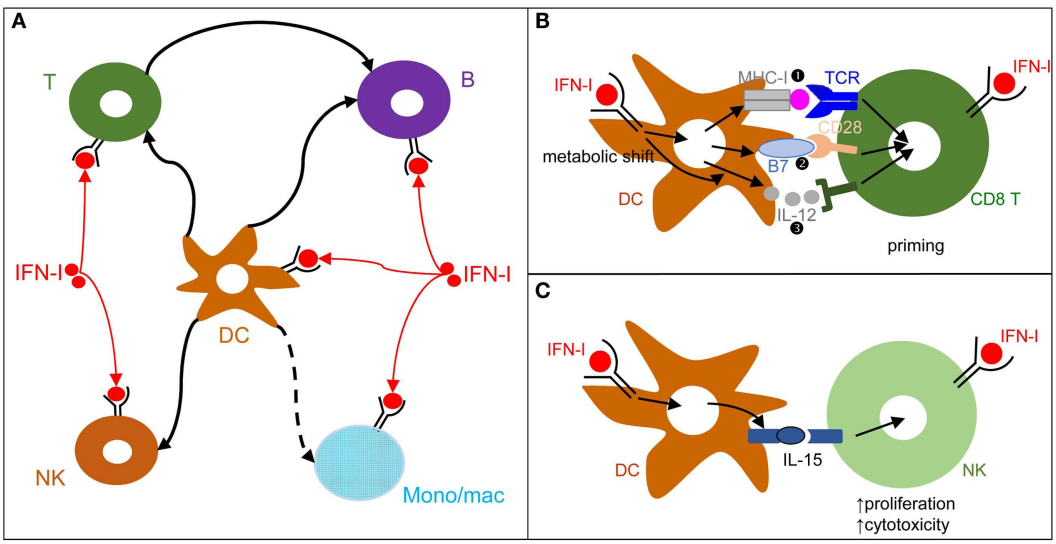

FIGURE 3 | DCs play a central role in IFN-I orchestration of innate and adaptive immune responses. (A) IFN-I exert cell-intrinsic as well as indirect effects on a variety of immune cell populations. DC responses to IFN-I play a major role in promoting protective activation and functional polarization of other innate and adaptive immune cells, not only during viral infections but also in other physiopathological situations including cancer. **(B)** DC cell-intrinsic responses to IFN-I endow them to deliver appropriate signals for T cell priming and functional polarization. IFN-I can modulate all three types of signals delivered by DC to T cells: MHC-I/antigenic peptide complexes (❶), co-stimulation (❷), and cytokines (❸). This depends both on IFN-I-dependent transcriptional induction in DC of some of the corresponding genes and on IFN-I-dependent metabolic reprogramming of DC. **(C)** DC cell-intrinsic responses to IFN-I endow them to deliver appropriate signals, in particular IL-15 trans-presentation, for NK cell activation. See main text for further details.

cytokines (signal 3). Upon activation, including early after viral infections *in vivo*, DCs up-regulate their expression of signal 1 and activating signal 2 and secrete T cell-activating cytokines. This process is called DC maturation. Gene expression profiling of DCs stimulated by microbial stimuli identified a core set of genes up-regulated in mature DCs irrespective of the stimulus they receive, irrespective of the subset they belong to, and conserved across evolution (95). Most of these genes are induced during DC maturation in part through cell-intrinsic IFN-I signaling (95). Consistently, cell-intrinsic IFNAR signaling in DCs is required in many circumstances for the induction of protective immunity, including efficient CD8 T cell responses during viral infection or tumor development (96–98), Th1 responses upon PolyI:C injection independently of IL-12 or IFN-γ effects (99, 100), as well as follicular helper T cell and humoral responses (101, 102). Mechanistically, IFN-I promote DC immunogenicity for efficient T cell activation through a variety of effects (**Figure 3B**). It drives DC up-regulation of signal 2 *in vivo* during viral infections (103) and boosts their capacity to cross-present antigens for increased delivery of signal 1 to CD8 T cells (96–98). It shapes their delivery of activating signal 3, in particular inducing IL-15 and promoting or inhibiting IL-12 depending on experimental conditions (58, 104). Finally, it is necessary to induce their metabolic shift from mitochondrial oxidative phosphorylation to aerobic glycolysis, which fuels the increased needs in energy and the expansion of the intracellular organelles required for the production and proper intracellular routing of the signal 1 and 2 proteins (100, 105). Selective inactivation of IFNAR on cDCs compromises mouse resistance to MCMV and MHV infections (103, 106). In contrast, IFNAR expression is not required on NK cells for protection against MCMV and on pDCs, T cells, and B cells for early control of MHV replication (103, 106). Although cell-intrinsic IFN-I signaling in NK cells can promote their activation (107) (**Figure 3A**), IFN-I-induced IL-15 trans-presentation by DCs plays a more prominent role for this function in many conditions including *in vivo* during MCMV infection (103, 108) (**Figure 3C**).

Cell-intrinsic IFN-I signaling in CD4 T cells (109), CD8 T cells (110, 111), and B cells (112) can also contribute to their efficient activation and functional polarization (**Figure 3**). This depends on experimental settings. CD8 T cell-intrinsic IFN-I responses are crucial for mounting efficient cytotoxic CD8 T cell responses against LCMV but are less critical against Vaccinia virus and vesicular stomatitis virus (110, 113, 114). Mechanistically, cell-intrinsic IFN-I signaling in CD8 T cells can promote their survival during their antigen-induced proliferation (110). Cell-intrinsic signaling in DCs and CD8 T cells may act in a synergistic manner. Indeed, conditional inactivation of IFN-I responsiveness was required to occur simultaneously in both of these two cell types to dramatically affect CD8 T cell expansion upon vaccination with a modified Ankara vaccinia virus (115).

In summary, IFN-I generally play a crucial, beneficial, role in immune defenses against viral infections, both through the induction of cell-intrinsic anti-viral defenses and through the orchestration of innate and adaptive immunity. However, if these responses are not properly regulated, they can contribute to diseases as we will now discuss.

DIFFERENT, AND SOMETIMES OPPOSITE, PROCESSES UNDERLIE DELETERIOUS IFN-I RESPONSES DEPENDING ON THE PHYSIOPATHOLOGICAL CONTEXTS

DELETERIOUS EFFECTS RESULTING FROM THE INDUCTION OF UNBRIDLED INFLAMMATORY RESPONSES CAUSING SEVERE TISSUE DAMAGE, AS EXEMPLIFIED IN AUTOIMMUNE DISEASES

A frequent side effect of IFN-I treatment against cancer or chronic viral infections is the induction of autoimmune reactions. Consistently, ISG expression is a hallmark of many spontaneous systemic or tissue-specific autoimmune diseases, including systemic lupus erythematosous (SLE), Sjogren's syndrome, psoriasis, and other skin disorders (11). The dysregulation of IFN-I responses observed in patients with these autoimmune diseases likely results from both genetic and environmental factors. Genome-wide association studies show that polymorphisms in genes involved in IFN-I responses strongly correlate with increased susceptibility to many autoimmune diseases (11). Diverse environmental factors can also contribute to the onset of autoimmune diseases. Microbial infections often precede first clinical manifestations of autoimmune diseases. Whether infections (116) and/or alterations in the commensal microbiota of the affected barrier tissues (117, 118) are the cause or rather the consequence of autoimmunity is still matter of debate. Infection- or dysbiosis-induced tissue damages and unbridled IFN-I responses can contribute to initiate autoimmune reactions. Gender is another prominent factor affecting susceptibility to autoimmune diseases. Women are more prone to autoimmunity, which may result from endocrine regulation of IFN-I responses. pDC IFN-I production is enhanced in human and mouse females, due at least in part to cell-intrinsic enhancement of TLR7/9 responses by the female hormone estradiol (119). In autoimmune diseases, different mechanisms could operate to initiate the dysregulation of immune responses leading to a vicious circle of reciprocal activation between innate IFN-I responses and adaptive self-reactive lymphocyte responses (**Figure 4**). Adaptive immune cells are educated to spare "self." This occurs through negative selection of potentially autoimmune B and T cells during their development in the bone marrow or thymus, respectively, a process called central tolerance. Self-reactive B or T cells that have escaped this pruning can be either deleted or functionally inactivated once they have egressed in secondary lymphoid organs or non-lymphoid tissues, a process called peripheral tolerance. In some individuals, polymorphisms in genes involved in the promotion of central or peripheral tolerance lead to a higher number, diversity, and/or responsiveness of self-reactive lymphocytes in the periphery, in particular of B cells secreting anti-DNA or anti-RNP antibodies (120, 121). Mammalian DNA or RNA are poor inducers of pDC IFN-I induction under normal conditions. However, pre-existing anti-DNA or anti-RNP autoantibodies can break this innate tolerance of pDC. Indeed, antibodies binding to self nucleic acids can protect them from degradation and compact them into nanoparticles that are very effective for the induction of IFN-I in pDC (**Figure 4**). DNA-containing immune complexes (ICs) are frequently found in the serum of SLE patients (SLE-ICs) and can activate pDC IFN-I production (122). In turn, pDC IFN-I activate cDCs, monocytes (123), and B cells, leading to a vicious circle of reciprocal activation between DCs and

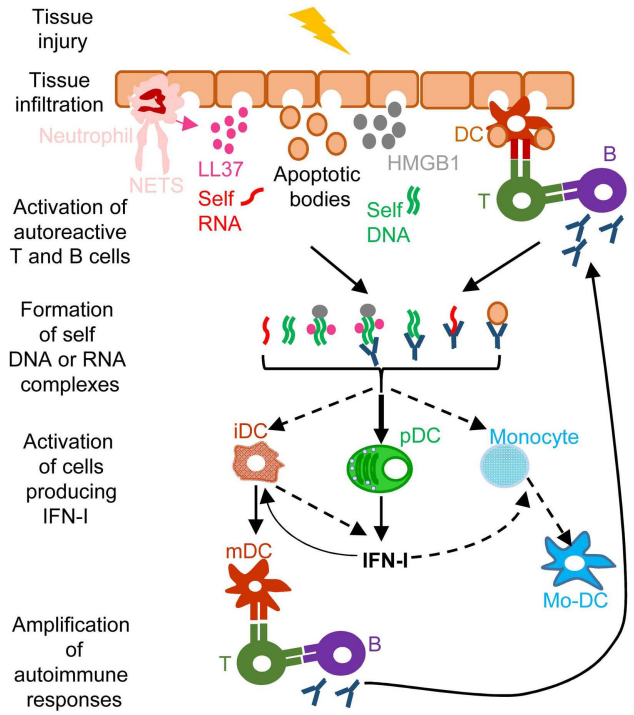

FIGURE 4 | A simplified model of the deleterious role of IFN-I in several autoimmune diseases. When exposed to different kinds of injuries (microbial infection, commensal microbiota dysbiosis, chemical or physical insults), healthy tissues can undergo cell damage and death. These events induce the release of apoptotic bodies encompassing self RNA or DNA. Neutrophil recruitment and activation in inflamed tissues can also constitute a potent source of self nucleic acids, through the release of neutrophil extracellular traps (NET). Self RNA or DNA can associate with cationic peptides (e.g., LL37) as shown in psoriatic patients or with inflammatory molecules (e.g., high mobility group box 1, HMGB1) to generate nanoparticles that are extremely efficient for IFN-I production by pDC and eventually other cell types. pDC can also be efficiently activated for IFN-I production by immune complexes (ICs) generated by the association between self nucleic acids and auto-antibodies as frequently found in the serum of systemic lupus erythematosus patients. IFN-I promote the differentiation and/or the maturation of antigen-presenting cells, in particular different subsets of DC. Activated DC can then present self-antigens for activation of auto-reactive T $CD4^+$ cells, including follicular helper lymphocytes, which in turn activate auto-reactive B cells for auto-antibody secretion, leading to a vicious circle of reciprocal activation between innate and auto-reactive adaptive immune cells. iDC, immature DC; mDC, mature DC; Mo-DC, monocyte-derived DC. See main text for further details.

self-reactive lymphocytes and to the exacerbation of autoimmune responses (**Figure 4**). Certain infections or dysbiosis of the commensal microbiota of the affected barrier tissues could promote chronic production of host amphiphatic peptides able to combine with eukaryotic DNA or RNA, likely released from dying cells, thus forming pDC-activating nanoparticles. Indeed, in psoriatic skin, both a high expression of LL37 and a massive infiltration of pDCs is observed (124) (**Figure 4**). Hence, to treat many autoimmune diseases, novel therapeutic strategies could be designed to target dysregulated pDC IFN-I production or B cell activation by IFN-I.

DELETERIOUS RESPONSES RESULTING FROM INAPPROPRIATE FUNCTIONAL POLARIZATION OF IMMUNE RESPONSES, AS EXEMPLIFIED IN FAILURE TO CONTROL SECONDARY BACTERIAL OR FUNGAL INFECTIONS

One of the most common complications of primary infections by many respiratory viruses, in particular influenza virus, is a life-threatening pneumonia due to secondary pulmonary infections by bacteria, such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, or *Haemophilus influenza* (125, 126). These pathologies affect especially infants, elderly, and immunocompromised patients. Retrospective studies indicate that secondary bacterial pneumonia was highly recurrent in lung tissues isolated from patients who died during last century influenza pandemics, independently of antibiotic availability (127, 128). Influenza virus induces high IFN-I responses in human beings and mice. In both hosts, secondary bacterial infections are lethal only when they occur in a limited time window following primary viral infection (3–7 days), around the peak of IFN-I responses, before complete virus clearance. Mouse models of viral/bacterial coinfections are being used to dissect disease mechanisms (129). IFNAR1-deficient mice appear more resistant to secondary pulmonary bacterial infections, showing that IFN-I responsiveness contributes to disease (130). Similarly, after lymphochoriomeningitis virus (LCMV) infection, wild-type but not IFNAR1-deficient mice are more susceptible to LPS-induced septic shock (131). Several mechanisms may contribute to the detrimental role of IFN-I in secondary bacterial infections (**Figure 5**). Early during viral infection, IFN-I decrease the host ability to control bacterial replication, by dominantly polarizing immune responses toward anti-viral functions, simultaneously inhibiting the development of the types of immune responses required for protection against most bacterial infections. IFN-I can inhibit the production of chemokines required for the recruitment to the respiratory tract of antibacterial effector innate immune cells, in particular neutrophils or monocytes/macrophages (132, 133) (**Figure 5**). Depending on the experimental models used, IFN-I can on the contrary induce a CCR2-dependant recruitment of classical monocytes (134). In infected tissue, IFN-I might skew the functional polarization of resident or infiltrating monocytic cells toward immunosuppression, because it does limit their antibacterial functions by inhibiting their IL-1 production (135–137) while it might promote their production of IL-10 and nitric oxygen intermediates. The exact nature of infiltrating monocytic cells is not clear and could correspond to activated classical monocytes, MoDCs, monocyte-derived macrophages, or myeloid-derived suppressor cells (MDSCs). The boundaries between these putatively different cell types are currently ill-defined (138). These cells could fuel local replication of monocyte/macrophage-tropic bacteria (134), be immunosuppressive (139) or contribute to local immunopathology (140). The role of IFN-I on monocytes/macrophages is complex and will require further investigations to determine when it is protective versus deleterious and what the underlying mechanisms are. Depending on the context, IFN-I can either promote or inhibit the induction of Th1 cytokines such as IL-12 and IFN-γ, and myeloid cell responses to IFN-γ (10, 141-143). IFN-I can also polarize CD4 T cell responses toward Th1 at the expense of Th17, while the Th17-type cytokines IL-17A and IL-22 are required for host defense against pulmonary

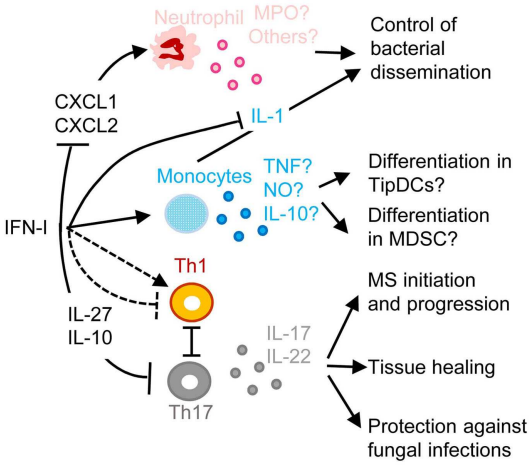

FIGURE 5 | A simplified model of the deleterious role of IFN-I in secondary pulmonary bacterial infections or in fungal infections and of their protective role in multiple sclerosis (MS). IFN-I-dependent signals can block CXCL1 and CXCL2 production, thus inhibiting the recruitment and activation of neutrophils in inflamed tissues, hampering their protective functions against secondary pulmonary bacterial infections or fungal infections. IFN-I-dependent signals can enhance CCL2 production, promoting the recruitment in inflamed tissues of $CCR2^{+}$ monocytes that can potentially differentiate into TipDC or into MDSC. This can contribute to disease either through enforcing T cell activation leading to immunopathology or on the contrary through suppressing anti-microbial immune defenses. IFN-I can either inhibit or promote Th1 responses, which in the latter case occurs at the expense of Th17 responses thus compromising the production of IL-17 and IL-22, which are respectively required for control of microbial replication and for tissue healing. IFN-I-induced IL-10 and IL-27 can directly inhibit Th17. In MS, inhibition of Th17 functions may contribute to the protective effects of IFN-β therapy. MPO, myeloperoxidase; TNF, tumor necrosis factor alpha; NO, nitric oxide. See main text for other abbreviations and further details.

bacteria by inducing the production of anti-microbial peptides and of tissue repair molecules (**Figure 5**) (141–143). IFN-I may not only affect host resistance to bacterial infection, but also host tolerance, i.e., the ability of the host to tolerate a given burden of pathogen without undergoing excessive tissue damages (143, 144). Hence, to counter IFN-I deleterious effects during secondary bacterial infections, it will be important to better delineate the respective contribution of lung tissue tolerance modulation and of immune-mediated resistance weakening.

Another well documented example of deleterious effects of IFN-I due to their inappropriate functional polarization of immune responses is the enhanced susceptibility to fungal infections of patients with genetically determined hyperactive IFN-I responses, as exemplified in the hereditary disease Chronic Mucocutaneous Candidiadis (CMC) (**Figure 5**) (145). Patients with CMC have a significant deficit in Th17 CD4 T cells, at least in part as a consequence of altered responsiveness to IL-6 or IL-21. Several STAT1 mutations were identified in patients with autosomal dominant CMC. Gain-of-function STAT1 mutations were found to hard wire CD4 T cell responses to cytokines toward STAT1 signaling, compromising their STAT3-dependent ability to produce IL-17 upon IL-6 or IL-21 stimulation. This was associated to induction of a global IFN-I transcriptomic signature in blood (145). Deleterious IFN-I effects on immunity to *Candida* might not only occur in CMC patients but also in other types of individuals upon secondary fungal infections occurring shortly after a primary viral infection, likewise to the situation discussed above for secondary bacterial infections. Indeed, PolyIC induced IFN-I abrogate innate immunity to systemic candidiasis in mice (146), and IFNAR-deficient mice can be more resistant to *Candida* infection under certain experimental settings (147). However, the role of IFN-I in the modulation of the ability of immunocompetent hosts to control fungal infection is disputed (148, 149).

The inhibition of Th17 responses by IFN-I could be protective in at least one important human pathology, MS (**Figure 5**). MS represents a striking exception to the previously discussed detrimental role of IFN-I in autoimmune diseases. Indeed, a large proportion of MS patients have low serum IFN-I activity and low ISG levels. These MS patients present a significant reduction of MS relapse upon IFN-β administration (150). The underlying mechanisms are not yet completely unraveled. However, in the experimental autoimmune encephalitis mouse model of MS, Th17 responses bear a major contribution to nervous system damages and are inhibited by the IL-10 and IL-27 induced upon IFN-I administration (151).

In summary, IFN-I responses can be deleterious in autoimmunity by promoting a vicious circle of reciprocal activation between innate immune cells and auto-reactive CD4 T or B lymphocytes. IFN-I responses can also be deleterious upon secondary bacterial or fungal infections in the lung or the kidneys occurring shortly after a primary viral infection, by compromising the recruitment of anti-microbial innate effector cells and/or by preventing the proper functional polarization of immune responses. We will now discuss how IFN-I responses can also compromise host immune defenses against certain viruses and promote chronic infections.

DELETERIOUS RESPONSES RESULTING FROM THE INDUCTION OF IMMUNOSUPPRESSION, AS EXEMPLIFIED IN CHRONIC LCMV INFECTION

Different LCMV strains such as Armstrong and clone-13 (Cl13), respectively, lead to acute versus chronic infections in mice. A hallmark of chronic LCMV infection is the loss of the proliferative potential and effector functions of anti-viral CD8 T cells, a process called exhaustion. Exhausted CD8 T cells are characterized by a high expression of the inhibitory receptors PD-1, CTLA4, and LAG-3 (152). *In vivo* blockade of these inhibitory receptors can reverse T cell exhaustion and allow resolution of the chronic infection (152). IFN-I and ISGs are induced early after infection with all strains of LCMV, albeit to lower levels with those leading to chronic infection. This early IFN-I production is critical to limit viral replication (3). In models of acute infection, IFN-I responses rapidly return to normal, undetectable, levels, before viral replication is completely controlled. In contrast, ISG induction is maintained in chronic infection, including the expression of PD-1 ligands on APCs and of the immunosuppressive IL-10 cytokine, consistent with a prolonged expression of IFN-I albeit at low levels (13, 14). *In vivo* neutralization of IFN-I by antibody administration promoted resolution of chronic LCMV Cl13 infection, allowing the

restoration of functional anti-viral CD8 T cell responses at least in part through CD4 T cell- and IFN-γ-dependent mechanisms (13, 14). During persistent LCMV Cl13 infection, chronic low level IFN-I production polarizes CD4 T cell responses toward T follicular helper (Tfh) rather than Th1 functions. Thus, chronic IFN-I responses promote enhanced anti-viral B cell responses but facilitate CD8 T cell exhaustion due to deficient CD4 T cell help, therefore contributing to host failure to prevent chronic infection (153). Strikingly, establishment of chronic infection by LCMV Cl13 could also be prevented by early administration of two shots of a high dose of exogenous IFN-I, at days 2 and 5 post-LCMV inoculation. This treatment allowed viral clearance by rescuing anti-viral CD8 T cell from exhaustion (154). Altogether, these studies show that the timing and duration of IFN-I production during viral infections is critical in determining how this response will impact the balance between the virus and the host. An early and robust but transient production of IFN-I promotes strong induction of cell-intrinsic viral restriction mechanisms as well as adequate polarization of adaptive anti-viral immune responses, which combined effects lead to viral clearance. In contrast, if the production of IFN-I is too low and/or too late, both viral replication and low IFN-I responses become chronic, their combined action leading to induction of immunosuppressive effects and to inadequate functional polarization of CD4 T cells. This results in CD8 T cell exhaustion and maintenance of chronic infection. Chronic viral replication and CD8 T cell exhaustion is also a hallmark of HIV-1 infection. We will now discuss the complex and disputed role of IFN-I in this disease.

THE COMPLEX AND DISPUTED ROLE OF IFN-I IN HIV-1 INFECTION

Both in HIV-1 infection and in its most relevant animal model, infection of non-human primates with simian immunodeficiency virus (SIV), disease progression after the acute phase of the infection is associated with high and chronic expression of ISGs while IFN-I production is inconsistently detected (155–157). In contrast, the individuals that do not progress toward disease despite persistent high viral loads show much lower immune activation, in particular low ISG expression, after the acute phase of the infection (158–161). Hence, chronic low levels of IFN-I are associated to disease progression independently of the level of viral replication. Therefore, an outstanding question still open for a better understanding of the physiopathology of HIV-1 infection is whether chronic IFN-I responses are merely a marker of progression, or whether they are implicated in driving disease development. In addition to mechanisms similar to those uncovered in the mouse model of chronic LCMV infection, during HIV-1 infection other effects of IFN-I could promote a vicious circle of reciprocal activation between chronic viral replication and sustained, deleterious immune responses (**Figure 6**). Very early after HIV-1 infection, in most individuals, IFN-I production might be too weak or too late to induce a combination of cell-intrinsic defense mechanisms and of immune responses efficient enough to prevent later establishment of chronic infection. On the contrary, as demonstrated in the case of the mouse model of LCMV infection, IFN-I responses could favor CD8 T cell exhaustion, either by direct cell-intrinsic effects on CD8 T cells (**Figure 6**, ❶) or by contributing to deprive them from CD4 T cell help (**Figure 6**, ❷). Several effects of IFN-I might compromise anti-HIV-1 Th1 responses or more generally contribute to the global depletion of CD4 T cells. These mechanisms include functional polarization of anti-HIV-1 CD4 T cells toward Tfh rather than Th1 responses, CXCL10 production leading to enhance recruitment of memory CD4 T cells to the sites of viral replication where they fuel chronic viral replication with new HIV-1 target cells (**Figure 6**, ❸ to ❻), direct pro-apoptotic and anti-proliferative effects on CD4 T cells (**Figure 6**, ❼), as well as TRAIL induction on pDCs licensing them for killing CD4 T cells irrespective of their infection (**Figure 6**, ❽) (162, 163). Altogether, these mechanisms entertain chronic viral replication and continuous depletion of CD4 T cells, leading to the dramatically enhanced susceptibility to opportunistic infections (**Figure 6**, ❾) characteristic of the acquired immunodeficiency syndrome (AIDS) (**Figure 6**, ❿). Other lines of evidences have been reported to support a deleterious role of pDC activation during HIV-1 infection. Women undergo faster HIV-1 disease progression than men with similar viral loads, which may result in part from the highest IFN-I production of women's pDCs including in response to HIV-1 stimulation (164). pDC recruitment and activation in the vaginal mucosa of female macaques early after local SIV inoculation contribute to attract and activate CD4 T cells, which can then be infected and promote virus dissemination from its portal of entry (165). However, *in vivo* blockade of pDC IFN-α production by administration of TLR7/9-antagonistic oligonucleotides early after SIV infection of macaques did not decrease T lymphocyte activation, which suggests that additional sources of IFN-I likely contribute to the immune dysfunction observed in SIV/HIV-1 infections. Targeting dysregulated IFN-I responses during HIV-1 infection might represent an interesting adjuvant therapeutic strategy to highly active antiretroviral treatments. Administration of IFN-I in the non-pathogenic SIV infection model of sooty mangabeys was not sufficient to switch it into a pathogenic model. No CD4 T cell depletion ensued, no hyperactivation of immune responses were observed. Viral loads were even significantly decreased. However, this could be consistent with the positive impact of early and high dose IFN-I administration in chronic LCMV infection (154). Indeed, during the review process of this manuscript, it was reported that, early during primary SIV infection in the pathogenic rhesus macaque model, a high dose injection of IFN-I was protective while neutralization of endogenous IFN-I was deleterious. In contrast, in the same animal model, prolonged IFN-I administration accelerated disease development in the chronic stage of the infection (166). In mice with a humanized immune system, pDC depletion strongly decreased ISG induction and enhanced viral replication both in the acute and chronic phases of HIV-1 infection. However, pDC depletion during chronic infection decreased infection-induced T cell apoptosis and increased T cell numbers in lymphoid organs (167). These results further emphasize the dual role of IFN-I and pDCs in the physiopathology of HIV-1 infection. A strong and transient production of IFN-I early after infection benefits the host by lowering the set-point of viral replication during chronic infection. Sustained production of low levels of IFN-I during chronic infection contributes to immune dysregulation and CD4 T cell depletion. Further studies will be necessary to examine whether complementing standard-of-use antiretroviral drugs with pDC

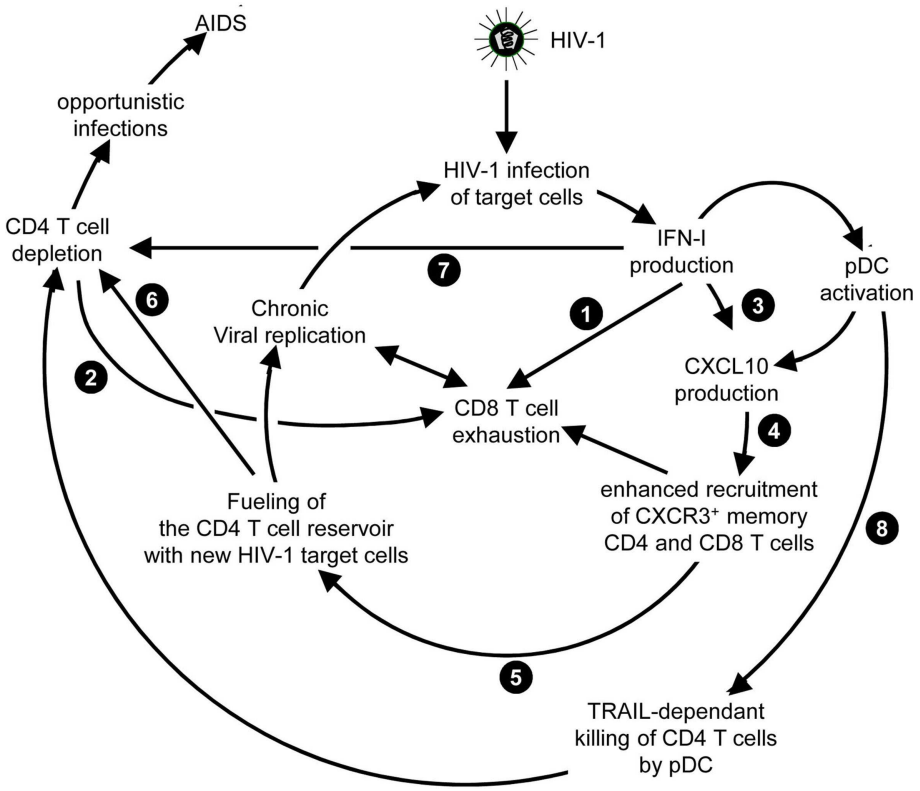

FIGURE 6 | Potential mechanisms through which chronic, low level IFN-I production might promote disease progression in HIV-1 infection. High and sustained expression of ISG in blood and lymphoid organs is a hallmark of progressive infection with immunodeficiency viruses both in human beings and in non-human primates, irrespective of the levels of viral replication. Several mechanisms summarized here have been proposed to explain how chronic, low level IFN-I production might promote disease progression in HIV-1 infection. These mechanisms include direct (❶) and indirect (❷) promotion of the exhaustion of anti-viral CD8 T cell responses, as well as direct (❼) and indirect (❸-to-❻, and ❽) promotion of CD4 T cell depletion with a proposed central role of pDC in this deleterious process. Altogether, these mechanisms may sustain a vicious circle of reciprocal activation between chronic viral replication and deleterious immune responses, driving the progressive depletion of all CD4 T cells ultimately causing the enhanced susceptibility to opportunistic infections characteristic of the acquired immunodeficiency syndrome (AIDS). See main text for further details.

depletion, IFN-I neutralization, or selective inhibition of T cell responses to IFN-I could yield additional benefits to chemotherapy in non-human primates during chronic SIV infection.

IFN-I administration has been used for many years to treat another human chronic viral disease, HCV infection. Roughly, half of the patients do not show sustained virological responses (SVR). The treatment causes severe side effects in many individuals. New chemotherapeutic drugs very potent at blocking HCV replication *in vivo* have recently become available. Hence, whether IFN-I administration still constitutes a viable treatment against chronic HCV infection is being questioned (168, 169). We will now discuss this issue.

IFN-I TREATMENT OF CHRONIC HCV INFECTION: BALANCING BENEFITS FOR VIRUS CONTROL WITH SIDE EFFECTS STRONGLY AFFECTING PATIENT'S QUALITY OF LIFE

Chronic HCV infection is the main cause of liver cirrhosis and hepatocellular carcinoma. There is currently no vaccine against HCV. The most common therapy for chronic HCV patients is the administration of recombinant pegylated IFN-α (Peg-IFN-α) combined with the anti-viral drug ribavirin. However, because of IFNAR pleiotropic expression, IFN-α administration induces severe side effects including flu-like syndrome, fever, fatigue, myalgia, and nervous depression (**Figure 7A**) (170). Moreover, only about half of treated patients harbor SVR (171). Prior-to-treatment high hepatic ISG expression is a negative predictor of SVR upon Peg-IFN-α therapy. High ISG expression in untreated patients likely results from chronic but low IFN-I production triggered by persistent HCV replication. Indeed, hepatocytes from non-responder patients were found to be infected at a greater frequency and to exhibit dampened antiviral and cell death responses (172). What the cellular sources of IFN-I production are and why they persist only in non-responder patients still remain to be established. In chronic HCV infection, cytotoxic effector lymphocytes may contribute to the development of hepatocarcinoma by causing low level but sustained hepatocyte death and renewal. In contrast, local production of IFN-γ in the liver by NK and T lymphocytes could promote resistance to disease through non-cytolytic control of viral replication. As discussed previously for LCMV and HIV-1, low chronic production of endogenous IFN-I in HCV patients could compromise both innate and adaptive anti-viral immune responses. Chronic exposure to IFN-I could dampen the ability of

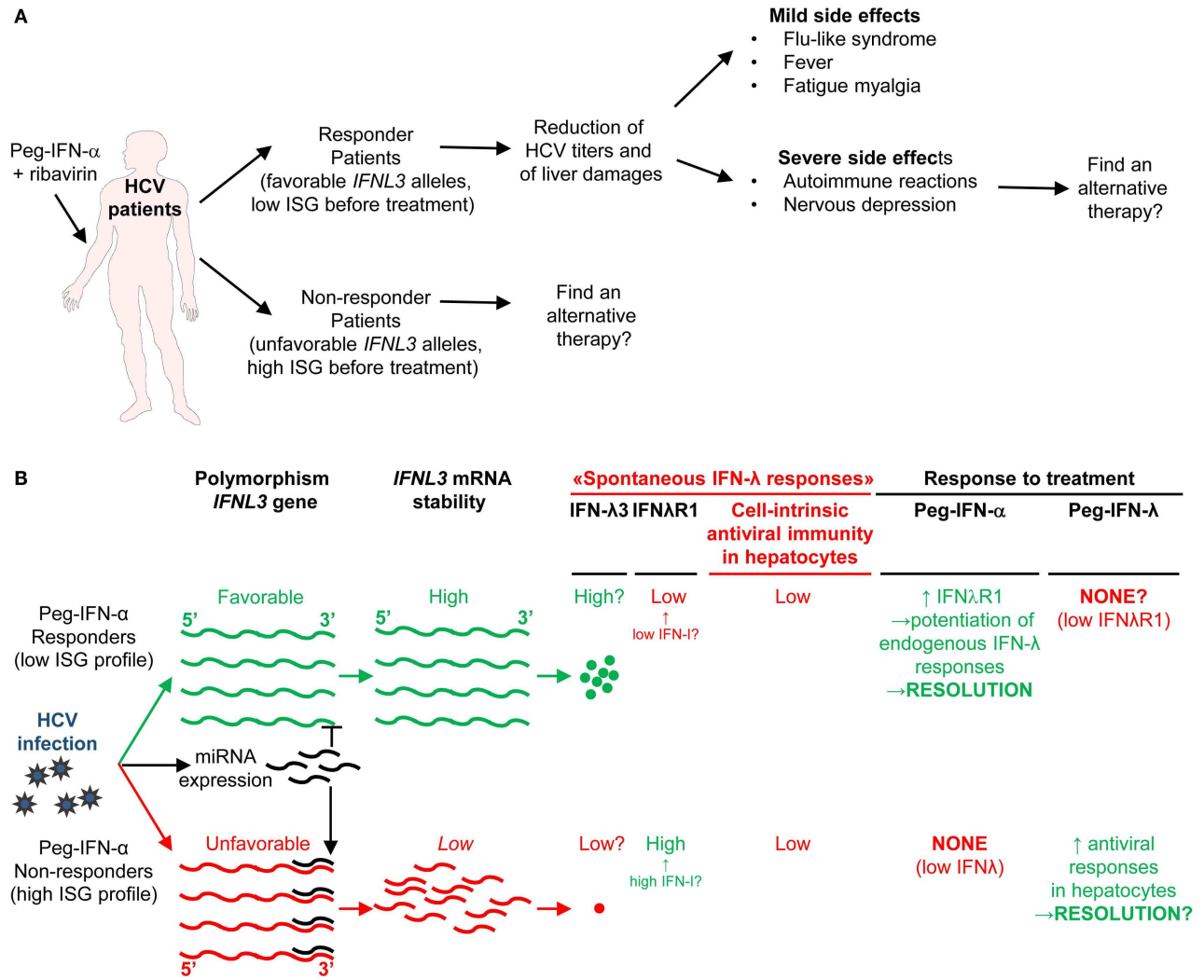

FIGURE 7 | A novel hypothetical model attempting to explain the respective roles of IFN-I and IFN-III in HCV infection. **(A)** Classification of patients suffering from chronic HCV infection and treated with PEG-IFN-α in non-responders and responders, and identification of *IFNL3* (*IL28B*) gene polymorphisms as the best predictors for treatment response. The side-effects induced by PEG-IFN-α treatment are also listed since they can severely affect the patient's quality of life and lead to treatment failure due lack of compliance or suicide. Hence, new approaches are needed to promote beneficial over deleterious effects of IFN-I administration in chronic HCV infection. **(B)** Proposal of a new hypothesis explaining the relationships between endogenous ISG levels in patients prior to treatment, *IFNL3* gene polymorphism, endogenous expression of IFN-I, IFN-λ, and IFNλR1, and responsiveness to IFN-I administration. Efficient control of HCV infection may depend on hepatocyte response to IFN-λ rather than IFN-α. Upon HCV infection, the virus induces the expression of host miRNA able to bind the 3′ UTR of *IFNL3* mRNA to promote their degradation. The favorable *IFNL3* allele associated with responsiveness to PEG-IFN-α treatment may allow endogenous expression of sufficient levels of IFNL3 for efficient induction of cell-intrinsic anti-viral defenses in hepatocytes. This process is, however, hampered by the limited expression of the receptor for this cytokine (IFNλR1) in these patients. PEG-IFN-α treatment might promote resolution of the infection by inducing IFNλR1 in these patients, potentiating their response to their endogenous production of IFNL3. In the patients that do not respond to PEG-IFN-α treatment, endogenous levels of IFNL3 are insufficient for efficient induction of cell-intrinsic anti-viral defenses in hepatocytes, due to the degradation of the corresponding mRNA in infected hepatocytes. In these patient's hepatocytes, however, IFNλR1 is already expressed to high levels prior to treatment due to their high endogenous IFN-I responses. Administration of exogenous IFN-λ might cure these patients. See main text for further details.

NK and CD8 T cells to produce IFN-γ (173, 174) and promote CD8 T cell exhaustion (175). It could also induce an antagonist form of CXCL10, a chemokine required for recruitment to the liver of anti-viral NK and CD8 T cell effectors (176). It may also polarize monocytes toward immunosuppressive functions (177). Therefore, better understanding IFN-I effects in HCV infection is critical to improve care of both responders and non-responder patients to Peg-IFN-α. For responder patients, the issue is to modify the treatment to favor beneficial antiviral and immunoactivating effects over side effects strongly affecting patient's quality of life (**Figure 7A**). This might be achieved by specific delivery of IFN-I to targeted cell types as discussed later. For non-responder

patients, the issue is to understand the mechanisms underlying treatment failure to determine whether alternative therapies could be designed (**Figure 7A**).

Genome-wide association studies identified various single nucleotide polymorphisms (SNP) in the gene encoding IL-28B/IFN-λ3, one of the IFN-III, as well in its 5′ and 3′ non-coding regions (178–181). One SNP, called rs12979860, is located 3 kb upstream of the *IFNL3* gene. Patients harboring the CC genotype have a favorable prognosis to IFN-I treatment. Patients with the TT genotype are at high risk of treatment failure (178, 179). In Europeans, the favorable CC genotype is the major, most common, *IFNL3* allele. The unfavorable TT SNP is the minor allele. The frequency of these alleles is reversed in Africans. The favorable allele allows escape of *IFNL3* mRNA from degradation by cellular microRNA induced upon HCV infection (181).

Until recently, *IFNL3* genotypes and hepatic ISG expression were considered as independent predictors of response to Peg-IFN-α treatment in HCV patients (171). Here, we propose a potential explanation, which integrates both factors in a relatively simple model (**Figure 7B**). Our main hypothesis is that efficient control of HCV infection depends on hepatocyte response to IFN-λ rather than IFN-α. This is supported by reports that IFN-λ induces a stronger and more sustained ISG expression in hepatocyte cell lines *in vitro* (182), and that PolyI:C-induced control of HCV replication in humanized liver in chimeric mice is correlated to the induction of IFN-λ but not IFN-I in human hepatocytes (183). Responder patients harboring the favorable *IFNL3* allele preventing the degradation of the corresponding RNA in infected cells might express significant levels of endogenous IFN-λ3, although this is disputed. However, they express only low levels of IFN-λR1, which limits IFNλ3 efficiency (**Figure 7B**) (184). How these patients benefit from Peg-IFN-α treatment could be that it induces IFN-λR1 expression on hepatocytes thus boosting endogenous IFN-λ3 effects (184). In contrast, high ISG-expressing non-responder patients harboring the unfavorable *IFNL3* allele might not express enough IFN-λ3 for virus control. However, they do express IFN-λR1 as a result of their endogenous production of IFN-I. Hence, Peg-IFN-α might be ineffective in these patients because they already express IFN-λR1 but fail to produce endogenous IFN-λ3 due to the degradation of its mRNA in infected hepatocytes (**Figure 7B**). These patients may be good candidates for Peg-IFN-λ therapy, currently undergoing clinical development. Since the expression of IFN-λR1 is mainly restricted to epithelial cells, melanocytes, and hepatocytes, some of the side effects related to IFN-I treatment might be strongly attenuated in Peg-IFN-λ therapy. However, as IFN-I are key to induce anti-viral immune responses, it will be critical to determine whether, beside viral clearance, Peg-IFN-λ therapy can also induce long-term immune protection against HCV.

CELLULAR AND MOLECULAR MECHANISMS DETERMINING THE BENEFICIAL VERSUS DELETERIOUS OUTCOME OF IFN-I EFFECTS

EXISTENCE OF DIFFERENT PATHWAYS DOWNSTREAM OF IFNAR SIGNALING

IFN-I transduce intracellular signals through a single receptor, IFNAR, but via a multitude of downstream signaling pathways. The Janus activated kinase (JAK)/STAT pathway was the first to be identified (185). IFNAR is composed of two distinct subunits, IFNAR1 and IFNAR2, which are constitutively associated with members of the JAK family, tyrosine kinase 2 (TYK2) and JAK1, respectively (186). The binding of IFN-I to their receptor leads to the phosphorylation of JAK1 and TYK2, which in turn induce the phosphorylation and activation of the STAT proteins (186).

Different STAT complexes can form upon triggering of IFNAR (**Figure 8**). A transcriptional complex that forms in most conditions of IFN-I stimulation and induces the expression of many molecules of cell-intrinsic anti-viral immunity is interferon-stimulated gene factor 3 (ISGF3), a heterotrimer composed of pSTAT1, pSTAT2, and IRF9 (187) (**Figure 8**). Following its translocation into the nucleus, ISGF3 binds to ISRE regulatory sequences in target genes. Many molecules playing a key role in the function of innate or adaptive immune leukocytes are also induced by ISGF3, including CD80, CD86, or IL-15 in DC, and Granzyme B in NK cells. ISGF3 is generally composed of STAT1 phosphorylated on Tyr701 and Ser727 and of STAT2 phosphorylated on Tyr689. However, alternative ISGF3 complexes have been described in various contexts which could participate to the diversity of IFN-I effects (188).

The pSTAT1 homodimer also plays a prominent role in cell-intrinsic IFN-I-dependent gene induction. It binds IFNγ-activated sequences (GAS) and controls the expression of many pro-inflammatory molecules (187). pSTAT1 homodimers can form upon stimulation with either IFN-I or IFN-γ. Many GAS-regulated genes can be induced by either cytokines.

Depending on cell types, JAK signaling downstream of IFNAR can lead to the activation of virtually all STAT proteins and to their combinatorial association into a variety of complexes with different affinities for specific GAS elements (189–191) (**Figure 8**). This diversity contributes to IFN-I induction of different transcriptional programs in distinct cell types (39). STAT complex formation depends in part on the relative abundance of STAT molecules in the cell (192). While STAT1, STAT2, STAT3, and STAT5 can be activated in most cell populations, STAT4 and STAT6 are mainly activated in lymphocytes (193). For example, quiescent NK cells express more STAT4 than STAT1, leading to constitutive association of IFNAR to STAT4 in these cells. Hence, quiescent NK cells mount pSTAT4 homodimer-dependent responses to IFN-I stimulation, including IFN-γ production and T-bet-driven proliferation (**Figure 8**) (194, 195). Changes in STAT levels can also occur upon the differentiation/activation of a given cell type and lead to a shift in its functional response to the cytokines (196). Upon activation, NK cells decrease their expression of STAT4 and increase that of STAT1, shifting their IFN-I response from STAT4-dependent in a quiescent state to STAT1-dependent in pre-activated cells. This translates into opposite IFN-I effects on IFN-γ production and proliferation for quiescent versus pre-activated NK cells (194). However, this outcome can be modulated by simultaneous exposure to other cytokines such as IL-15 or IL-12/18. A reverse STAT1-to-STAT4 shift occurs in DC during their maturation, shifting their functional responses from inhibition to activation of IL-12 production in response to combined stimulations with IFN-I and CD40L (197). This

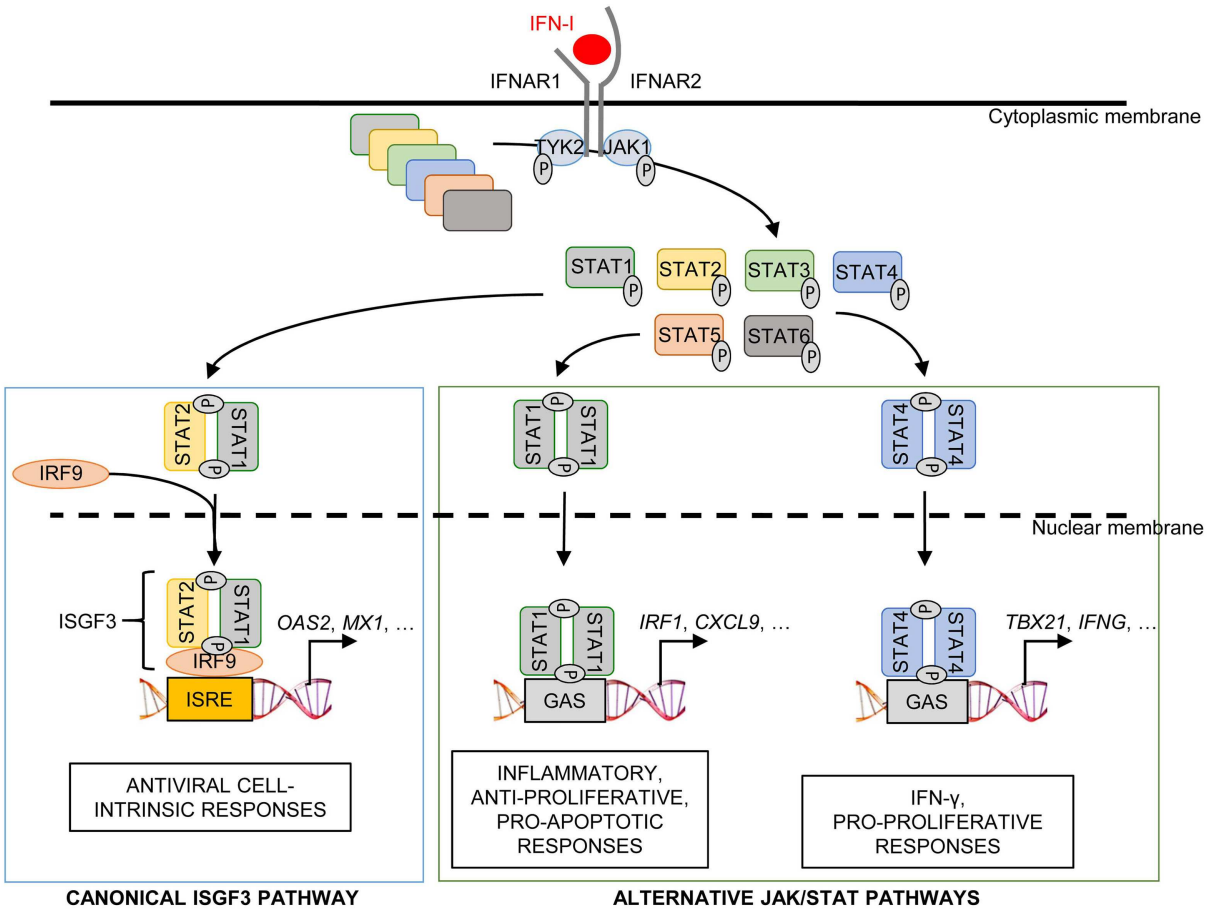

FIGURE 8 | Schematic representation of the ISGF3 and alternative JAK/STAT signaling pathways induced by IFN-I. The receptor for IFN-I, IFNAR, is composed of two chains, IFNAR1 and IFNAR2, which are respectively associated with the JAK family kinases TYK2 and JAK1. IFN-I binding to IFNAR triggers the phosphorylation of TYK2 and JAK1, which in turn phosphorylate a variety of STAT proteins. Activated STATs are able to form complexes, as homo- or hetero-dimers. The heterodimer STAT1-STAT2 binds to a third partner, IFN-regulatory factor 9 (IRF9), in order to form the ISGF3 complex. This complex translocates into the nucleus and binds to specific regulatory sequences, IFN-stimulated response elements (ISRE), to activate the expression of many interferon-stimulated genes (ISGs). In particular, ISGF3 induces most, if not all, of the ISGs encoding effector molecules of cell-intrinsic anti-viral defenses such as OAS or MX1. Alternative JAK/STAT pathways include the formation of STAT1 or STAT4 homodimers, which may drive different functional responses to IFN-I. STAT1 homodimers bind to IFNγ-activated sequences (GAS) in the promoter of certain ISGs, which may promote inflammatory, anti-proliferative, and pro-apoptotic responses. STAT4 homodimers also bind to GAS but promote IFN-γ production and pro-proliferative responses.

enables mature DC to efficiently activate CD8 T cells. Other yet unknown mechanisms control the formation of different STAT complexes in distinct cell types. The nature and dynamics of the signaling pathways triggered by IFN-α or -β were evaluated in bulk cultures of human blood leukocytes using flow cytometry (191) or high throughput mass cytometry (190). A diversity of phosphorylation patterns of STAT1/3/5 was observed upon IFN-I stimulation. IFN-α activation induced phosphorylation of STAT1, STAT3, and STAT5 in most cell types, peaking at 15 min (190). IFN-β-induced STAT1 phosphorylation was found to be poor in B cells as compared to monocytes and T cells (191). However, the underlying mechanism remains to be identified since B cells did not express lower amount of IFNAR2 or STAT1 or enhanced levels of the inhibitory SOCS1 molecule. The high STAT1 activation in monocytes led to their induction of IFN-I-dependent pro-apoptotic genes while this was not the case in B cells. These results strikingly differ from those obtained in the other study upon IFN-α stimulation, where STAT1 phosphorylation was on the contrary lower in $CD14^+$ monocytes and was prolonged in B cells and NK cells (190). The differences between these two studies might have resulted from the use of different subsets and doses of IFN-I. In any case, both studies consistently reported that CD4 T cells showed the highest activation of STAT5. All CD4 T cells but not all CD8 T cells activated STAT5 and for a longer time (190). IFN-β activation of STAT3 was delayed in CD4 T cells and B cells as compared to CD8 T cells and monocytes (191). Different STAT complexes may lead to distinct transcriptional programs linked to different biological functions (**Figure 8**). More systematic studies are needed to understand this complexity. Besides changing STAT levels between cell types or

activation states, the processes controlling differential formation of STAT complexes downstream of IFNAR triggering remain to be identified.

In addition to JAK/STAT signaling, other pathways can be activated downstream of IFNAR, including those involving the phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinases (MAPK), and the CRK adaptor molecules (39, 198). This leads to the activation of other transcription factors such as IRF, NF-κB, or PU.1, which contribute to orchestrate cell responses to the cytokines by regulating both distinct and overlapping sets of genes as compared to STAT (199, 200).

In summary, IFNAR signals through a remarkable diversity of pathways, including but not limited to diverse combinations and kinetics of STAT phosphorylations. This explains at least in part the diversity of IFN-I effects, including their induction of opposite responses depending on the physiopathological contexts and/or the nature of the principal responding cell types (200, 201). IFN-III induce the same signaling pathways as IFN-I, although they engage a different heterodimeric receptor, composed of the IL-28RA and IL-10RB chains and preferentially expressed on epithelial cells including hepatocytes.

THE DIFFERENT IFN-I SUBTYPES HAVE DIFFERENT AFFINITIES FOR THEIR RECEPTOR LEADING TO THEIR INDUCTION OF DISTINCT SIGNALING

In mice and human beings, numerous IFN-I subtypes exist. Functional and population genetic analyses showed that these IFN-I subtypes significantly differ in their functions (202–207). Hence, one of most extraordinary feature of IFN-I biology is how IFN-I subtypes can elicit so many pleiotropic and diverse functions by interacting with the same receptor complex (208).

Both IFNAR1 and IFNAR2 are required for the initiation of IFN-I-dependent signals, as mice deficient in either one are highly susceptible to viral infections (3, 5). The assembling of the IFN-receptor ternary complex is a two-step process. First, a binary complex is formed by the binding of one side of the IFN molecule to IFNAR2. Then, a single binary complex interacts with IFNAR1 via the other side of the IFN molecule. The stability of the ternary complex will be determined in part by the association and dissociation kinetics between the cytokine and the two receptor chains, as well as by IFNAR expression levels since the cell surface concentrations of the receptor subunits are relatively low. Hence, both the affinity of IFN-I subsets for IFNAR and the amounts of IFN-I, IFNAR1, and IFNAR2 will regulate their biological effects (**Figure 9A**) (209, 210). Cell membrane density of IFNAR1 and IFNAR2 is also involved in differential IFN-β- versus IFN-α-induced functional activities, such as anti-proliferative function (211). A variety of cell-intrinsic parameters can also impact the lifetime of the IFN-receptor ternary complex, such as the rate of endocytosis/degradation/recycling of signaling complexes, and negative ISG regulators such as USP18 that decrease the affinity of IFN-IFNAR1 binding (203, 212).

Based on a definition of a prototypic cytokine-receptor binding module and by analogy with the EPO receptor system, IFN-I subtypes were originally postulated to form ternary complexes of differing architectures, resulting in distinct geometry and assembling of intracellular signaling components (213). Experimental evidence rejected this hypothesis. Rather, the differential activities of IFN-I subtypes are determined by the stability of the ligand/receptor ternary complex (207, 212). Differential affinities of the IFN-I subtypes for IFNAR1 and IFNAR2 extracellular domains generate subtype-specific signaling cascades and biological outcomes (**Figure 9A**) (210, 214). Crystal structure of ternary IFN-I/IFNAR1/IFNAR2 complex illuminated the biochemical complexity of IFN-I interaction with their cognate receptors (215). The main conformational features of IFN-I/IFNAR1/IFNAR2 ternary complexes are conserved among the different IFN-I, but are quite different from the other cytokine receptors (214, 215). In the formation of the binary IFN-I/IFNAR2 complex, IFN-I ligand discrimination resides on differential energetics during the interaction of anchor points with IFNAR2, shared by all IFN-I, as well as on key amino acid substitution among IFN-I subtypes (215). IFNAR1 then performs major conformational changes to interact with IFN-I associated in the binary complex, thus displaying an optimized functional plasticity (215). These differences in the chemistry of IFN-I subtype interaction with IFNAR2 and IFNAR1 thus explain the different affinities of IFN-α versus IFN-β within ternary complex and their differential activities (210).

CELL-INTRINSIC RESPONSES OF DISTINCT CELL TYPES DIFFERENTIALLY CONTRIBUTE TO IFN-I EFFECTS IN VARIOUS PHYSIOPATHOLOGICAL CONDITIONS

The functions regulated by IFN-I strongly depend on the main responding cell types (**Figure 9B**). This has been studied *in vitro* by examining the functional consequences of the stimulation of different cell types with IFN-I, and *in vivo* by determining the contribution of cell-intrinsic IFN-I responses of different cell types to resistance or susceptibility to various diseases. An emerging concept is the central role of DC responses to IFN-I for induction of protective immunity against viral infections or tumors (**Figure 3**). The development of mutant mice allowing conditional genetic inactivation of *Ifnar1* in a cell-type specific manner using the Cre-lox system (216) has been instrumental in accelerating our understanding of how different cell types respond to IFN-I *in vivo* and what their respective contribution is to protective or deleterious IFN-I responses. This has been investigated most extensively in viral infections (106, 111, 112, 115, 217) but also in cancer (97, 98), bacterial infections (218), autoimmunity (216, 219), sepsis (220), or inflammatory diseases (221). Efforts are being pursued to better understand which cell types respond to IFN-I in a manner promoting protective versus deleterious effects in different physiopathological settings. That knowledge will considerably help to develop novel strategies to modulate IFN-I functions for promoting health over disease. The development of mutant mice allowing conditional genetic inactivation of *Stat1*, *Stat3*, and *Stat5* (222–226) will help better understanding how different signaling pathways in different cell types determine the outcome of IFN-I response *in vivo* in various conditions. This knowledge might lead to the development of strategies aiming at targeting a given cell type with a specific subset of IFN-I, or in the presence of antagonists of certain signaling pathways, to surgically tune IFN-I responses *in vivo* toward the most desirable outcome.

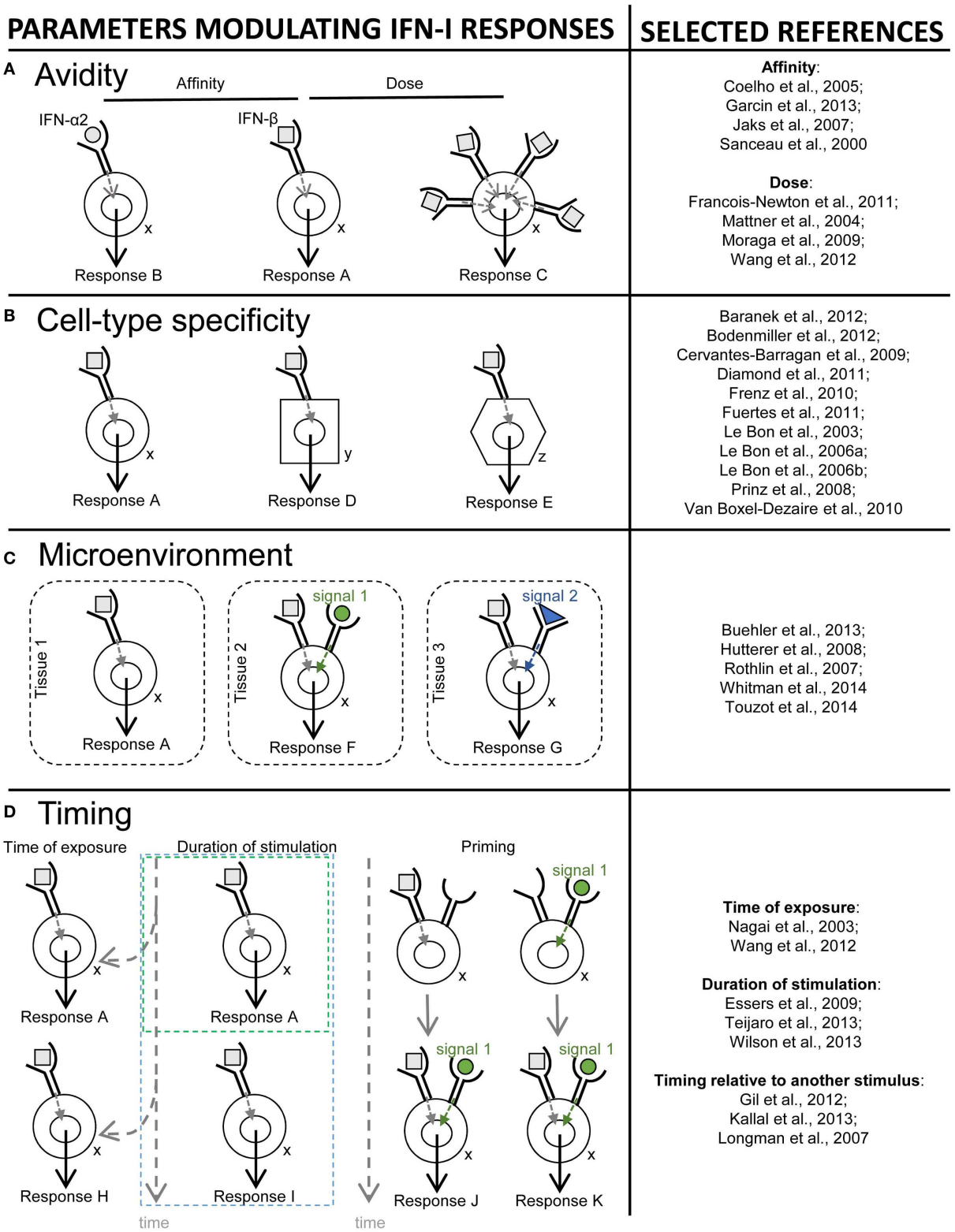

FIGURE 9 | Schematic illustration of different mechanisms controlling the diversity of IFN-I effects. Different parameters that contribute to promote and control the diversity of IFN-I responses are depicted on the left side of the figure. References of papers illustrating each mechanism are given on the right side of the figure. **(A)** Avidity (a combination of affinity and dose). For example, the affinity of IFN-β for IFNAR1 is 100-times higher than that of IFN-α2, and IFNβ is much more potent in inhibiting cellular proliferation or

(*Continued*)

FIGURE 9 | Continued
monocyte differentiation into osteoclasts (response A), while both IFN-I subtypes are equipotent in establishing an anti-viral state (response B). The same subset of IFN-I can also exert different biological effects at low versus high doses. For example, low, but not high, doses of IFN-β protect BALB/c mice from progressive cutaneous and fatal visceral disease after *Leishmania major* infection. **(B)** Cell type specificity. Mouse DC but not NK cells are strong responders to IFN-I, and cell-intrinsic responses to IFN-I are critical in DC but not in some other cell types for immune defenses against viral infections or tumors. **(C)** Tissue microenvironment. The response of a given cell type to a given dose of a specific subset of IFN-I can also be modulated by the microenvironment of the cell. For example, in cancer, protective IFN-I effects on infiltrating DC or other immune cells might be dampened by inhibitors of IFNAR signaling locally produced by the tumor, such as ligands of the TAM receptor tyrosine kinases. **(D)** Timing. Differences in the time and duration of exposure to IFN-I can also determine distinct functional outcomes. For example, during viral infections, early and transient high levels of IFN-I promote protective DC and T cell responses, while delayed, chronic and low level IFN-I production compromises host immune defenses and promotes chronic viral infections. Within a given cell type, the outcome of IFN-I stimulation also depends on time of exposure to these cytokines relative to other modulatory signals (timing relative to other stimuli). For example, in naïve CD8 T cells, TCR signaling prior to IFN-I stimulation leads to increased expression of STAT4 and promotes IFN-γ production and proliferation, while IFN-I stimulation prior to TCR triggering leads to STAT1-dependent anti-proliferative and pro-apoptotic effects.

THE ANATOMICAL LOCATION AND TIMING OF CELL EXPOSURE TO IFN-I MIGHT ALSO BE MAJOR PARAMETERS CONTROLLING THE EFFECTS OF THE CYTOKINES

The formation of specific STAT complexes is a highly dynamic process. It depends not only on the cell type but also on its specific state at the time it sees IFN-I. Hence, major parameters controlling the effects of IFN-I in a given cell type also include its microenvironment (**Figure 9C**) and the timing of its exposure to the cytokines both in terms of duration of the stimulation and of previous activation history (**Figure 9D**).

The TAM receptor ligand Gas6 is expressed within tumor cells in various solid cancers (227, 228). Elevated Gas6 expression is of bad prognosis in different cancers (228, 229). In a mouse model of ovarian cancer, early during tumorigenesis tumor-infiltrating DCs were found to be immunogenic and promote antitumor immunity, but they were later altered in the course of tumor development to acquire immunosuppressive properties beneficial to the tumor (230). One may thus hypothesize that expression of TAM soluble ligands in certain tumors and of TAM receptors on tumor-infiltrating DCs might contribute to dampen DC response to IFN-I and therefore facilitate their polarization by the tumor microenvironment into immunosuppressive cells (**Figure 9C**).

Acute versus chronic exposure to IFN-I can lead to strikingly opposite effects on a given cell type (13, 14, 231). In addition to duration, the time when a cell is exposed to IFN-I can also dramatically impact its functional response, depending on its previous activation history (**Figure 9D**). *In vitro* stimulation of DCs with IFN-β can lead to opposite outcomes depending whether it occurs simultaneously to, or after, TNFα-induced maturation. IFN-β polarizes DCs toward Th1 induction in the former case, and toward IL-10-secreting T cells in the latter case. These opposite effects result at least in part from the differential expression of IL-12/18 by DCs (232). Similarly, IFN-I effect on the functional polarization of CD4 T cells is strongly modulated by the other cytokines present in the lymphocyte microenvironment at the same time (233). IFN-I can also mediate opposite effects on CD8 T cells depending whether it occurs before or after cognate engagement of the T cell receptor. Indeed, while CD8 T cells have the potential to respond to IFN-I by inducing both STAT1- and STAT4-dependent genes, this depends upon their activation history. Naïve CD8 T cells respond mostly to IFN-I through STAT1 signaling, leading to the inhibition of their proliferation and eventually to the induction of their apoptosis. However, cognate triggering of the T cell receptor causes a decrease in STAT1 and an increase in STAT4 expression in CD8 T cells. This leads to a shift of their IFN-I response from STAT1-to-STAT4 signaling, resulting in the promotion of their proliferation and IFN-γ production. During LCMV infection, this mechanism promotes STAT4-dependant expansion of anti-viral CD8 T cells, but STAT1-dependant inhibition of naïve CD8 T cell proliferation (234).

INNOVATIVE BIOCHEMICAL ENGINEERING APPROACHES TO TUNE IFN-I EFFECTS

Since the late 70s the clinical potential of IFN-I for the treatment of patients suffering of viral infection or cancer diseases has been widely acknowledged (235). Today, this expectation is tempered because IFN-I treatment can induce severe side effects and sufficient doses cannot be administered in patients. Therefore, there is a strong need to create tuned IFN molecules devoid of side effects. Based on our current understanding of IFN-I responses as reviewed above, many parameters could be tuned individually or in a combined manner to modulate IFN-I activity to promote their beneficial effects over the deleterious ones in a number of diseases. These parameters include modifying the affinity of IFN-I for its receptor, playing with the local quantity/concentration of IFN-I and with the duration of its delivery, and modulating the nature of the cells that are responding to IFN-I. We will discuss here novel strategies being developed to deliver IFN-I to, or block IFN-I responsiveness of, a specific target cell type *in vivo* (**Figure 10**).

MODULATING IFN-I FUNCTIONS BY ALTERING THEIR BINDING TO THEIR RECEPTOR THROUGH ADVANCED BIOCHEMICAL ENGINEERING

If IFN-I-induced side effects are a consequence of the pleiotropic nature of IFN-I, and if the bioactivities mediating deleterious effects have some degree of independence from those mediating beneficial effects, one could mutate the IFN-I molecules in order to skew their activity toward a desired bioactivity. Indeed, introducing key mutation in IFN-α2 allowed increasing its affinity to IFNAR1 by a factor of 100. Accordingly, this IFN-α2 mutant is 100-times more potent in inhibiting cell proliferation, but as potent as WT IFN-α2 in inducing an anti-viral state (236–238). Hence, it is possible to tune IFN activity by modifying its binding to IFNAR. However, translating such an approach for the design of molecules for clinical application is severely hampered by the poor understanding we have on the IFN-I bioactivities mediating the side effects. Furthermore, we are far from having established the list of bioactivities that could be differentially modulated by changing the stability of the IFN-I/IFNAR complex. We know more about the

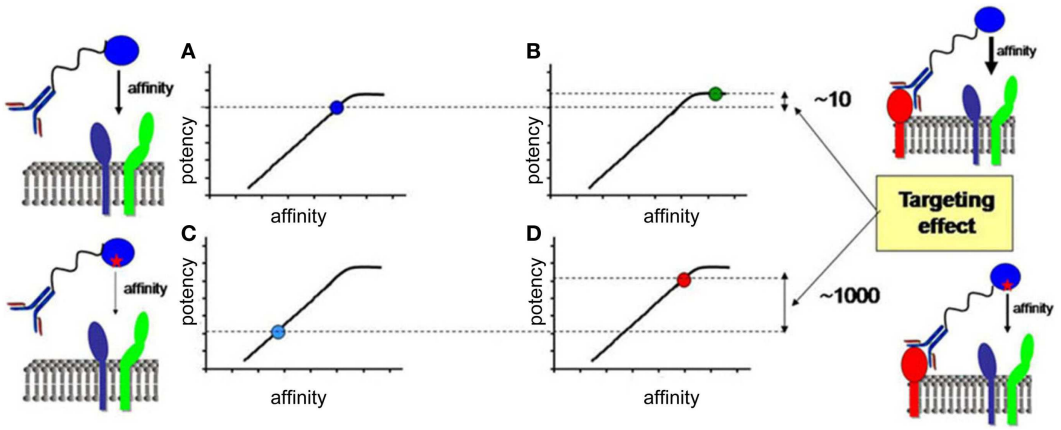

FIGURE 10 | Strategy for high efficiency cell type-specific targeting of cytokine activity. **(A)** Most cytokines have evolved to exhibit optimized specific activities. **(B)** When the antibody moiety of a classical immunocytokine binds its cellular target, the ensuing increase of cytokine-receptor avidity translates into a modest increase of cytokine potency. **(C,D)** By introducing a mutation that decreases the affinity of the cytokine for its receptor, the activity of the mutated immunocytokine is now focused with a very high efficiency on target cells.

cell types that mediate beneficial versus deleterious IFN responses in various diseases. Hence, we will now discuss strategies aimed at focusing IFN activity to specific cell types to promote health over disease.

CELL SPECIFIC TARGETING OF EXOGENOUS IFN-I

Several strategies have been developed to specifically target IFNs on tumor cells, tumor-infiltrated immune cells or infected tissues. These strategies include intra-lesional injection (239, 240), adenoviral-mediated gene transfer (241–243), engineered tumor-infiltrating monocytes (244), and fusion of IFNs with a cleavable protecting shell (245). Another strategy to increase cytokine accumulation within the tumor or infected tissue is antibody-mediated targeting of cytokine delivery, where a cytokine moiety is fused to an antibody directed against a specific cell surface marker (**Figure 10**). The fusion molecule retains both antigen-binding and IFN-I bioactivities, and is enriched at the targeted site upon *in vivo* injection (246–249). When targeted to human CD20, IFN-I inhibited the proliferation of lymphoma cells engrafted in immunodeficient mice (250). An IFN-I targeted to a tumor antigen can also amplify the therapeutic effect of the antibody by acting on tumor-infiltrated DCs, thus increasing antigen cross-presentation and antitumor cytotoxic T cell responses (249). On non-targeted cells, the antibody conjugation negatively impacts IFN-I potency, but only modestly (18, 248, 251) (**Figure 10A**). Fusion molecules generally retain full IFN-I biological activity on the cells expressing the antibody target (**Figure 10B**). Hence, this difference only leads to a modest ratio between the IFN-I specific activity measured on target and non-target cells (**Figure 10B**). Such a targeting efficiency is definitely too low to reduce the toxic effect of IFN-I administration, because it will not specifically focus IFN-I activities on "beneficial cells" without stimulating "deleterious cells."

The engineering of immuno-IFN-I must be improved to reach the very high targeting efficacy required to significantly diminish the treatment side effects. We recently reported an innovative strategy reaching this goal (18). It is based on the postulate that the antibody moiety of an immuno-IFN-I stabilizes the IFN-I/receptor-complex by avidity. It also takes into account the fact that the biological potency of an IFN-I is proportional to the stability of the IFN-I/receptor complex up to a certain threshold beyond which increasing the stability does not increase its potency (238, 252). IFN-α2 and IFN-β are used in most immuno-IFN-I studies. They have evolved to retain close to maximal potency. Hence, their targeting by an antibody that only provides a modest gain in terms of biological potency. However, it is expected that decreasing the affinity of the IFN-I for its receptor, by introducing a mutation, would increase the targeting effect of the antibody (**Figures 10 C,D**). This is indeed the case. Using an IFN-I with a single point mutation that dramatically decreases its affinity for IFNAR2 (**Figure 10C**) allows engineering immuno-IFNs that are up to 1000-fold more potent on cells expressing the antibody target (**Figure 10D**). The three log targeting efficiency of these novel types of immuno-IFNs is found for various activities measured *in vitro* or *in vivo* when delivered in mice. If the toxic side effect experienced by the patients treated with IFN-I is due to systemic IFN-I activity, this targeting technology may find considerable clinical applications since such engineered immuno-IFNs are virtually inactive while "en route" and are activated only after binding of the fused antibody to the desired target. It remains to define the useful targets according to pathologies, for example, tumor cells themselves and professional cross-presenting XCR1$^+$ DCs for cancer (97, 98, 249), or hepatocytes for chronic HCV infection.

CELL SPECIFIC BLOCKADE OF ENDOGENOUS IFN-I

To treat autoimmune diseases, novel therapeutics targeting IFN-I have been developed, including two IFN-α-neutralizing monoclonal antibodies currently in clinical trials (Sifalimumab and Rontalizumab) (253, 254). However, long-term systemic neutralization of IFN-I activity may increase susceptibility to viral infection and tumor development. Alternative strategies are needed to specifically inhibit IFN-I deleterious effects in these diseases without globally compromising IFN-I anti-viral and

anti-tumoral functions. The sequential nature of the assembling of the IFN-I/receptor complex opens the possibility to design IFN-I antagonists specifically targeting the cell subsets responsible for IFN-I deleterious effects.

An IFN-α2 carrying a single amino acid substitution that blocks the IFN-I/IFNAR1 interaction engages IFNAR2 in a complex, which cannot bind IFNAR1 (255). Since the binary IFN-I/IFNAR2 complex is devoid of any IFN-I activity, such mutant behaves as a potent IFN-I antagonist. When linked to an antibody specific for a cell surface marker, the antagonistic activity of the mutant IFN-I should be significantly reinforced specifically on the cells expressing the target. Hence, it should be possible to design and construct targeted antagonists that inhibit responsiveness to endogenous IFN-I specifically on the cell subsets on which the cytokines act to promote autoimmunity or severe side effects, leaving the other cells fully responsive. For example, in chronic HCV patients treated with Peg-IFN-α, one of the most deleterious side effects is nervous depression, which might be prevented by co-administration of an IFN-I antagonist specifically targeting neurons or other cells of the central nervous system.

CONCLUSION

In the last decade, several major technological breakthroughs and the generation of novel animal models have remarkably advanced our understanding of the mode of action of IFNs. *In vitro* high throughput screening allowed systematically studying the functions of ISGs by ectopic expression or knock-down. Advance biophysical investigation of the interactions between IFN-I and the IFN-I receptor allowed to rigorously investigate the mechanistic basis for the differential bioactivities of IFN-I subtypes. The analyses of the responses of different cell types to IFNs or to viral infection, *in vitro* but also *in vivo* in various pathologies, demonstrated that IFN-I often mediate beneficial versus deleterious roles by acting on different cell types. From integrative analysis of these data, a picture is now emerging suggesting that it will be possible to segregate protective from deleterious IFN-I effects, based (i) on their differential induction depending on IFN-I subsets or on the magnitude/timing of IFN-I production, (ii) on their conditioning in different tissues, (iii) or on their occurrence in different cell types. Hence, innovative immunotherapeutic treatments are being designed to tune IFN-I activity toward desired effects in order to promote health over disease in a manner adapted to each physiopathological condition. In particular, a proof-of-concept has been made *in vitro* that it will be possible to target IFN-I activity on given cell types or tissues to administer to patients sufficiently high doses of the cytokine at the site of interest while limiting unwanted effects in other tissues or cell types. The next steps will be to demonstrate efficacy of this strategy *in vivo* in preclinical animal models. Importantly, to foster the development of these innovative immunotherapies, major efforts are still warranted to continue delineating which cell types are mainly responsible for the protective versus deleterious effects of IFN-I in different diseases. Combining high throughput technologies and systems biology approaches will also advance our understanding of the molecular mechanisms dynamically controlling IFN-I responses in health and diseases, which should reveal potentially novel therapeutic targets.

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