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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 phosporylation 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 (Platanias, 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; Shahangian 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-1α 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* (Bellocchio 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-1 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-1 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-1α 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, monocytes 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-1 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 *Ifnar1*-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-α, 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-α 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(deoxyadenylic-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

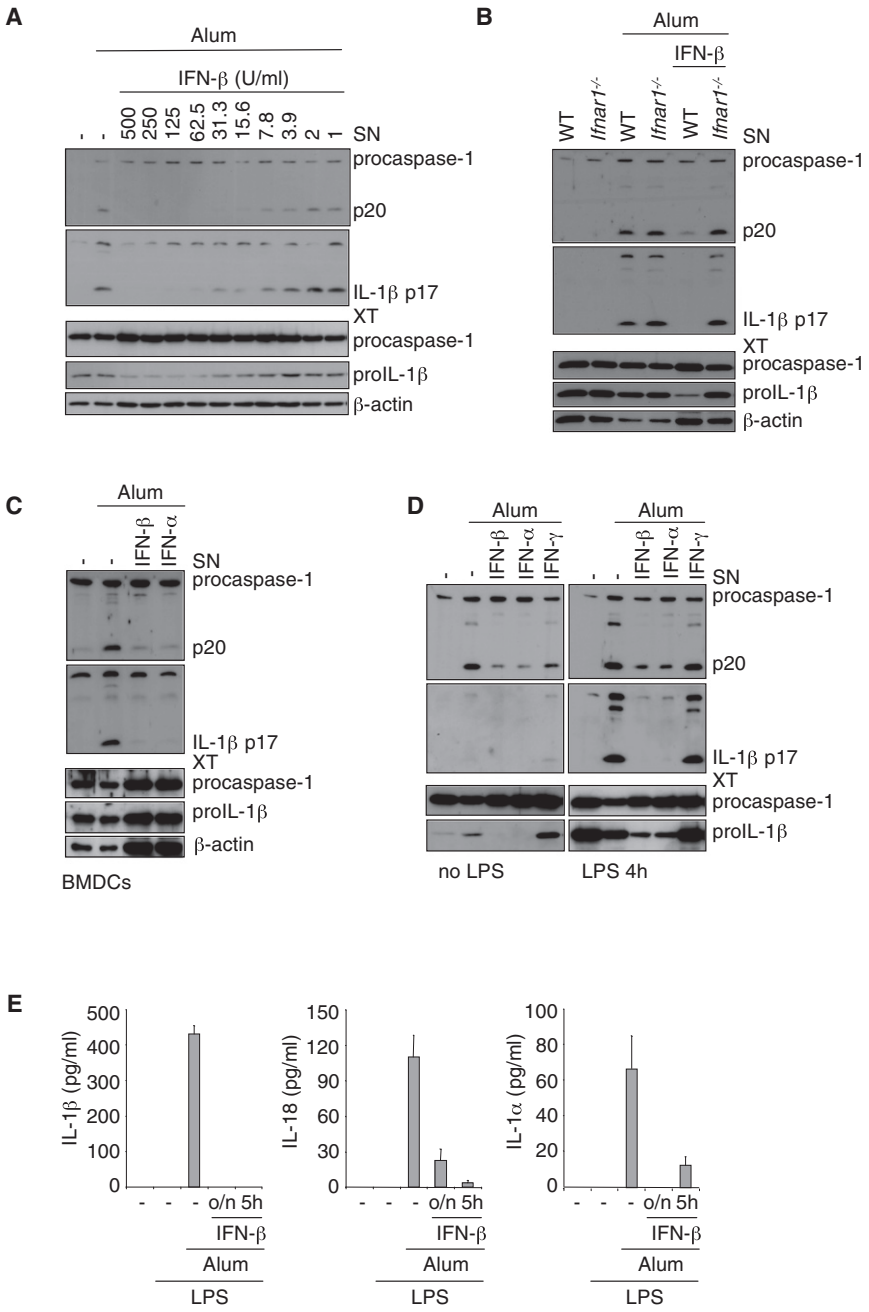

Figure 1. IFN-β Decreases Inflammasome Activity and Pro-IL-1β

(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.

decreased amounts of intracellular pro-IL-1β (Figure 2C and data not shown). Thus, IFN-β specifically inhibits the activity of NLRP1b and NLRP3 inflammasomes.

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 *Il10*$^{-/-}$ 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 expresssion 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).

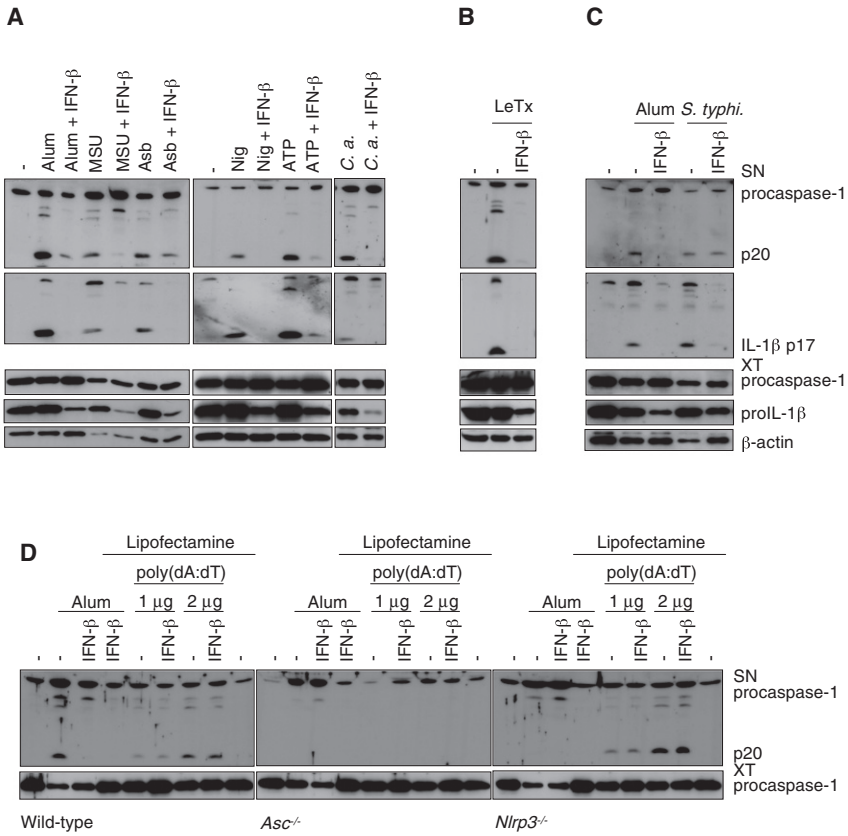

Figure 2. IFN-β Inhibits NLRP3 and NLRP1b Inflammasomes

BMDMs were cultured overnight in the presence or absence of IFN-β. LPS was added to prime the BMDMs during the last 4 hr preceding inflammasome stimulation. Data are representative of at least three individual experiments.

(A–C) Caspase-1 activation and IL-1β release in supernatants after stimulation with alum, MSU, asbestos (Asb), nigericin (Nig), ATP, and *C. albicans* (*C.a.*) (A), after LeTx stimulation (B), or after *S. typhimurium* (*S. typhi.*) infection (C).

(D) Caspase-1 release by BMDMs of WT, *Asc*$^{-/-}$, and *Nlrp3*$^{-/-}$ origin after poly(dA:dT) transfection.

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 (Platanias, 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 (*Stat1*$_{S727A}$)). 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 pretreated 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^{+}

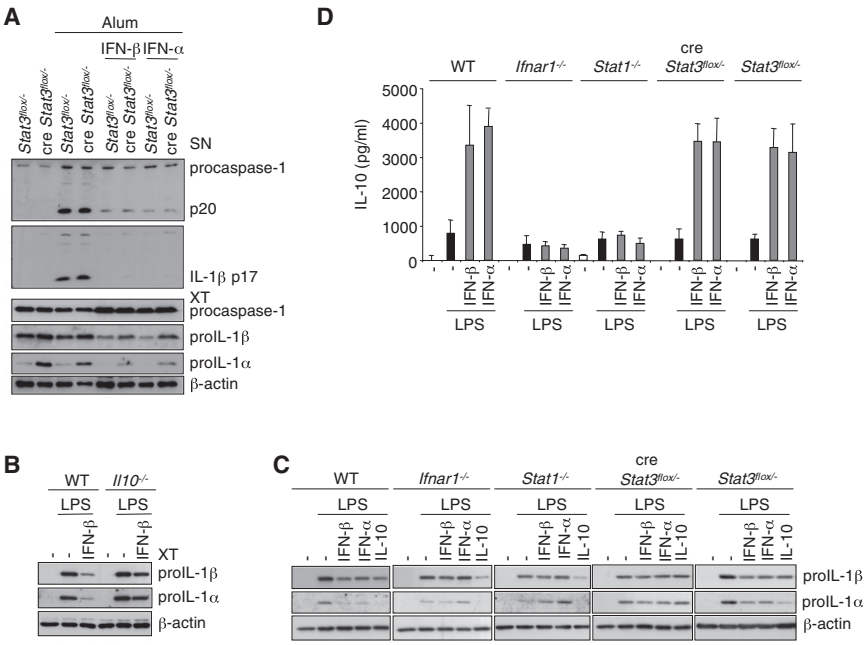

Figure 3. Type I IFN Regulates Pro-IL-1 Levels via IL-10 and STAT3

(A) BMDMs from control (*Stat3*$^{flox/-}$) or LysMcre *Stat3*$^{flox/-}$ (cre *Stat3*$^{flox/-}$) mice were incubated 12 hr with IFN-β or IFN-α. Thereafter, LPS was added to prime the BMDMs and, 4 hr later, alum stimulation was performed. Caspase-1 activation and IL-1β release are shown in supernatants, procaspase-1, pro-IL-1β, 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 *Stat3*$^{flox/-}$, or control (*Stat3*$^{flox/-}$) mice were incubated 5 hr with IFN-β, IFN-α (C and D), 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 (C), IL-10 levels by ELISA (D). Data represent mean ± SD of three individual experimental points (D).

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 5C–5E; Dostert et al., 2009; Martinon et al., 2006). In this case, PECs, neutrophils, and (to a lesser extent) Ly6C^{+} monocytes were still recruited to the peritoneum despite the poly(I:C) pretreatment, further demonstrating that poly(I:C) suppresses alum-induced peritonitis by blocking the generation of IL-1.

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^{+} 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; Kanneganti 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

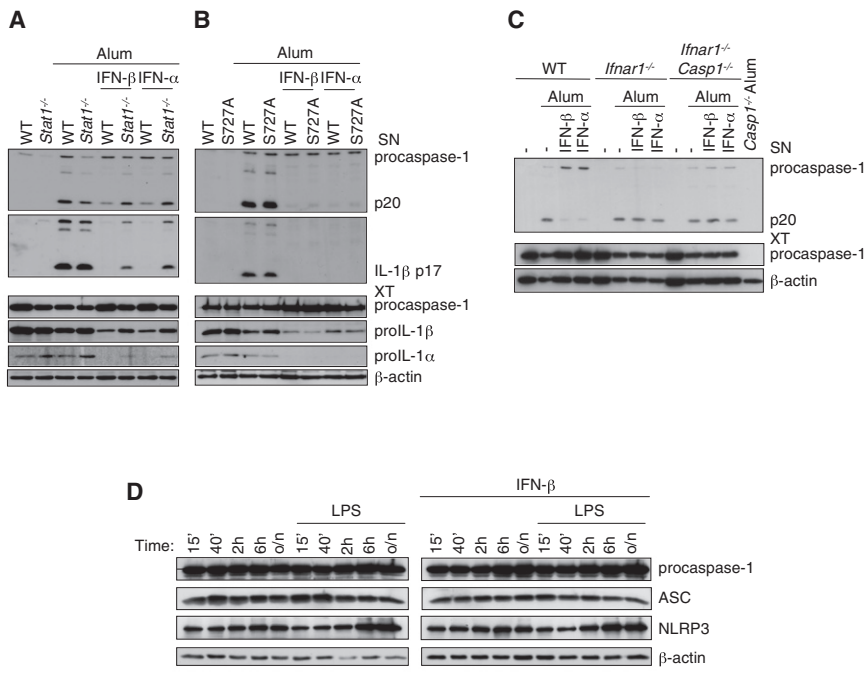

Figure 4. Inflammasome Inhibition by Type I IFN Is STAT1 Dependent

(A and B) C57BL/6 (WT), *Stat1*$^{-/-}$ (A), and *Stat1*$_{S727A}$ 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, alum stimulation was performed. Caspase-1 activation and IL-1β release are shown in supernatants, procaspase-1, pro-IL-1β, and pro-IL-1α levels in cell lysates.

(C) BMDMs from C57BL/6 (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 alum 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.

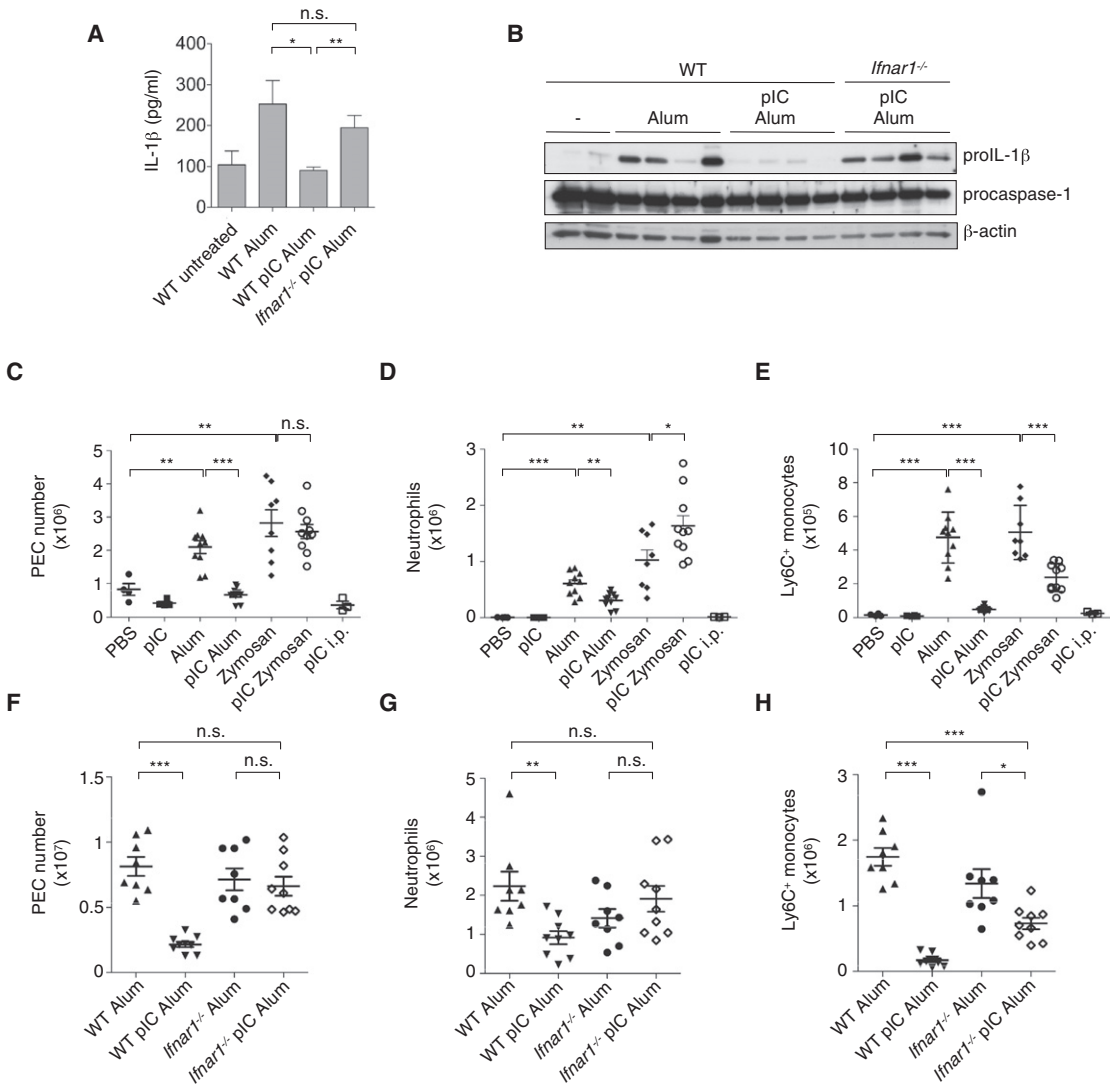

Figure 5. Type I IFN Decreases the Inflammatory Response to Alum

C57BL/6 or *Ifnar1*$^{-/-}$ mice were injected with poly(I:C) (pIC) i.v. or left untreated as a control. 5 hr later, mice were challenged with alum, zymosan, or poly(I:C) (pIC i.p.) i.p.

(A and B) 2 hr after alum injection, peritoneal cavities were lavaged. IL-1β content in the lavage fluid was measured by ELISA (A), while PECs recovered were lysed and analyzed for their expression of pro-IL-1β by immunoblot (B). Values are the mean ± SEM of five to eight mice per group, pooled from two independent experiments (A), while in (B) individual mice are represented in each lane.

(C–E) 12–14 hr after alum or zymosan injection, mice were sacrificed and peritoneal lavages performed. Absolute numbers of PECs, neutrophils, or Ly6C^{+} monocytes recruited to the peritoneum are depicted.

(F–H) 12–14 hr after alum injection, C57BL/6 or *Ifnar1*$^{-/-}$ mice were sacrificed and peritoneal lavages performed. Absolute numbers of PECs, neutrophils, or Ly6C^{+} monocytes recruited to the peritoneum are depicted.

(C–H) Data represent mean ± SEM of the pool of two independent experiments, and results for individual mice are illustrated.

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 Hasenclever, 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

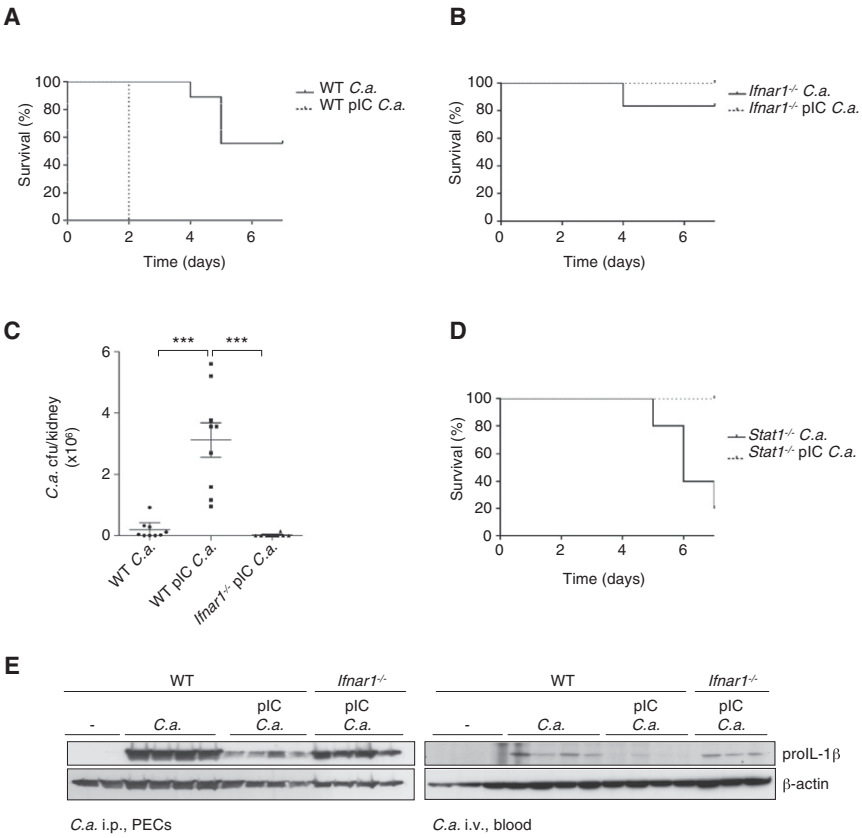

Figure 6. Type I IFN Worsens Susceptibility to *C. albicans* Infection

(A–D) WT, *Ifnar1*$^{-/-}$, or *Stat1*$^{-/-}$ mice were treated or not with poly(I:C) (pIC) i.v. and 5 hr later, mice were infected i.v. with *C. albicans* (*C.a.*). On day 1, poly(I:C)-treated mice were injected a second time with poly(I:C).

(A, B, D) Survival of the different cohorts of mice was monitored for 7 days postinfection. Data are representative of at least two independent experiments (per condition, n = 9–10 for WT, n = 4–6 for *Ifnar1*$^{-/-}$ or *Stat1*$^{-/-}$ mice).

(C) *C. albicans* load was assessed in the kidneys of 2 day infected mice by counting *C. albicans* cfu/kidney in the indicated groups. Cfu per kidney are represented as mean ± SEM of the pool of two experiments (9–10 mice per group).

(E) WT or *Ifnar1*$^{-/-}$ mice were treated or not with poly(I:C) i.v. and 5 hr later, mice were infected i.p. with 10^6 or i.v. with 10^7 *C. albicans* fungal cells. 3 hr after i.p. injection and 5 hr after i.v. injection, PECs or blood were harvested, respectively. Intracellular content of pro-IL-1β was then assessed by immunoblot.

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 IFN-β, primed with LPS, and then stimulated with alum. In line with findings from mice, incubation with IFN-β effectively diminished alum-induced IL-1β secretion and caspase-1 cleavage (Figures 7A and 7B). As shown in Figure 7C, even at low concentrations IFN-β significantly suppressed IL-1β production. Similar to what was observed in mouse BMDMs, a striking decrease in pro-IL-1β accompanied by IL-10 induction was noted (Figure 7B; Figure S6A). Thus, type I IFN plays a crucial role in the negative regulation of both caspase-1 activity and pro-IL-1β production in human primary monocytes.

IFN-β is currently used in the management of several diseases including MS. We therefore asked whether IFN-β treatment in RR-MS patients similarly suppresses IL-1β production. First, we confirmed that the recombinant IFN-β preparations administered to the patients (IFN-β-1a, Rebif, produced in mammalian cells, and IFN-β-1b Betaferon, produced in bacteria) had the same in vitro effects on IL-1β production as the IFN-β used for research purposes (Figure S6B). Finally, monocytes isolated from blood of MS patients on IFN-β therapy were challenged ex vivo with alum in the presence or the absence of LPS priming or left untreated (Figure 7D). IL-1β production by monocytes derived from both Rebif- and Betaferon-treated patients released significantly less IL-1β than monocytes derived from HDs. Taken together, these results suggest that a possible mode of action for the success of IFN-β treatment for MS might indeed rely on suppressing IL-1-mediated inflammation.

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

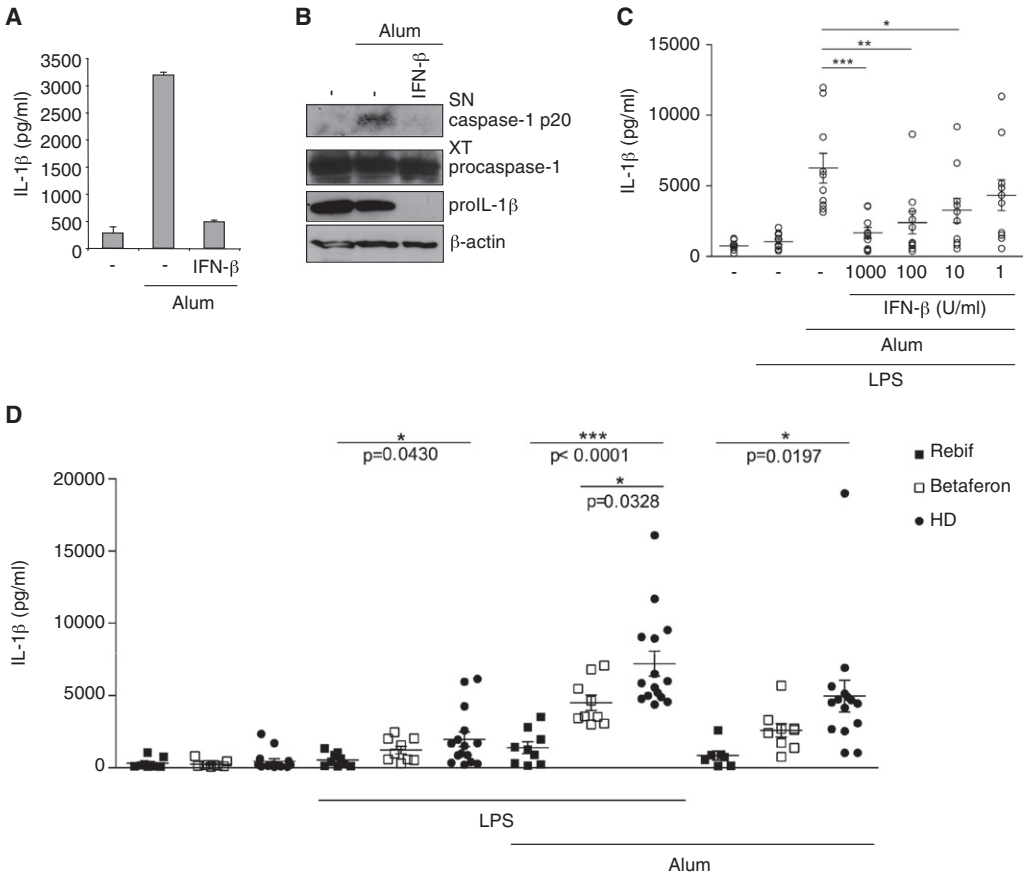

Figure 7. IFN-β Suppresses IL-1β Production by Human Primary Monocytes

(A–C) Human primary monocytes were cultured overnight in the presence of 1000 U/ml (A, B) or the indicated doses of human IFN-β (C). LPS was added to prime the monocytes for 3 hr, followed by stimulation with alum. IL-1β release was measured by ELISA and data represent mean ± SD of 3 experimental replicates (A) or mean ± SEM of 10 individual donors (C). Caspase-1 activation in the supernatants after stimulation with alum and pro-IL-1β levels in cell lysates are shown in (B). (D) Monocytes from RR-MS patients treated with Rebif or Betaferon, and in parallel, from age- and gender-matched HDs were primed with 3 hr LPS exposure or left unprimed. Then, cells were stimulated with alum. IL-1β release was measured by ELISA and data represent mean ± SEM of 9 Rebif-treated RR-MS patients, 9 Betaferon-treated RR-MS patients, and 15 untreated healthy donors.

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 NLRP3 inflammasome activity, whereas the serine 727 modification was dispensable. Tyrosine phosphorylation is required and sufficient for STAT1 nuclear translocation and DNA binding to target promotors, 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; Kanneganti 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 (Bellocchio 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-1β and interleukin-1 receptor antagonist gene polymorphisms were found to be associated with disease severity (Kantarci et al., 2002). Although more experimental evidence is required, it is tempting to speculate that the successful treatment of MS with IFN is mediated, at least in part, by its ability to suppress the IL-1-inflammasome axis. Such a notion is supported by our observation that inflammasome activity is suppressed in monocytes from patients under IFN therapy. Our data retrospectively shed light on some aspects of the widespread use of IFN in the treatment of autoimmune and/or inflammatory diseases and suggest that inflammasome inhibitors may complement current treatment regimes.

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 × 10^4 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-α11 (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 (Imject-alum). ATP stimulations were performed for 45 min, other stimulations for 150 min (unless otherwise indicated). *C. albicans* was put on BMDMs at a multiplicity of infection of 1 for 150 min. *B. anthracis* LeTx stimulation and *S. typhimurium* infection were carried out as previously described (Guarda et al., 2009). For the stimulation of the AIM-2 inflammasome, poly(dA:dT) (purchased from Amersham) was admixed at the indicated concentrations to Lipofectamine 2000 (from Invitrogen) according to manufacturer's instructions and cells were stimulated for 6 hr.

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 Betaferon 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 PECs, 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 PECs 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), followed by a second i.v. injection of 2.5 × 10^5 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 scoresheet, 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 $p \leq 0.05$ (*), very significant when $p \leq 0.01$ (**), and extremely significant when $p \leq 0.001$ (***).

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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