Type I Interferon Inhibits Interleukin-1 Production and Inflammasome Activation

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SUMMARY
Type I interferon (IFN) is a common therapy for autoimmune and inflammatory disorders, yet the mechanisms of action are largely unknown. Here we showed that type I IFN inhibited interleukin-1 (IL-1) production through two distinct mechanisms. Type I IFN signaling, via the STAT1 transcription factor, repressed the activity of the NLRP1 and NLRP3 inflammasomes, thereby suppressing caspase-1-dependent IL-1β maturation. In addition, type I IFN induced IL-10 in a STAT1-dependent manner; autocrine IL-10 then signaled via STAT3 to reduce the abundance of pro-IL-1α and pro-IL-1β. In vivo, poly(I:C)-induced type I IFN diminished IL-1β production in response to alum and Candida albicans, thus increasing susceptibility to this fungal pathogen. Importantly, monocytes from multiple sclerosis patients undergoing IFN-β treatment produced substantially less IL-1β than monocytes derived from healthy donors. Our findings may thus explain the effectiveness of type I IFN in the treatment of inflammatory diseases but also the observed “weakening” of the immune system after viral infection.

INTRODUCTION
In response to cytosolic viral invasion, activation of intracellular RIG-I-like receptors triggers interferon-β (IFN-β) production (Meylan et al., 2006). Specialized immune cells can also produce type I IFN (both IFN-α and IFN-β) in response to extracellular stimuli of viral or bacterial origin by toll-like receptor (TLR) engagement. For example, conventional dendritic cells (cDCs) and macrophages produce IFN-α and IFN-β in response to TLR3 and TLR4 stimulation. In addition, plasmacytoid dendritic cells (pDCs), a specialized type of IFN-producing antigen-presenting cell (APC), can also produce type I IFN in response to TLR-7 and TLR-9 ligands (Meylan et al., 2006; Stetson and Medzhitov, 2006).

Type I IFNs bind to a common receptor, the type I IFN receptor (IFNAR), composed of two subunits (namely IFNAR1 and IFNAR2), which are associated with the Janus kinases (JAKs) tyrosine kinase 2 (TYK2) and JAK1. Activation of TYK2 and JAK1 causes phosphorylation of signal transducers and activators of transcription-1 (STAT1) and STAT2, leading to the formation of a trimeric complex composed of phosphorylated STAT1 (pSTAT1), pSTAT2, and IFN regulatory factor 9 (IRF9). This complex translocates to the nucleus where it binds to IFN-stimulated response elements (ISREs), motifs present in promoters and enhancers of interferon-stimulated genes (ISGs), to regulate transcription. Moreover, pSTAT1 homodimers, STAT3, and STAT5 can play a role downstream of IFNAR (Platianis, 2005).

Particular attention has been paid to the anti-inflammatory effects exerted by type I IFN, demonstrated by two lines of evidence (Billiau, 2006; Theofilopoulos et al., 2005). First, patients recovering from a primary viral infection are often more susceptible to secondary infection. In such circumstances, an immunosuppressive role of α- and β-IFNs was suggested by several reports (Decker et al., 2005; Jensen et al., 1992; Shahan-gian et al., 2009). Second, a number of studies show the effectiveness of this family of cytokines in reducing inflammation in different experimental settings and, most importantly, type I IFN is successfully used in the clinic, not only for the treatment of diseases of viral origin but also for the management of diseases such as multiple sclerosis (MS) (Barkhof et al., 2007; Billiau, 2006; Comi et al., 2001; Giovannoni and Miller, 1999). For patients suffering from MS in the relapsing-remitting phase (RR-MS), IFN-β markedly attenuates the course and the severity of the disease, contributing to the maintenance of the blood-brain barrier integrity and reducing the occurrence of relapses. More recently, IFN treatment has also been successfully used for patients suffering from familiar Mediterranean fever (FMF) and from Behcet’s syndrome, two inflammatory disorders linked to IL-1 overproduction (Kötter et al., 2004; Tweezer-Zaks et al., 2008).

IL-1β and IL-1α are two potent proinflammatory cytokines, which share a common IL-1 receptor (IL-1R). IL-1β is synthesized as an inactive precursor, pro-IL-1β, which requires cleavage by caspase-1 in order to attain its active form (indicated as p17, because of its molecular weight of 17 kDa), whereas
IL-1β does not require processing for activity (Mosley et al., 1987). Caspase-1 activation is accomplished within a protein complex known as “inflammasome,” which comprises, beside caspase-1 and its substrate pro-IL-1β, a sensor protein (Martinon et al., 2002). To date, four different sensors able to induce inflammasome platform assembly have been identified, NLRP1b, NLRP3, IPAF (NLRC4), and AIM2, which trigger the formation of the inflammasome in response to distinct stimuli (Martinon et al., 2009; Schroder et al., 2009).

Though IL-1β contributes to the control of several pathogenic infections, such as Salmonella typhimurium and Candida albicans (Belloccchio et al., 2004; Gross et al., 2009; Lara-Tejero et al., 2006), an excessive production of this cytokine has been associated with several autoinflammatory and/or autoimmune conditions such as FMF or cryopyrin-associated periodic syndromes (McDermott and Tschopp, 2007).

Despite the growing use of type I IFN in the clinic, the mechanisms underlying its protective effects in autoimmune and inflammatory disorders are still poorly understood. Some reports propose a role for IFN-β in altering integrin and matrix metalloproteinase expression or activity, thereby reducing leukocyte infiltration to the site of inflammation (Billiau, 2006). IFN-β was also shown to alter the production of several cytokines involved in T cell polarization or in inflammation, including IL-1β (Billiau, 2006; Coclet-Ninin et al., 1997; Huang et al., 1995; Masters et al., 2010; Zang et al., 2004). However, the mechanisms explaining this observation and their relevance to the clinic have never been thoroughly assessed. Specifically, the effects of type I IFN on inflammasome-dependent cytokine maturation have not been investigated. We therefore examined the possible role of type I IFN in controlling inflammasome activity and IL-1β production, which might account for both the efficacy of IFNs in therapeutic settings and the immunosuppressive effects of this cytokine after viral infection. We indeed found that type I IFN strongly suppressed IL-1β production. This was due to STAT1-dependent inhibition of NLRP3 and NLRP1 inflammasome activity. In addition, in bone marrow-derived macrophages (BMDMs), type I IFN enhanced the production of IL-10, which in turn decreased the levels of pro-IL-1β and pro-IL-1β. In vivo, pro-IL-1β induced by aluminum salts and Candida albicans was suppressed by type I IFN, rendering mice highly susceptible to Candida infection. Moreover, monocyes derived from IFN-β-treated MS patients showed decreased IL-1β production in response to inflammasome stimulation, recapitulating the suppressive effects of type I IFN observed in vitro and in vivo in the murine model.

RESULTS

IFN-β Suppresses Both Pro-IL-1β Availability and IL-1β Maturation

To assess the anti-inflammatory effects of type I IFN on IL-1β production, we incubated BMDMs for 12 hr with IFN-β. Cells were then primed with lipopolysaccharide (LPS) for 4 hr in order to induce pro-IL-1β synthesis and stimulated with alum to activate IL-1β maturation via the NLRP3 inflammasome. We found that IFN-β blocked the secretion of IL-1β by suppressing the activation of both caspase-1 and the intracellular pro-IL-1β pool (Figure 1A). Inflammasome inhibition and pro-IL-1β reduction appeared within a few hours of IFN-β stimulation (Figure S1A available online) and could not be ascribed to cell death (Figure S1B). Furthermore, by using fn1r1-deficient BMDMs, we ensured that the effects of IFN-β on inflammasome activity and pro-IL-1β were specific to its receptor (Figure 1B). We next asked whether type I IFN could exert similar effects also on bone marrow-derived dendritic cells (BMDCs) and found that caspase-1 activation was inhibited by both IFN-β and IFN-γ, whereas the suppressive effect on pro-IL-1β was not observed in this cell type (Figure 1C). Thus, depending on the cell type, type I IFN can regulate the production of the proinflammatory cytokine IL-1β at two levels: by inhibiting inflammasome function and by reducing the pool of intracellular pro-IL-1β.

In order to rule out the possibility that IFN-β-dependent inflammasome suppression was due to interference with LPS priming, NLRP3 inflammasome activity was assayed in cells without prior priming and in cells that were primed with LPS for 4 hr. As shown in Figure 1D, inflammasome-mediated caspase-1 activation was inhibited by IFN-γ and -β independently of priming, whereas IFN-γ failed to decrease inflammasome function and intracellular levels of pro-IL-1β under these conditions. Nevertheless, when overnight LPS priming was employed, suppression by type I IFN was most prominent and IFN-γ also displayed inhibitory activity (Figure S1C). In an attempt to determine the mechanisms mediating the observed cross talk between LPS and IFNs, we assessed IFN receptor-proximal signaling and the amounts of signal transducing factors (Figure S1D). No significant differences were observed, suggesting that the diverging responses to type I and II IFNs observed upon different priming regimes are due to downstream integration events of LPS and IFN-β signaling pathways.

Not surprisingly, the secretion of the caspase-1-dependent cytokines IL-1β, IL-18, and IL-1α was strongly diminished when BMDM were pretreated with IFN-β (Figure 1E). In contrast, the amounts of secreted TNF were not significantly altered (Figure S1E), whereas the expression of CD40 and CD86 were even enhanced by IFN-β (Figure S1F). Together, these results show that IFN-β decreases IL-1 and IL-18 production, though it does not affect BMDM activation in a general way.

IFN-β Inhibits NLRP1- and NLRP3-Triggered Inflammasome Activity

To determine whether the suppression exerted by IFN-β specifically affected alum-dependent NLRP3 inflammasome activity, we tested other NLRP3 agonists and found that NLRP3 inflammasome activity after monosodium urate crystals (MSU), asbestos, nigericin, ATP, and C. albicans was repressed by IFN-β, similar to alum (Figure 2A).

In order to define whether the effect of IFN-β were confined to NLRP3 or extended to other inflammasome types, we assessed the effects of IFN-β pretreatment on the NLRP1b, IPAF, and AIM2 inflammasomes by activating these inflammasomes with B. anthracis lethal toxin (LeTx), S. typhimurium, or intracellular delivery of poly(deoxyadenylc-thymidylic) acid (poly(dA:dT)), respectively. Similar to the NLRP3, NLRP1b-dependent inflammasome activity was inhibited by IFN-β (Figure 2B), whereas IPAF-inflammasome and ASC-dependent but NLRP3-independent AIM2-inflammasome were unaffected by IFN-β pretreatment (Figures 2C and 2D). As expected, in all cases mature IL-1β amounts were diminished by IFN-β, reflecting the
Type I IFN Inhibits IL-1 Production

![Image](70x359 to 351x627)

**Figure 1. IFN-β Decreases Inflammasome Activity and Pro-IL-1β**

(A and B) BMDMs were incubated 12 hr with the indicated doses of IFN-β. Thereafter, LPS was added to prime BMDMs and, 4 hr later, alum stimulation was performed. IL-1β and caspase-1 activation and release were assessed by immunoblot. (B) BMDMs of wild-type (WT) and of Ifnar1−/− origin were used. (C) As for (A), but with BMDCs instead of BMDMs and IFN-β or IFN-α.

(D) BMDMs were incubated overnight with IFN-β, IFN-α, or IFN-γ. LPS was either not added, or added the last 4 hr preceding alum stimulation. (E) BMDMs were treated with IFN-β overnight for 12 (o/n) or for 5 hr (5h) and primed with LPS for 4 hr. Cells were then stimulated with alum and cytokines measured by ELISA. Data represent mean ± SD of three individual experimental points.

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<th>IFN-β (U/ml)</th>
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<td><a href="#">Alum</a></td>
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**Type I IFN-Induced IL-10 Controls IL-1β and IL-1α Precursor Levels**

A potential role for STAT3 in the suppressive effects downstream of IFNAR was suggested by the inflammatory phenotype of Stat3 myeloid-specific conditional deleted mice (LysMcre Stat3^flox/−^) (Takeda et al., 1999). Though type I IFN-dependent caspase-1 inhibition was unaltered by Stat3 deletion when compared to control Stat3^flox/−^ BMDMs (Figure 3A), the reduction of intracellular pro-IL-1β and pro-IL-1α was much less prominent in Stat3-deficient cells (Figure 3A). This suggested that type I IFN induces an inhibitory factor, which is likely to signal via STAT3, that diminishes IL-1β and IL-1α precursor amounts.

Given that the evidence for STAT3 involvement in IFNAR signaling is not compelling and that STAT3 is known to be involved in signaling downstream of IL-10 receptor (IL-10R), we tested the contribution of the anti-inflammatory cytokine IL-10 to the reduction of IL-1 precursor protein. We found that the suppression of LPS-induced pro-IL-1β and pro-IL-1α amounts induced by 5 hr or overnight incubation with IFN-β was strongly abolished in Ifnar1−/− BMDMs (Figure 3B; Figure S2). In line with this, exogenous IL-10 decreased the abundance of IL-1β and IL-1α precursor proteins in wild-type (WT) cells (Figure 3C), whereas this was not the case in LysMcre Stat3^flox/−^ BMDMs, suggesting a crucial role for STAT3 in the IL-10-mediated reduction of pro-IL-1β and pro-IL-1α amounts. Ifnar1−/− and BMDMs lacking the IFN key transcription factor STAT1 (Stat1−/−) diminished IL-1 precursor amounts in response to IL-10, but failed to do so upon type I IFN (Figure 3C), indicating that IL-10 production is downstream of IFNAR-triggered pathway. Taken together, these results suggest a model in which type I IFN induces secretion of IL-10, which subsequently downregulates pro-IL-1 expression through activation of IL-10R and STAT3.

In support of such a mechanism, we found strong IL-10 secretion by WT and conditional LysMcre Stat3^flox/−^ BMDMs upon type I IFN plus subsequent LPS exposure, which was not apparent in Ifnar1−/− and Stat1−/− cells (Figure 3D).

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**Table:**

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Type I IFN Inhibits NLRP3 Inflammasome Activation in a STAT1-Dependent Manner

Next, we tested whether inflammasome inhibition by type I IFN was mediated via the IFNAR-STAT1 pathway. Indeed in Stat1−/− cells, type I IFN could no longer inhibit caspase-1 activation (Figure 4A).

STAT1 can be phosphorylated at residues tyrosine 701 or serine 727. Tyrosine phosphorylation is required for nuclear translocation of STAT1 and transcription factor function (Platania, 2005). We therefore tested whether type I IFN efficiently suppressed the inflammasome in BMDMs in which STAT1 can be exclusively phosphorylated at the tyrosine residue (in which serine 727 is substituted with an alanine residue (Stat1S727A)). Our results indicate that serine phosphorylation is dispensable (Figure 4B), suggesting that tyrosine phosphorylation is sufficient for inflammasome inhibition.

Next, we asked whether the inflammasome inhibitory factor was secreted. We tried to rescue type I IFN-dependent inflammasome inhibition in Ifnar1−/− BMDMs by coculturing these cells with caspase-1-deficient (Casp1−/−) BMDMs, which should normally respond to IFN. As shown in Figure 4C, Casp1−/− cells were unable to restore inflammasome inhibition in Ifnar1−/− cells, suggesting that the inhibitory factor is unlikely to be secreted. Because the known inflammasome components NLRP3, ASC, and caspase-1 also appeared not to be downregulated by type I IFN (Figure 4D), we hypothesized that an intracellular negative regulator of the inflammasome may be induced by STAT1. Several proteins have been suggested to function as caspase-1 or inflammasome inhibitors, including the proteinase inhibitors serpin peptidase inhibitor, clade B, member 9 (Serpinb9, also called SPI-6) and Serpinb2 (also known as PAI-2), caspase-12, pyrin (also called mediterranean fever), and the Bcl family members Bcl-2 and Bcl-XL (also known as BCL2-like 1) (Guarda and So, 2010). We examined whether these candidates were upregulated by type I IFN (by using as a control the IFN-inducible chemokine CXCL10) or whether their deletion affected inflammasome activity (Figures S3A and S3B). However, none of these proteins were significantly induced by type I IFN or were essential for IFN-mediated inhibition of the inflammasome.

Taken together, our results indicate that STAT1 inhibits NLRP3-mediated caspase-1 activation by an as yet undefined mechanism.

Type I IFN Suppresses IL-1-Dependent Inflammatory Cell Recruitment In Vivo

We next asked whether the effects of in vitro IFN treatment on IL-1 production were similar in vivo. We pretreated mice intravenously (i.v.) with polyinosinic-polycytidylic acid (poly(I:C)), a synthetic RNA analog that strongly induces type I IFN production, and subsequently injected them with alum intraperitoneally (i.p.), thus inducing an IL-1-dependent peritonitis that is lost in Il1r1−/− mice, as illustrated in Figure S4A. Peritoneal exudate cells (PECs) were collected and cytokine content in the lavages was measured. We found that alum challenge upregulated mature IL-1β secretion in the lavage fluid and that this was prevented by poly(I:C) pretreatment (Figure 5A). We also observed that pro-IL-1β amounts were reduced in PEC extracts from mice treated with poly(I:C) (Figure 5B), whereas pro-IL-1α was undetectable (data not shown). All of the aforementioned effects were not observed in Ifnar1−/− animals (Figures 5A and 5B), indicating that the effects elicited by poly(I:C) treatment are dependent on type I IFN.

We consequently analyzed alum-induced recruitment of inflammatory cells in control mice or in mice pretreated i.v. with poly(I:C). The number of total PECs recruited upon alum challenge was strongly reduced in mice pretreated with poly(I:C) (Figure 5C). In line with this observation, neutrophil and Ly6C"
monocyte recruitment was significantly blocked by poly(I:C) pretreatment (Figures 5D and 5E). In order to exclude the probability that the observed reduction was due to a general defect induced by poly(I:C) on migratory cells, zymosan, which is known to induce a peritonitis independently of IL-1, was used in parallel (Figures 5D and 5E). In order to exclude the possibility that the observed reduction was due to a general defect induced by poly(I:C) on migratory cells, zymosan, which is known to induce a peritonitis independently of IL-1, was used in parallel (Figures 5D and 5E). In order to exclude the possibility that the observed reduction was due to a general defect induced by poly(I:C) on migratory cells, zymosan, which is known to induce a peritonitis independently of IL-1, was used in parallel (Figures 5D and 5E).

Again, the effects of poly(I:C) were specific to type I IFN, as shown by the fact that poly(I:C) pretreatment did not alter the recruitment of total PECs and neutrophils and affected Ly6C+ monocytes were still recruited to the peritoneum despite the poly(I:C) pretreatment, further demonstrating that poly(I:C) suppresses monocyte influx only in a minor proportion in Ifnar1−/− mice (Figures 5F–5H).

It was reported that poly(I:C) itself can induce IL-1β through activation of the NLRP3 inflammasome (Allen et al., 2009; Kangnegati et al., 2006). Although we can confirm that poly(I:C) primes cells for pro-IL-1β synthesis, particularly in vitro, we found no evidence for direct inflammasome activation by IFN-β

Figure 4. Inflammasome Inhibition by Type I IFN Is STAT1 Dependent
(A and B) C57BL/6 (WT), Stat1−/− (A), and Stat1S727A knockin (S727A) (B) BMDMs were incubated 12 hr with IFN-β or IFN-α. Thereafter, LPS was added to prime the BMDMs and, 4 hr later, alun stimulation was performed. Caspase-1 activation and IL-1β release were shown in supernatants, pro-IL-1β, pro-IL-1α, and pro-IL-1α levels in cell lysates.

(C) BMDMs from WT, Ifnar1−/−, or Caspase1−/− (Casp1−/−) were cultured alone or cocultured as indicated. Cells were incubated 12 hr with IFN-β or IFN-α, followed by 4 hr LPS and alun stimulation. Caspase-1 activation was assessed by immunoblot in culture supernatants.

(D) BMDMs were stimulated for the indicated times with LPS, IFN-β, or a combination of the two. Expression of inflammasome components was assessed by immunoblot in cell lysates.

Data are representative of two to four individual experiments.

Figure 3. Type I IFN Regulates Pro-IL-1β Levels via IL-10 and STAT3
(A) BMDMs from control (Stat3flox/−) or LysMcre (cre) mice were incubated 12 hr with IFN-β or IFN-α. Thereafter, LPS was added to prime the BMDMs and, 4 hr later, alun stimulation was performed. Caspase-1 activation and IL-1β release are shown in supernatants, pro-IL-1β, pro-IL-1α, or pro-IL-1α levels in cell lysates.

(B) BMDMs from WT or IL10−/− mice were incubated 5 hr with IFN-β, followed by 4 hr LPS treatment. Pro-IL-1β and pro-IL-1α levels were assessed in cell lysates.

(C and D) BMDMs from WT, Ifnar1−/−, Stat1−/−, LysMcre (cre) mice were incubated 5 hr with IFN-β, IFN-α, or IL-10 (C), followed by 4 hr LPS treatment. Pro-IL-1β and pro-IL-1α levels in cell lysates were measured by immunoblot (O). IL-10 levels by ELISA (D). Data represent mean ± SD of three individual experimental points (D).

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Type I IFN Inhibits IL-1 Production

Figure 2. Inflammasome Inhibition by Type I IFN Is STAT3 Dependent
(A) C57BL/6 (WT), Stat3−/−, or Stat3flox/− (cre) mice were incubated 12 hr with IFN-β or IFN-α. Thereafter, LPS was added to prime the BMDMs and, 4 hr later, alun stimulation was performed. Caspase-1 activation and IL-1β release were shown in supernatants, pro-IL-1β, pro-IL-1α, or pro-IL-1α levels in cell lysates.

(B) BMDMs from WT, Ifnar1−/−, Stat1−/−, LysMcre (cre) mice were incubated 5 hr with IFN-β, IFN-α, or IL-10 (C), followed by 4 hr LPS treatment. Pro-IL-1β and pro-IL-1α levels in cell lysates were measured by immunoblot (O). IL-10 levels by ELISA (D). Data represent mean ± SD of three individual experimental points (D).

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poly(I:C) in vitro (Figures S4B and S4C). We also saw no evidence for poly(I:C)-dependent inflammasome activation in vivo (Figure 5D), because i.p. injection of poly(I:C) did not trigger neutrophil influx, a hallmark of IL-1 production (Chen et al., 2006).

**Type I IFN Increases Susceptibility to C. albicans Infection**

Treatment of mice with poly(I:C) impairs their survival to C. albicans infection (Jensen et al., 1992; Worthington and Hascenclever, 1972). Given the importance of IL-1 in the control of this infection (Figure S5A; Bellocchio et al., 2004), we wondered whether under those circumstances, poly(I:C)-induced type I IFN might decrease IL-1, thus explaining increased susceptibility to C. albicans. We compared survival after C. albicans challenge of naive or poly(I:C)-pretreated WT mice and Ifnar1−/− mice. Poly(I:C)-primed WT mice showed dramatically increased susceptibility to C. albicans infection as compared to unprimed mice (Figure 6A), whereas poly(I:C)-primed Ifnar1−/− mice survived C. albicans challenge similar to unprimed animals (Figure 6B). The premature death of WT poly(I:C)-treated animals was found...
to be due to a defective control of C. albicans, as indicated by the fact that kidneys of these animals contained significantly more colony forming units (CFU) than the kidneys of unprimed WT mice and the kidneys of poly(I:C)-treated Ifnar1-/- animals (Figure 6C). We also tested the outcome of poly(I:C) priming on C. albicans infection in Stat1-/- mice (Figure 6D). As expected, Stat1-/- mice resisted the detrimental effects of poly(I:C) priming on candidiasis progression. Intriguingly, we noticed that poly(I:C)-primed Stat1-/- mice and, to a lesser extent, poly(I:C)-primed Ifnar1-/- animals showed a tendency to slightly better survive C. albicans infection compared to their unprimed counterparts (Figures 6B and 6D). We also assessed the role of IFN-γ in STAT1-mediated C. albicans susceptibility and found, as predicted from our in vitro data, that type II IFN is not playing a dominant role (Figure S5B).

We then verified whether poly(I:C)-induced type I IFN was indeed reducing the IL-1 response to C. albicans in vivo. We challenged poly(I:C)-primed or -unprimed WT and Ifnar1-/- animals with C. albicans i.p. or i.v. and then analyzed the levels of pro-IL-1β in PECs and blood, respectively. As shown in Figure 6E, poly(I:C)-induced type I IFN clearly decreased the ability of the mice to produce pro-IL-1β upon C. albicans challenge.

**IFN-β Suppresses IL-1β Production in Human Primary Monocytes**

To investigate whether type I IFN exerts the same anti-inflammatory effects in human cells, primary monocytes were isolated from blood of healthy donors (HDs) and cultured overnight with

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**DISCUSSION**

In addition to its antiviral effects, type I IFN is acknowledged as an immunomodulatory cytokine, though the underlying mechanisms are poorly understood. Our work suggests that
the anti-inflammatory effects exerted by type I IFN may be due at least partially to its ability to control IL-1 production. IL-1α, which does not require processing by caspase-1, is reduced in BMDMs upon incubation with type I IFN, whereas secretion of active IL-1β is abolished by two distinct means: through the reduction of pro-IL-1β protein and via suppression of NLRP1b and NLRP3 inflammasome-dependent caspase-1 activation. Such inflammasome inhibition by IFN also provides a mechanism for the observed interferon-dependent suppression of IL-18 maturation, as shown by the fact that IL-18 maturation also depends on inflammasome activity.

The predominant mechanism leading to type I IFN-dependent suppression of pro-IL-1β and pro-IL-1α is based on the STAT1-mediated synergistic action of type I IFN and LPS to induce IL-10. IL-10, in turn, regulates pro-IL-1β and pro-IL-1α amounts in a STAT3-dependent manner. In agreement with a previous report (Hu et al., 2006), the ability to induce IL-10 is specific to type I IFN, thus nicely supporting our observation that IFN-γ does not significantly decrease the levels of pro-IL-1β and pro-IL-1α. The mechanism by which IL-10 exerts its anti-inflammatory effects is still the object of intense research, but the reduction of IL-1 precursor levels may represent one important pathway.

STAT1 is crucial for the inhibition of NLRP3 inflammasome activity by type I IFNs. We found that phosphorylation at tyrosine 701 was sufficient for the suppression of NLRP1b and NLRP3 inflammasome-dependent caspase-1 activation. Tyrosine phosphorylation is required and sufficient for STAT1 nuclear translocation and DNA binding to target promoters, whereas modification of serine 727 appears to enhance STAT1 transactivator function. This suggests that transcriptional induction of a target gene was required for the inflammasome-suppressing activity of type I IFN. Our results also indicate that the inhibitory pathway is likely to be cell intrinsic, but future studies will be needed to further characterize this pathway.

IL-1β has been reported to play a role in controlling viral infections such as influenza. Moreover, viral RNA and poly(I:C) were reported to induce release of IL-1β through activation of the NLRP3 inflammasome (Allen et al., 2009; Kaneganti et al., 2006). Interestingly, we observed an increase in intracellular pro-IL-1β upon poly(I:C) exposure, in particular in vitro. However,
we could detect neither activation of caspase-1 nor release of mature IL-1β under these circumstances. In vivo, poly(I:C) triggered strong induction of type I IFN that efficiently prevented further pro-IL-1β induction by exogenous inflammasome agonists. Hence, depending on the type of infection, the balance between the induction of type I IFN and pro-IL-1β could indeed dictate the final level of mature IL-1β.

The capacity of type I IFNs to suppress inflammasome activity and IL-1 production is relevant to the phenomenon of superinfections. It is well established that although infections with viruses such as influenza are very common, mortalities purely attributable to the primary viral infection are relatively rare. Many influenza-related deaths are caused by secondary bacterial infections, which present a unique clinical challenge resulting from the emergence of antibiotic resistance and increased severity of infection. Influenza infections impair innate immune function and several mechanisms for the increased susceptibility to secondary bacterial infections have been proposed, some of which are dependent on type I IFNs (Shahangian et al., 2009). Our data suggest that the ability of type I IFN to suppress inflammasome activity and IL-1 secretion may contribute to the increased risk of infection after viral exposure. In line with this, we showed that poly(I:C)-induced type I IFN rendered WT mice as susceptible to C. albicans infection as Il1r1−/− mice (Bellochio et al., 2004; Kullberg et al., 1990; Van’t Wout et al., 1988; Vonk et al., 2006). To further corroborate our hypothesis, we showed that poly(I:C)-induced type I IFN suppressed an effective IL-1 response to C. albicans infection. In an analogous manner, Medzhitov’s laboratory delineated a mechanism by which influenza virus leads to suppression of the host immune response and to increased susceptibility to secondary bacterial infection through glucocorticoid induction (Jamieson et al., 2010). Interestingly, IL-1β is inhibited by glucocorticoid hormones in vivo and in vitro (Fantuzzi and Ghezzi, 1993).

The contributions of IL-1 and IL-18 to the development of inflammatory and autoimmune manifestations are well described (Chae et al., 2009; Furlan et al., 1999a, 1999b; Kantarci et al., 2002). These include MS, where interleukin-1 and interleukin-1 receptor antagonist gene polymorphisms were intercorrelated (chiro et al., 2004; Kullberg et al., 1990; Van’t Wout et al., 1988; Vonk et al., 2006). To further corroborate our hypothesis, we showed that poly(I:C)-induced type I IFN suppressed an effective IL-1 response to C. albicans infection. In an analogous manner, Medzhitov’s laboratory delineated a mechanism by which influenza virus leads to suppression of the host immune response and to increased susceptibility to secondary bacterial infection through glucocorticoid induction (Jamieson et al., 2010). Interestingly, IL-1β is inhibited by glucocorticoid hormones in vivo and in vitro (Fantuzzi and Ghezzi, 1993).

**EXPERIMENTAL PROCEDURES**

**Mice**

Mice were treated in accordance with the Swiss Federal Veterinary Office guidelines.

**In Vitro Stimulation Experiments**

BMDMs and BMDCs were differentiated as previously described (Guarda et al., 2009). 5 x 10⁴ differentiated BMDMs or BMDCs were incubated for 12 hr or the indicated times in the presence of 500 U/ml IFN-β, 500 U/ml IFN-x11 (both from PBL Interferon Source), 20 ng/ml IFN-γ (Calbiochem), or 20 ng/ml IL-10 (Peprotech) unless otherwise specified. According to what was indicated, cells were primed with 10 ng/ml ultrapure LPS (Invivogen) either for 12 hr (at the same time as IFN treatment), for the 4–6 hr preceding inflammasome stimulations, or left unprimed. Then, stimulations were carried out. ATP (500 μM), nigericin (0.4 μM), and MSU crystals (300 μg/ml) were from Sigma. Asbestos (300 μg/ml) was from SPI-CHEM and alum (300 μg/ml) from Pierce Biochemicals (Injec-alum). ATP stimulations were performed for 45 min, other stimulations for 150 min (unless otherwise indicated).

**Isolation of Human Primary Monocytes**

We obtained buffy coats from the Lausanne Blood Transfusion Center. Additionally, healthy volunteers and RR-MS patients on Rebif or Betaseron treatment (receiving their last IFN-β injection at the same day or up to 3 days prior to blood collection) donated 40 ml of blood after informed consent. Blood draw for this study was accepted by our institution’s ethical commission and all subjects gave their written consent according to review board guidelines.

**Stimulation of Human Primary Monocytes**

Monocytes from RR-MS patients or healthy donors were plated at 150,000 cells/well and left untreated or primed with 1 ng/ml LPS (Invivogen) for 3 hr. Unprimed or primed monocytes were subsequently stimulated for 6 hr with 500 μg/ml alum (Pierce). For in vitro IFN-β treatment, CD14+ monocytes were incubated with the indicated amounts of IFN-β (Peprotech) for 12 hr followed by 3 hr LPS priming and 6 hr alum stimulation. To test the production of IL-10 upon IFN-β exposure, CD14+ monocytes were incubated in the presence of IFN-β for 12 hr and then stimulated with LPS for 4 hr or left unstimulated.

**In Vivo Peritonitis and Candida albicans Infection Experiments**

For peritonitis, mice were injected i.v., unless otherwise indicated, with 200 μg poly(I:C) (Invivogen) followed by a second i.p. injection of alum 5 hr later. For the analysis of PECSs, 350 μg alum (Pierce) or 350 μg zymosan (Invivogen) as control were injected. 12–14 hr after alum injection, mice were sacrificed and peritoneal cavities were washed with 6 ml PBS. PECS were analyzed by FACS. For the analysis of IL-1β in the peritoneal cavity, 5 hr after i.v. injection of 200 μg poly(I:C), mice were injected i.p. with 700 μg alum. Lavages and PECSs were harvested 2 hr later, and peritoneal fluids were concentrated for ELISA analysis with Amicon Ultra 10k from Millipore.

C. albicans was cultured on Chromagar Candida plates (BD Bioscience). For C. albicans infections, mice were injected i.v., with 200 μg poly(I:C) (Invivogen) followed by a second i.v. injection of 2.5 x 10⁵ fungal cells 5 hr later. The day after, mice treated with poly(I:C) were injected again i.v. with 100 μg poly(I:C). For analysis of C. albicans load, mice were sacrificed 48 hr after infection, and homogenized kidneys were plated on Chromagar Candida plates to determine colony forming units. For survival assay, mice were monitored with a score-sheet, in accordance with local guidelines for animal experimentation.

**Statistical Analysis**

Statistical differences were calculated with an unpaired Student’s t test, two-tailed (GraphPad Prism version 5.0). Differences were considered significant when p < 0.05 (*), very significant when p < 0.01 (**), and extremely significant when p < 0.001 (***).

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at doi:10.1016/j.immuni.2011.02.006.
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