Mycobacterium tuberculosis Triggers Host Type I IFN Signaling To Regulate IL-1 β Production in Human Macrophages

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Mycobacterium tuberculosis is a virulent intracellular pathogen that survives in macrophages even in the presence of an intact adaptive immune response. Type I IFNs have been shown to exacerbate tuberculosis in mice and to be associated with disease progression in infected humans. Nevertheless, the mechanisms by which type I IFNs regulate the host response to M. tuberculosis infection are poorly understood. In this study, we show that M. tuberculosis induces an IFN-related gene expression signature in infected primary human macrophages, which is dependent on host type I IFN signaling as well as the mycobacterial virulence factor, region of difference-1. We further demonstrate that type I IFNs selectively limit the production of IL-1β, a critical mediator of immunity to M. tuberculosis. This regulation occurs at the level of IL1B mRNA expression, rather than caspase-1 activation or autocrine IL-1 amplification and appears to be preferentially used by virulent mycobacteria since avirulent M. bovis bacillus Calmette-Guérin (BCG) fails to trigger significant expression of type I IFNs or release of mature IL-1β protein. The latter property is associated with decreased caspase-1-dependent IL-1β maturation in the BCG-infected macrophages. Interestingly, human monocytes in contrast to macrophages produce comparable levels of IL-1β in response to either M. tuberculosis or BCG. Taken together, these findings demonstrate that virulent and avirulent mycobacteria employ distinct pathways for regulating IL-1β production in human macrophages and reveal that in the case of M. tuberculosis infection the induction of type I IFNs is a major mechanism used for this purpose.

levels of type I IFNs in vivo, and in most studies mice deficient in type I IFN signaling display significantly reduced bacterial loads following infection (18–20). Similarly, intranasal administration of polyriboinosinic-polyribocytidylic acid, a type I IFN-inducing agent, exacerbates pulmonary tuberculosis (TB) in wild-type (WT) but not IFN-α/β receptor-deficient mice (21).

The role of type I IFNs in the regulation of human M. tuberculosis infection is poorly understood. Nevertheless, a recent clinical study revealed that IFN-inducible genes are highly expressed in the leukocytes of active but not latent TB patients (22), strongly arguing for a contributing role of type I IFNs in TB progression in humans. Previous in vitro studies have also shown that M. tuberculosis infection significantly upregulates the expression of a number of type I IFN-inducible genes (23, 24). In this study, we report that this induction of a type I IFN signature in human macrophages is a property of live virulent mycobacteria and is not triggered by either BCG or an RD1-deficient M. tuberculosis mutant. Additionally, we report that IL-1β, also preferentially induced by virulent M. tuberculosis, is negatively regulated by type I IFNs. These findings demonstrate a functional intersection between type I IFN and IL-1β signaling in M. tuberculosis-infected human macrophages.

Materials and Methods

Cell cultures and infection

Peripheral blood-derived monocytes were isolated from healthy donors by counterflow centrifugal elutriation under protocols approved by the Institutional Review Boards of both the National Institute of Allergy and Infectious Diseases and the Department of Transfusion Medicine of the National Institutes of Health for these studies after appropriate informed consent. Monocytes were differentiated as previously described (23). Briefly, cells were cultured in antibiotic-free RPMI 1640 medium containing 10% FCS, 2 mM glutamate, 50 μM 2-ME, and 10 mM HEPES buffer in 24-well plates (Corning) at a density of 1 × 10^6 cells/ml for 7 d. Recombinant human M-CSF (10 ng/ml; PeproTech) was added on days 0, 2, and 4. For differentiation of THP-1 cells, the cell line (1 × 10^6 cells/well) was stimulated with PMA (10 ng/ml) in 24-well plates for 2 d. The cells were then rested in fresh complete medium for 4 h before infection.

For stimulation with mycobacteria, differentiated macrophages were exposed to live BCG or RD1 mutant (gift from Dr. S. Derrick, U.S. Food and Drug Administration) or WT M. tuberculosis H37Rv at a multiplicity of infection of 5, or with irradiated M. tuberculosis at 50 μg/ml. In some experiments, a mouse mAb to human IFN-α/β receptor-2 (10 μg/ml) was included. Recombinant human IFN-β or IFN-γ was purchased from PeproTech, and IL-27 was obtained from R&D Systems. All cytokines were used at a final concentration of 10 ng/ml. In some experiments, IL-27R/Fc (2 μg/ml) was purchased from R&D Systems) and anakinra (30 μg/ml; R&D Systems) and anakinra (30 μg/ml; Agen) were added to the cultures.

Measurement of gene expression

Total RNA was isolated using the RNeasy Mini kit (Qiagen, Valencia, CA), and residual DNA was digested using RNase-free DNase (Qiagen). The individual RNA samples were reversely transcribed using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). Unless otherwise indicated, gene expression measurements were performed using Green-based real-time quantitative PCR (25) in which 18S mRNA was used as the housekeeping gene. The oligonucleotide primers used were: 18S, forward, 5′-CGATGCTGGTGGGACTTTTCTTC-3′ and reverse, 5′-TGGAGAACACCACTTGTTGCT-3′; TNF, forward, 5′-CAGCCGCTCCCTCTCTCTCT-3′ and reverse, 5′-CACGGGGCGGATCTGCGAT-3′; IL-6, forward, 5′-TTAATGGCAGTCCTCCTTTCT-3′ and 5′-TGCTCTTAACGGCTCATACT-3′; TGF-β, forward, 5′-CAGCAGTCTCCTGCTCTGCTG-3′ and reverse, 5′-GCCAGAAGGCGGATGAG-3′; IL-1β, forward, 5′-ATTTGCGGGCAGCTTTCAGG-3′ and reverse, 5′-TCCTGCTTTGCTTTGCTTTG-3′; IL-10, forward, 5′-AGCAGTCCAGATCAGATTG-3′ and reverse, 5′-GAGGAAATGACGAGCGTGG-3′; IFNB, forward, 5′-GAGGAAATGACGAGCGTGG-3′ and reverse, 5′-TCCTGCTTTGCTTTGCTTTG-3′; IL-12, forward, 5′-AGCAGTCCAGATCAGATTG-3′ and reverse, 5′-GAGGAAATGACGAGCGTGG-3′; and β-actin, forward, 5′-TACTGCTGCTGGGCAACATTCAAC-3′ and reverse, 5′-GACCGGCCGTCGTTTCGCTTT-3′. For immunoblotting, supernatants were precipitated with methanol/chloroform and the resulting protein pellet was resuspended in reducing sample buffer (Thermo Fisher Scientific). Cells were lysed using cell lysis buffer (Cell Signaling Technology) supplemented with a protease inhibitor mixture (Calbiochem) and PMSF (Sigma-Aldrich). The samples were next separated by SDS-PAGE on a 12% polyacrylamide gel and then transferred onto a polyvinylidene difluoride membrane. The membranes were then sequentially probed with polyclonal Abs for caspase-1 (Cell Signaling Technology) or IL-1β (R&D Systems or Cell Signaling Technology).

Statistics

A Wilcoxon ranked sum test was used in experiments involving pairwise comparisons. A p value of <0.05 was considered statistically significant.

Results

Virulent M. tuberculosis induces a subset of type I IFNs in human macrophages

We first determined whether M. tuberculosis triggers IFNB mRNA expression in human macrophages at 4 and/or 24 h postinfection using a SYBR Green-based quantitative PCR assay and found that the gene expression was significantly elevated in infected primary human macrophages (Fig. 1A) and THP-1 cells (Fig. 1B). As expected, the induction of IFNB was closely correlated with the expression of the IFN-inducible gene CXCL10 in M. tuberculosis-infected primary macrophages (Fig. 1C) and THP-1 cells (Fig. 1D). In addition to IFN-β, there are 12 closely related IFN-α subtypes in the human type I IFN family (28). To profile the subtypes of IFN-α induced by M. tuberculosis in infected macrophages, we next quantified the copy number of IFN-β and IFN-α family members using a TaqMan-based PCR at 24 h postinfection (Fig. 1E, IF). This independent assay confirmed the induction of IFNB expression and further revealed that among all IFN-α subtypes examined, only IFNA1 is specifically upregulated by M. tuberculosis, suggesting a restricted IFN-α subtype usage in M. tuberculosis-infected human macrophages.
Regulation of M. tuberculosis-induced cytokine expression in human macrophages by type I IFNs

To identify innate inflammatory cytokines that are regulated by type I IFN signaling, we treated M. tuberculosis-infected macrophages with a mouse mAb specific for IFN-α/β receptor 2 (IFN-ABR2), thereby blocking signaling of all members of the type I IFN family. We then analyzed the effect of the signaling blockade on the expression of 64 genes that are known to be critical in mediating NF-κB- or IFN-dependent cellular responses to microbial stimulation using a novel nCounter analysis system. We found that M. tuberculosis strongly induced both NF-κB- and IFN-related genes (Fig. 2A, left panel). Importantly, the expression of IFN-inducible genes such as IFT1, IFIT2, MX1, MX2, and IL27 was significantly reduced in the presence of the mAb to IFNABR2 (Fig. 2A, right panel), thus establishing that the induction of IFN-related genes in M. tuberculosis-infected macrophages is dependent on endogenously produced type I IFNs. Additionally, we observed that blocking type I IFN signaling led to increased expression in a small group of genes (Fig. 2A, right panel). Among these, IL1B and IL1A were the most highly upregulated.

To further determine whether type I IFNs are a regulator of IL1B mRNA expression in M. tuberculosis infection, we extended our study to a larger cohort of healthy donors and compared the expression of IL1B in the infected macrophage cultures with or without IFNABR2 blockade. In addition to reducing the expression of the IFN-dependent CXCL10 (data not shown), blockade of type I IFN signaling significantly increased IL1B mRNA levels when compared with the cultures infected with M. tuberculosis alone (Fig. 2B). In contrast, expression of other NF-κB-dependent genes, such as IL6 and TNF, was not significantly altered by the mAb, suggesting a selective regulation of IL1B expression by type I IFNs.

Downregulation of M. tuberculosis-induced IL1B by type I IFNs occurs independently of alterations in IL-1R signaling, IL-27R signaling, and caspase-1 activation

Type I IFNs have been shown to modulate IL-1 receptor expression (29) and it is well established that IL-1 can autoamplify its production (30, 31) through interaction with its own receptors. Therefore, it was possible that the increase in IL1B expression in our mAb blocking experiments was due to the release of type I IFN-dependent inhibition of IL-1R signaling and subsequent autocrine stimulation of IL-1 production. To test this, we first analyzed the effect of endogenous type I IFNs on the expression of the signaling receptors IL1RI and IL1RAcp, the inhibitory receptor IL27R, and the receptor antagonist IL1Ra in M. tuberculosis-infected primary macrophages. We found that relative to uninfected controls, M. tuberculosis-infected macrophages displayed reduced expression of the IL1RI but increased levels of IL1RAcp, IL1RII, and IL1Ra (Fig. 3A). Nevertheless, blockade of type I IFN signaling failed to significantly alter the expression of any IL-1R species.

To further exclude the possibility that endogenous type I IFNs regulate IL-1–induced IL1B expression, we compared gene expression in M. tuberculosis-infected macrophages cultured with mAb to IFNABR2 or anakinra, a recombinant IL-1Ra competing with IL-1 for IL-1RI binding, or both. Addition of anakinra did not significantly reduce IL1B expression in macrophages infected with M. tuberculosis alone or in combination with the mAb to IFNABR2 (Fig. 3B). Taken together, these findings suggest that the IL-1R-dependent positive feedback loop does not appear to contribute significantly to M. tuberculosis-induced IL-1B production in human macrophages and that the augmented IL1B expression following blockade of type I IFN signaling is not due to enhanced IL-1R signaling.

Because IL-27, an IFN-inducible regulatory cytokine, has also been implicated in suppressing IL-1β production in macrophages through the regulation of IL-1 receptor expression (32), we examined its possible involvement in regulating M. tuberculosis-induced IL-1β production. We found that the addition of exogenous IL-27 or neutralization of endogenous IL-27 activity with a soluble IL-27R/Fc chimera did not significantly alter M. tuberculosis-induced IL-1β production (Fig. 3C), thus arguing against a role for this cytokine in regulating IL-1β production in infected macrophages.

In addition to mRNA induction, mature IL-1β release requires caspase-1 activation and cleavage of pro–IL-1β. To determine whether type I IFNs regulate caspase-1 activation in M. tubercu-
Regulation of the macrophage cytokine response to *M. tuberculosis* by type I IFN signaling. A. Primary human macrophages from five individual healthy donors were left unstimulated or infected with *M. tuberculosis* for 24 h in the presence or absence of a blocking mAb to IFNAR2 and gene expression analyzed using an nCounter gene expression assay. Data are expressed as the log2 ratio (fold change) of mRNA counts. Left panel, *M. tuberculosis* infected relative to uninfected. Right panel, *M. tuberculosis* plus IFNAR2 mAb relative to *M. tuberculosis* alone. Known IFN-dependent genes are shown in boxed areas. *False discovery rate < 0.5.

B. Increased IL1B expression in human macrophages infected with *M. tuberculosis* following blockade of endogenous type I IFN measured by quantitative PCR at 24 h. The data shown are fold change over noninfected cultures. Each paired data set represents an individual donor.

*FIGURE 2.*

Regulation of IL-1β by *M. tuberculosis*-induced type I IFN is independent of altered IL-1R and IL-27R signaling and caspase-1 activation. A. Expression of IL-1R components and IL-1Ra in the presence or absence of IFNAR2 mAb and (B) IL1B levels in the presence of anakinra and/or IFNAR2 mAb was measured in *M. tuberculosis*-infected human macrophages at 24 h using quantitative PCR. Data shown are fold change over noninfected cultures. Each symbol represents an individual donor. C. IL-1β secretion in macrophage cultures incubated with recombinant IL-27 or IL-27R/Fc protein measured by ELISA at 24 h postinfection. Each line represents an individual donor. D. IL1B mRNA expression, caspase-1 activation, and IL-1β processing in THP-1 cells infected with *M. tuberculosis* alone or in combination with exogenous IFN-β or IFNAR2 mAb at 24 h. IL1B mRNA in lysates was measured using quantitative PCR, whereas cleaved caspase-1 and mature IL-1β in culture supernatants were assayed by Western blotting involving sequential probing of the same membrane with Abs against each product. The mRNA data shown are fold change over nonstimulated cultures and data shown are the means (±SD) of three independent experiments with similar results.

*FIGURE 3.*

Differential effects of type I IFN on IL-1β production in *M. tuberculosis*-infected macrophages and monocytes. A. Increased IL-1β protein secretion by *M. tuberculosis*-infected macrophages and monocytes. B. IL-1β limits *M. tuberculosis*-induced IL-1β secretion by macrophages but not monocytes. Primary human macrophages and monocytes from the same donors were infected with *M. tuberculosis* in the presence or absence of exogenous IFN-β or INF-γ. The cytokines (10 ng/ml) were added to the cultures at the beginning of the infection. The quantity of secreted IL-1β protein was measured at 24 h by ELISA. Each symbol represents an individual donor.
response of monocytes and macrophages to *M. tuberculosis* is differentially regulated by IFNs.

**Virulent mycobacteria induce type I IFN expression in an RD1-dependent fashion**

To determine whether the ability to induce type I IFNs is a common property of all mycobacteria, we compared expression of type I IFN-related genes in macrophages exposed to virulent versus avirulent or inactivated mycobacteria. We found that BCG and irradiated *M. tuberculosis* stimulated significantly weaker expression of IFNA1, IFNB, and CXCL10 compared with live *M. tuberculosis* (Fig. 5A), indicating a preferential induction of type I IFNs by virulent mycobacteria in human macrophages.

Because the RD1 locus, which is present only in virulent *M. tuberculosis*, has been implicated in the induction of type I IFNs in mice (4, 13), we compared IFN-related gene expression in primary human macrophages infected with an RD1-deficient mutant (ΔRD1) or WT *M. tuberculosis*. Compared to WT *M. tuberculosis*, the ΔRD1 mutant was significantly less effective at inducing the expression of the IFN-inducible IFIT1 and MX1 while being capable of upregulating TNF, IL6, and IL1B mRNA (Fig. 5B), indicating a requirement for the mycobacterial virulence determinant RD1 in inducing type I IFNs in human macrophages.

**Virulent *M. tuberculosis* induces higher levels of CXCL10 and IL-1β protein compared with BCG**

To compare the cytokine response of human macrophages to virulent and avirulent mycobacteria at the protein level, primary human macrophages were infected with either *M. tuberculosis* or BCG, and secreted inflammatory cytokines/chemokines were quantified using a multiplex or a conventional ELISA assay. Whereas a low level of IFN-β protein was detected in *M. tuberculosis*-infected macrophage cultures of some but not all donors (data not shown), large quantities of IFN-inducible CXCL10 were induced (Fig. 6A) in every donor tested. Consistent with the analysis of mRNA expression (Fig. 5A), multiplex cytokine measurements showed that *M. tuberculosis*-infected macrophages secreted a significantly higher quantity of CXCL10 as well as IL-1β when compared with those infected with BCG. In contrast, the levels of other NF-κB–dependent proinflammatory cytokines were comparable. This difference in IL-1β protein secretion was subsequently confirmed using a conventional IL-1β ELISA assay showing that *M. tuberculosis* stimulated primary macrophages release ~10-fold more IL-1β protein than either BCG- or irradiated *M. tuberculosis*-stimulated cells (Fig. 6B, left panel). The difference was not due to a failure of avirulent mycobacteria to activate the innate system since all bacteria induced a comparable level of IL1B mRNA in the same infected macrophage cultures (Fig. 6B, right panel). Similar to primary macrophages, *M. tuberculosis*-infected differentiated THP-1 cells also consistently produced higher levels of secreted IL-1β protein than those stimulated with BCG (Fig. 6C).
M. tuberculosis is more efficient than BCG in activating caspase-1 in infected macrophages

Given that type I IFNs negatively regulate IL1B mRNA expression in M. tuberculosis-infected macrophages and BCG fails to induce type I IFN expression, it was surprising that BCG-infected macrophages produce decreased rather than increased IL-1β production. To determine whether M. tuberculosis and BCG differentially regulate IL-1β production at the posttranscriptional level, we analyzed levels of cleaved caspase-1 and IL-1β in the culture supernatants as well as procaspase-1 in the cell lysates of infected primary human macrophages. We found that virulent M. tuberculosis is more efficient than avirulent BCG and RDI-deficient M. tuberculosis in activating caspase-1 and triggering the secretion of mature IL-1β protein (Fig. 6D).

Type I IFNs have been suggested to activate the inflammasome in the Francisella infection model (33). Therefore, it was possible that compared with M. tuberculosis-infected cells, the weaker caspase-1 activation observed in BCG-infected culture is due to the lower level of type I IFNs expressed (Fig. 5A). However, the addition of exogenous IFN-β failed to increase caspase-1 cleavage to the level observed in M. tuberculosis-infected cells (Fig. 5E). Moreover, consistent with our earlier finding with M. tuberculosis (Fig. 4B), exogenous IFN-β reduced the quantity of BCG-induced IL-1β to undetectable levels in primary macrophage cultures (Fig. 5F), arguing that type I IFNs do not regulate mycobacteria-induced IL-1β production by altering caspase-1 activation. Taken together, these observations suggested that the difference in IL-1β production by BCG- versus M. tuberculosis-infected human macrophages is due to differential caspase-1 activation rather than type I IFN induction by these bacteria.

Monocytes, in contrast to macrophages, mount equivalent IL-1β response to M. tuberculosis and BCG

We next examined the IL-1β response of human monocytes to mycobacterial infection, as it has been reported previously that the production of this cytokine is differentially regulated in the two mononuclear cell populations (34, 35). In contrast to macrophages (Fig. 6B), the levels of IL1B mRNA and secreted IL-1β protein were comparable in monocyte cultures infected with M. tuberculosis, BCG, or irradiated M. tuberculosis (Fig. 6G), arguing that only differentiated macrophages are capable of distinguishing virulent from avirulent mycobacteria with regard to IL-1β release. Therefore, the IL-1β response to mycobacteria is influenced both by properties of the pathogen as well as the host cell type that is infected.

Discussion

Although essential for resistance to viral infections, type I IFNs have been shown to contribute to pathogenesis of intracellular bacterial infections (36, 37). In the case of M. tuberculosis, the cytokines have been shown to impair host resistance in mice (4, 18) and to be associated with TB disease progression in infected humans (22). However, the mechanisms by which type I IFNs regulate the host response to M. tuberculosis infection are poorly understood in both mice and humans. In this report, we show that type I IFNs are preferentially induced by virulent mycobacteria and function as regulators of IL-1β production in infected human macrophages through suppression of IL1B mRNA expression. This host cytokine-mediated, transcriptional regulation of IL-1β production is thus distinct from a previously described mechanism in murine macrophages involving direct inhibition of inflammasome activation by the mycobacterial product zmp1 (16). Taken together, our findings reveal a second pathway through which M. tuberculosis can manipulate host IL-1β production indirectly by inducing type I IFNs (Fig. 7). Although the molecular mechanisms required for the induction of type I IFNs by M. tuberculosis have yet to be fully elucidated, it is interesting to note that live M. tuberculosis selectively induces only a subset of type I IFNs in macrophages, as shown in this study, and dendritic cells (24). Therefore, a detailed comparison of the M. tuberculosis-induced IFN signature with that known to be triggered by viral infection may provide important clues in understanding the “probacterial” versus antiviral effects of this important cytokine family.

Our findings reveal that type I IFNs selectively inhibit IL-1β mRNA induction but not caspase-1 activation or IL-1R signaling in M. tuberculosis-infected human macrophages. A similar suppressive effect of IFN-β on IL-1β gene expression has been reported in dendritic cells isolated from multiple sclerosis patients before treatment (38). Nevertheless, type I IFNs have also been shown to regulate IL-1β production at the posttranscriptional level. For example, it has been reported that type I IFNs positively regulate IL-1β release by enhancing inflammasome activation in F. tularensis-infected murine macrophages (33), although in vivo type I IFN signaling has been shown to impair host control of Francisella infection (39). Moreover, although a previous study indicated that type I IFN/Tyk2 signaling regulates LPS-induced IL-1β production at the translational level in murine macrophages (40), a more recent paper reported that type I IFNs regulate LPS production by BCG- versus M. tuberculosis-infected human macrophages (Fig. 6B), levels of IL1B mRNA and secreted IL-1β protein were comparable in monocyte cultures infected with M. tuberculosis, BCG, or irradiated M. tuberculosis (Fig. 6G), arguing that only differentiated macrophages are capable of distinguishing virulent from avirulent mycobacteria with regard to IL-1β release. Therefore, the IL-1β response to mycobacteria is influenced both by properties of the pathogen as well as the host cell type that is infected.

![Figure 7](http://www.jimmunol.org/)
and alum triggered IL-1β production at the levels of both pro–IL-1β protein synthesis and inflammasome activation (41). These discordant findings have yet to be reconciled. One possibility is that the different mechanisms observed may result from variations in the strength, duration, and/or the combination of stimuli used for triggering pro–IL-1β synthesis and mature IL-1β release. Differences in the timing and duration of exogenous type I IFN exposure employed in the different studies may also have contributed to the distinct results obtained.

Interestingly, although IFN-α/β and IFN-γ share related signaling pathways, analyses performed on a subset of donors suggest that IFN-γ does not exhibit the same effects on IL-1β production by M. tuberculosis-infected human macrophages. Moreover, IFN-γ, a known macrophage-stimulating cytokine, is unable to antagonize the suppressive effects of endogenous type I IFNs on infected human macrophages, suggesting a dominant effect of type I over type II IFN signaling in regulating the IL-1β response to M. tuberculosis in human macrophages. This distinction may be due partly to the known ability of M. tuberculosis to interfere with IFN-γ signaling in macrophages, which has been proposed as a mechanism for evasion of adaptive immunity by the pathogen (42, 43).

The functions of the type I IFN and IL-1β pathways in human TB are poorly understood. Studies in the murine model have suggested that two cytokines play opposite roles in host resistance to M. tuberculosis, and the present study demonstrates that type I IFNs clearly limit IL-1β production in human macrophages infected with the pathogen. Because IL-1β is host protective in murine TB, restriction of IL-1β production by type I IFNs might be expected to prevent host clearance of the organism. However, due to the known proinflammatory effects of IL-1β on cell recruitment, it is also possible that downregulation of its activity in some settings could be host protective through dampening excessive IL-1β–mediated tissue pathology induced by virulent mycobacteria (44) and/or even preventing the establishment of the infection by restricting macrophage recruitment to granulomas, a process that has recently been proposed to support rather than limit mycobacterial survival in vivo (45). The impact of such regulation on the establishment and maintenance of chronic M. tuberculosis infection in humans remains to be elucidated.

Regardless of the exact mechanisms involved, the balance between IL-1β and type I IFN responses may be pivotal in ensuring the survival of host as well as pathogen, thereby contributing to the maintenance of persistent M. tuberculosis infection. Disturbing this balance may be detrimental for host control of the infection, as evidenced by our previous finding that polyriboinosinic-polyribocytidylic acid induction of type I IFNs is not due to a defect in the induction of pro–IL-1β message by BCG but rather the inability of the organism to trigger caspase-1–dependent processing and secretion of mature IL-1β. A likely explanation for this caspase-1 activation defect is the different intracellular trafficking of virulent versus avirulent mycobacteria in macrophages. Thus, virulent M. tuberculosis (which expresses the ESX-1/RD1 secretion system) has been proposed to translocate bacterial products from the phagolysosome into the cytoplasm where, as previously demonstrated in murine macrophages, they trigger host inflammasome activation and mature IL-1β protein release (10–12, 44). In contrast, avirulent BCG and other RD1-deficient mycobacteria lack this secretion system and are retained within the phagolysosomal compartment (Fig. 7). Therefore, regardless of the regulation of IL1B transcription by type I IFNs, avirulent mycobacteria remain incapable of stimulating the production of mature bioactive IL-1β protein. For this reason, it can be concluded that in macrophages infected with virulent versus avirulent mycobacteria IL-1β production is governed by both overlapping and distinct mechanisms. In addition to highlighting distinctions between M. tuberculosis and BCG, our data reveal major differences between human macrophages and monocytes in the regulation of IL-1β production following mycobacterial infection. It has been previously demonstrated that following pattern recognition receptor stimulation, human monocytes do not require a second signal for IL-1β release because caspase-1 is constitutively activated (34, 35). The latter distinction is the likely explanation of our observation that, in direct contrast to infected macrophages, M. tuberculosis- and BCG-infected monocytes secrete comparable levels of IL-1β. This cell-specific difference in IL-1β regulation in monocellular phagocytes may have important in vivo implications. Thus, if the mycobacterial infection is not contained locally within tissue macrophages and disseminates systemically, interaction of circulating monocytes with mycobacteria will trigger the robust production of IL-1β without the requirement for additional inflammasome activation signals, thereby preventing further spread of the pathogen.

M. tuberculosis has evolved multiple strategies to counteract host defense mechanisms in infected macrophage by regulating host cytokine signaling, cell survival, Ag presentation, and microbicidal activities (48, 49). In this study, we have demonstrated a cytokine regulatory loop by which M. tuberculosis can manipulate host IL-1 production through the induction of type I IFNs. These findings have important implications for the understanding of M. tuberculosis virulence, persistence, and pathogenesis. Because type I IFNs are readily triggered by numerous viral and other pathogens, these pathways may be of particular relevance in delineating the mechanisms by which coinfections may contribute to TB progression. The extent to which type I IFN and IL-1β induction and signaling influence the outcome of M. tuberculosis infection in humans is thus an important topic for future investigation.

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