

Regulation of Innate and Adaptive Immune Response to *Mycobacterium tuberculosis* by Interferons Type I, II and III

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ABSTRACT

Tuberculosis (**TB**) is one of the most common infections worldwide, and in 2012 around 8.6 million people developed TB and 1.3 million died from the disease.

The aim of this chapter is to present the previously known and new findings about the role of interferons type I and II, and recently discovered type III in regulation of innate and adaptive immune response to *Mycobacterium tuberculosis* (*M. tuberculosis*).

Infection of various cell types with *M. tuberculosis* induce both IFN- α and IFN- β synthesis. The majority of the studies support the findings that IFN type I actually promote infection with *M. tuberculosis*. It has been well establish that IFN- γ has protective function against *M. tuberculosis* and the other mycobacteria and that the primary source of this cytokine are CD4+ and CD8+ T cells. Recently it has been shown that also the innate lymphocytes, $\gamma\delta$ T cells, Natural Killer (**NK**) T cells and NK cells can also be the source of IFN- γ in response to mycobacterial infection.

Several studies have shown that CD4+ T cells protect mice against *M. tuberculosis* independently of IFN- γ . The balance between IFN- γ and different cytokines such as IL-10 and other Th2 cell cytokines is likely to influence disease outcome. Type I IFN appears to be detrimental through at least 3 separate, but overlapping, type I IFN-mediated mechanisms: induction of excessive apoptosis, specific suppression of Th1 and IFN- γ responses, and dampening of the immune response by strong IL-10 induction. Recently it has been found that *M. tuberculosis* infection in A549 lung epithelial cells stimulate up-regulation of IFN- λ genes *in vitro*. IFN- λ s also have a role in modulation of Th1/Th2 response. IFN- λ s are not essential for *M. tuberculosis* infection control, but can give some contribution in immune response to this pathogen.

Keywords: Interferon; Interferon lambda; *Mycobacterium tuberculosis*

INTRODUCTION

The causative agent of human tuberculosis is bacilli belonging to *Mycobacterium tuberculosis* (*M. tuberculosis*) complex, refers to group of species: *M. tuberculosis*, *M. canettii*, *M. africanum*, *M. microti*, *M. bovis*, *M. caprae* and *M. pinnipedi* that are genetically very similar. Among them, the most common cause of human tuberculosis is *M. tuberculosis*. Tuberculosis remains a major public health problem predominantly affecting low- and middle-income countries. Tuberculosis (TB) is one of the most common infections worldwide, and in 2012 around 8.6 million people developed TB and 1.3 million died from the disease [1]. The current tuberculosis epidemic is being sustained by two important factors: the Human Immunodeficiency Virus (HIV) infection and its association with development of the active tuberculosis and increasing resistance of *M. tuberculosis* strains to the first line anti-tuberculosis drugs [1].

Although human immune system can control the infection caused by *M. tuberculosis*, control does not invariably lead to sterilization. Similar as other intracellular bacteria, *M. tuberculosis* is able to avoid most of the antibacterial effects by living and growing inside macrophages. Although progression to symptomatic disease can sometimes be attributed to acquired immune deficiency (for example, AIDS, administration of TNF antagonists, corticosteroids) in many cases immunocompetent individuals also develops active tuberculosis, which indicates failure of their immune system to control the infection [2].

It has been widely accepted that interferons type I and II are important for host resistance to mycobacterial infection [2,3]. During the past ten years several studies that support the findings that IFN production by the innate immune system is an important component of the immune response to *M. tuberculosis* have been published. In a recently published study, it has been shown that IFN-inducible genes are highly expressed in the leukocytes of active, but not latent tuberculosis patients, strongly arguing for a contributing role of type I IFN in development of the disease [4]. Several studies have shown that the induction of the type I IFN genes in human macrophages is possible only with the live virulent mycobacteria and is not triggered either by BCG or RD1 deficient strains of *M. tuberculosis* [4,5].

Interferons (**IFNs**) are cytokines released by host cells in response to the presence of pathogens or tumor cells. The classification of human IFNs has been done on basis of the cognate receptors through which they signal gene sequence similarity and chromosomal location [6]. IFN type I include 17 subtypes (13 subtypes of IFN- α , and IFNs $\beta/\epsilon/\kappa/\omega$). They bind to a receptor complex composed of two chains, IFNAR1 and IFNAR2 [7]. IFN type II has only one member, IFN- γ , which signals through the receptor complex composed of the IFN γ R1 and IFN- γ R2 subunits [6].

Interferons are part of the large family of class II cytokines that includes also 6 cytokines related to IL-10 (IL-19, IL-20, IL-22, IL-24 and IL-26) [8,9,10]. IFNs and the IL-10 related cytokines can be grouped into the same family because they all signal through the receptors that share common motifs in their extracellular domains [10].

During 2003, new cytokine family classified as type III interferon has been discovered [8,9]. In humans there are three members of this family; designated as IFN- λ 1 (IL-29), IFN- λ 2 (IL-28A) and IFN- λ 3 (IL-28B). Recently, a new member of this family has been described, IFN- λ 4 [11]. IFN type III represent an evolutionary link between type I interferon and IL-10 family. IFN- λ 2 and IFN- λ 3 are virtually identical, sharing 96% amino acid identity, since IFN- λ 1 has 81% homology to IFN- λ 2/3 [9]. IFN- λ 4 has similar structure as IFN- λ 3 [11].

The aim of this review is to present the former and recent findings about the role of interferons type I and II, and recently discovered type III in *Mycobacterium tuberculosis* (*M. tuberculosis*) infection control.

For the understanding of this topic, it is necessary to describe the basic mechanism of the innate and the adaptive immune response to *M. tuberculosis* infection.

INNATE AND ADAPTIVE IMMUNE RESPONSE TO *M. TUBERCULOSIS*

The main sources of the infection are active disease patients with sputum smear positive pulmonary tuberculosis. Primary infection leads to clinical disease in less than 10% infected persons. In the remaining cases, the strong immune response arrests further growth of bacteria [2,12].

M. tuberculosis is exquisitely adapted obligate human pathogen that has the ability to survive and replicate inside a modified phagosomal compartment of the host macrophages. In this environment, *M. tuberculosis* is able to persist for decades [13]. A strong cell-mediated immune response effectively inhibits bacterial replication in approximately 90% of otherwise healthy individuals (latently infected individuals) [12]. In minority of cases, some bacilli escape killing by modifying the different mechanisms of immune cells (such as phagosome-lysosome fusion, antigen presentation by MHC class I, class II and CD1 molecules, nitric oxide and other nitrogen intermediates production) and remain in non-replicating (dormant or latent) state in old lesions [14,15]. The impairment of the immune response results in progressive bacterial replication, necrosis of infected lung tissue and spreading of infection. Similar as other pathogens that cause

chronic infections, the long term survival of *M. tuberculosis*, depends of the delicate balance between bacterial virulence and the host immunity [2,16]. *M. tuberculosis* is an intracellular pathogen that survives primarily in macrophage phagosomes. Infection with *M. tuberculosis* starts with phagocytosis of the bacilli by phagocytic antigen presenting cells in the lung including alveolar macrophages and Dendritic Cells (DC) [17]. Initial recognition is mediated by Pattern Recognition Receptors (PRR), which include Toll-Like Receptors (TLRs) and Nucleotide Binding Oligomerization Domain (NOD) –Like Receptors (NLRs) [18]. These receptors recognize conserved microbial structures, known as Pathogen Associated Molecular Patterns (PAMPs) [19]. The initial recognition elicits both the innate and adaptive responses of the host [20].

The control of the infection with *M. tuberculosis* depends on the development of efficient innate and adaptive responses [20]. T cells (CD4+, CD8+ and $\gamma\delta$ T cells) play the important role in the protection [2,3]. The main sources of immune mediators are CD4+ T cells that produce mainly IFN- γ , IL-2 and lymphotoxin- α [12,18]. CD8+ T cells and $\gamma\delta$ T cells are important source of granzymes and perforins that have a direct effect on mycobacteria and infected cells through Fas ligand- dependent and independent mechanisms [12,15].

It has been well known that DCs are crucial for the priming of T cells because they efficiently pick up antigen in the periphery and traffic to the lymph nodes, where T cell priming occurs. In the draining lymph nodes, thousands of naïve T cells transiently interact with the DC. If a naïve T cell's TCR recognizes a peptide antigen presented by the DC, a high affinity and longer lasting interaction occurs [21]. During such a cognate interaction, the accessory and co-stimulatory molecules expressed by the DC bind their counter-receptors on the T cell forming an immune synapse. The end result of this interaction is activation of the naïve T cell. Once primed, activated CD8+ T cells proliferate; exit the lymph node and home to sites of inflammation [22].

Significant progress has been made in elucidating the events leading to priming of CD8+ T cells specific for *M. tuberculosis*. There were suggested two possible mechanisms leading to the presentation of mycobacterial antigens to naïve CD8+ T cells. In the first possibility, infected DC in the lung traffic to the lymph nodes where they directly prime CD8+ T cells. The second possibility is that uninfected DC acquire mycobacterial antigens from dying cells and then migrate to the lymph node where they cross-present the antigens to CD8+ T cells. In both cases, the mycobacterial antigens still need to be transferred from the phagosomal compartment into the cytosolic compartment, which is sampled by the class I MHC pathway [22].

It has been known that *M. tuberculosis* efficiently infects murine and human DC *in vitro* [23]. How antigen is transferred from the phagosomal compartment to the class I MHC pathway in *M. tuberculosis* infected DC is unknown; however, two mechanisms have recently been described. First, there are the evidence that *M. tuberculosis* translocates from the phagosome to the cytosol in both human macrophages and DC [24]. In this case, the class I MHC pathway could directly sample bacterial products. An argument against this process is the late kinetics of translocation (days) compared to presentation of antigens (hours) [25].

Today it has been known that translocation is dependent on the bacterial ESX-1 type VII secretion system; however, priming of *M. tuberculosis* specific CD8+ T cells can occur independently of ESX-1 [26]. It is possible, that ESX-1 or related proteins, damages the phagosomal membrane allowing bacterial proteins to leak into the cytosol of the infected macrophage. Both of these mechanisms would be consistent with the experimental finding that proteins secreted by *M. tuberculosis* are over-represented as antigens recognized by CD8+ T cells [27].

Whether *M. tuberculosis* infects DC *in vivo* is less clear. However, in contrast to macrophages, DC infected with *M. tuberculosis* express co-stimulatory molecules, a sign of activation and an indication that they are competent to prime T cells [22].

M. tuberculosis, as a successful pathogen, has developed many ways to evade, or inhibit, immunity. Given the important role of DC in T cell priming and transporting *M. tuberculosis* from the lung to the lymph node, it is not surprising that *M. tuberculosis* interrupts this process by impairing activation of DC and their subsequent trafficking to the lymph nodes [28].

Samstein and contributors from the Pamers' group have found that *M. tuberculosis* transport from the lung to the draining lymph node following monocyte depletion 7-11 days after the infection. That might delay priming of *M. tuberculosis* specific CD4 T cells. Their research was made on the mice model and suggests that the dissemination of *M. tuberculosis* to the draining lymph nodes for initiating of T cell immunity requires *M. tuberculosis* infected monocytes but not DC [29].

Published studies have been shown that the development of T cell effectors responses requires the dissemination of live mycobacteria or mycobacterial antigens presented by DC [3,15]. After the presentation, several chemokine molecules are upregulated, mostly CCR7. This induction leads to migration of the infected cells into the draining lymph nodes [30,31]. The proliferation of naïve T cells specific for the *M. tuberculosis* occurs first in the draining lymph node in response to this dissemination [32]. The role of CD4+ and CD8+ T cells in immunity against *M. tuberculosis* has been well established, but there is growing evidence that also $\gamma\delta$ T cells play a significant role in the cellular immunity against *M. tuberculosis* [12,18]. The exact mechanisms are not completely understood, but recently it has been shown that $\gamma\delta$ T cells are significant source of IL-17 and IFN- γ . They are the important cytokines in the proinflammatory responses and chemokine regulation [15].

Interestingly, a large fraction of the transcriptional response to *M. tuberculosis*, which includes chemokines RANTES and IP-10, along with the inducible nitric oxide synthase enzyme NOS2, is induced independently of TLR2 and TLR4 and adapter proteins MyD88, MAL and TRIF. These responses rely on autocrine or paracrine signaling via type I interferons (IFN- α and IFN- β), which are induced through mostly yet unidentified pathways [33].

M. tuberculosis possesses a specific secretion system ESX1, which has been suggested to play a key role in mycobacterial virulence by facilitating the releasing of *M. tuberculosis* products into the macrophage cytoplasm [34]. It is interesting that ESX1 has been implicated in type I IFN

induction and in activation of the inflammasome. The signaling pathways for the IL-1 and IFN type I play opposite roles in immune response to *M. tuberculosis*. In contrast to IL-1 β , type I IFN seems to promote infection with *M. tuberculosis* in mice. It has been shown that virulent strains of *M. tuberculosis* induce high levels of IFN type I *in vivo* both in humans and mice [5].

The cytokines TNF, GM-CSF and IL-1 β and vitamins C and D are all implicated as mediators that activate macrophages to control the growth of *M. tuberculosis*. The widespread use of TNF blockers in therapy of autoimmune disease, such as rheumatoid arthritis, has resulted in increasing number of reactivated latent tuberculosis cases [35].

Neutrophils have an early protective role against *M. tuberculosis* in the lungs by producing IL-1 β , TNF, defensins, cathelicidins, lipocalin, NADPH oxidase and superoxides [36].

In a study in mice model it has been shown that dominant locus *Ipr1* is preferentially expressed by macrophages and alters their death modality toward apoptosis or necrosis [37]. This is an interesting insight, since some people might develop active tuberculosis because their macrophages are unable to control intracellular growth of *M. tuberculosis*, rather than because they have dysfunctional T cells.

Although there are no general rules, it is now clear that apoptosis is an innate defense mechanism that the host uses to limit the infection. In contrary, virulent *M. tuberculosis* strains subverts host pathways to inhibit apoptosis and instead induce necrosis, which allows the bacterium to evade immune response and to infect other cells. The regulation of the death modality is complex and now is clear that in a wild type host infected with virulent *M. tuberculosis*, both process of cell death occurs, depending on the microenvironment of the infected cell [22].

In peripheral blood of patients with active pulmonary tuberculosis, type I and type II interferon inducible genes are upregulated. Surprisingly, this gene signature is mostly accounted for by changes in neutrophil gene expression [38].

Although the epithelium can become infected, macrophages in the alveoli are the primary target for *M. tuberculosis* once it enters the lung. The interaction between *M. tuberculosis* and these cells actually defines the progression of infection [39].

Virulent *Mycobacterium tuberculosis* inhibits apoptosis and triggers necrosis of host macrophages and cause delay in the initiation of adaptive immunity. Necrosis is a mechanism used by bacteria to exit macrophage, evade the host defenses, and disseminate. In contrary apoptosis is associated with diminished pathogen viability. The balance between PGE2 and LXA4 determines whether macrophages infected with *M. tuberculosis* undergo apoptosis or necrosis and this balance determines the outcome of infection [40].

IFN TYPE I IN *M. TUBERCULOSIS* INFECTION CONTROL

Initiation of IFN Type I Production

Type I interferons expression is not detectable under steady state conditions *in vivo* using classical methods such as gene expression analysis by RT-PCR or protein titration by ELISA or bioassays. However, extremely small or localized but functionally relevant quantities of IFN-I are produced under steady state conditions [41]. Steady state IFN-I responses are promoted by gut commensals [42].

Pattern Recognition Receptors (**PRRs**) are important in the innate immune detection of pathogenic infection by recognizing different molecular motifs termed “Pathogen Associated Molecular Patterns” (**PAMPs**) [19]. After this initiation, PRR activate production of many cytokines and chemokines for supporting immune response. Type I IFNs are responsible for inducing transcription of a large group of genes that play a role in host resistance in viral infections [4]. This transcription activate the key components of the innate and adaptive immune response including Antigen Presentation Cells (**APC**) maturation as well releasing the cytokines involved in T cell activation [43].

It is important to note that type I INFs are transcriptionally regulated and are induced after recognition of typical PAMPs during infection [41]. There is a classical positive feedback-production loop for IFN- β . The transcription is firstly activated by signals that induce cooperative binding of the transcription factors c-Jun/ATF-2, NF- κ B and interferon regulatory factor (IRF3) to the IFN- β promoter. IRF3 is expressed constitutively in all cells and is present in the cytoplasm of unstimulated cells [41]. Upon induction, IRF3 becomes phosphorylated by the serine-threonine kinases TANK-binding kinase-1 (TBK1/T2K/NAK) or the inducible I κ B kinase [44,45]. IRF3 in this process forms the dimmers, translocates in the nucleus and combines with the co-activator molecule CBP/P300 to activate IFN- β [46]. After that, IFN- β initiates a positive feedback loop by the binding to IFNAR which activates AJK protein tyrosine kinases (JAK1 and Tyk2). These kinases phosphorylate STAT1 and STAT2 [46]. The complex STAT1 and STAT2 together with IRF9 form a transcription factor complex named IFN-Stimulated Gene Factor 3 (**ISGF3**) [41]. Then ISGF3 translocates in the nucleus and binds IFN-Stimulated Response Elements (**ISRE**) in order to induce expression of a large number of “IFN-inducible” genes, like IRF7 [41]. This mechanism of action is shown in Figure 1. IRF7 molecule can be expressed primary in lymphoid cells and it is transcriptionally induced in different cell type upon a type I IFN signaling [46].

The Production of Type I IFN Mediated by TLR

Toll-Like Receptors (**TLRs**) are a family of PRRs consisting of 12 members in mammals [19]. These molecules are expressed in the surface of the cell membrane or on the endocytic vesicles membrane of many type immune cells including macrophages and Dendritic Cells (**DC**). In the past decade it has been shown that TLRs recognize PAMPs from a broad array of pathogens and

that play a key role in activation of the innate and adaptive immune responses [47]. Characteristic feature of TLR is to contain extracellular Leucine-Rich Repeat (**LRR**) domains which participate in ligand recognition and intracellular TIR (Toll-interleukin IL-1 receptor) domains. Together they transmit downstream signals through the interactions with TIR-domain-containing adaptor molecules including myeloid differentiation factor 88 (MyD88), TIR-Associated Protein (**TIRAP/MAL**) and few other adaptor molecules [48].

It has been found that several members of TLR family activate production of type I IFN following ligand stimulation in various cell types [48]. TLR3 and TLR4, which recognize viral dsRNA and Gram negative bacterial lipopolysaccharide, can induce certain signaling pathways, including type I IFN production [49]. MyD88 plays an important role in the initiation of the innate immune response to *M. tuberculosis*. On reception to this molecule, TLR4 can induce intracellular signals through a pathway which is mediated by the adaptor molecule Toll/IL-1R (**TIR**) domain-containing adapter inducing IFN- β (**TRIF**). TLR4 induced autophagy has an important role in the phagosome-lysosome fusion, a process impaired by *M. tuberculosis* [50].

Of the particularly importance is a MyD88 dependent pathway activated by TLR7, TLR8 and TLR 9 leading to type I IFN production. This induction seems to occur in certain dendritic cell subtypes, for instance plasmacytoid dendritic cells, also known as interferon-producing cells [51].

After the interaction of specific mycobacterial components with TLRs, signaling pathways involving MyD88 are triggered [52]. After this initiation, IL-1 Receptor-Associated Kinases (**IRAK**), TNF Receptor-Associated Factor (**TRAF**) 6, TGF- β -Activated Protein Kinase 1 (**TAK1**) and Mitogen-Activated Protein (**MAP**) kinase, activate signal cascade leading to activation and nuclear translocation of NF- κ B [53]. The activation of NF- κ B leads to transcription of genes involved in the activation of the innate immune response, and to the production of proinflammatory cytokines like TNF, IL-1 β , IL-12 and nitric oxide [54].

In the activation of the innate immune response to *M. tuberculosis* important role have TLR2, TLR4 and TLR9 and possibly TLR8 [17,55]. *In vitro* studies showed that IL-12 release induced by *M. tuberculosis* components in dendritic cells was TLR9 dependent [12].

Recently it was confirmed that IFN type I contribute to protection in many bacterial infections [56], but there is growing evidence that it can impair the protection against some intracellular bacteria. In previously published study it has been shown that addition of exogenous IFN type I or its induction through TLR3 agonist polyI:C lead to decreasing of bacterial infection control and to increasing susceptibility to disease [57].

IFN Type I in Immune Response to *M. tuberculosis*

Infection of various cell types with *M. tuberculosis* has been shown to induce both IFN- α and IFN- β synthesis [15]. It has been found that infection with hyper virulent strain of *M. tuberculosis* induces higher level of IFN- α than normal strain and kills infected mice more faster [57]. Although

the production of Th1 cytokines was lower in mice infected with the hyper virulent strain, the simultaneous administration of IFN- γ in infected animals had no effects on survival. The administration of type I IFNs further enhanced death caused by the *M. tuberculosis* [57].

It has been observed that early death of infected mice induced by virulent *M. tuberculosis* strain may be partially due to the failure to induce a proper Th1 response, but it can be also partially due to production of type I IFNs [57].

Till today there is a lack of consensus if the interferons type I have any antimicrobial activity against *M. tuberculosis*. The majority of the studies support the findings that IFN type I actually promote infection with *M. tuberculosis* [58,59]. Antonelli and contributors have shown that type I IFN receptor deficient mice chronically infected with various different *M. tuberculosis* strains displayed reduced bacterial loads when compared with infected animals without this impairment [60].

Despite the ability of TLR4 to trigger transcription of the genes for type I IFN, existing evidence indicates that this response requires the recognition of bacterial products in the cytosol [18]. During the *M. tuberculosis* infection, the bacilli must disrupt the phagosomal membrane and to escape into the cytosol in order to trigger the type I IFN response in resting macrophages [19]. The induction of the IFN type I after infection with *M. tuberculosis* virulent strains has been evaluated in lot of studies [61,62]. The comparison of the gene expression profiles in the mouse macrophages infected with the virulent strains of *M. tuberculosis* with the infection with a virulent strain (with the inactive ESX-1 secretion system), revealed the selective induction of type I associated genes and IFN- β by the virulent strain [34]. This induction was not observed in infection with the a virulent strain. This response of IFN type I upon infection with virulent strains was independent of the TLR adaptor TRIF and RIP2, the adaptor for NOD1 and NOD2 signaling, but it seems to need TBK (a kinase necessary for type I IFN induction in viral infections and infections with *Listeria monocytogenes*) [34]. The findings of another study were different in arguing that NOD1, NOD2 and RIP2 are actually required for type I IFN induction by *M. tuberculosis* virulent strains [33]. Recently published study has suggested that type I IFN induction is an important factor that determines the outcome of *M. tuberculosis* infection in humans [4]. In this study the gene expression in whole blood samples collected from the patients with active tuberculosis and from the latently infected individuals has been investigated. They found that the majority of the patients with the active tuberculosis displayed an expression signature dominated by IFN-induced genes in neutrophil granulocyte. The similar profile was present in less than 10% of persons who were latently infected with *M. tuberculosis*, suggesting that these persons are at the highest risk for the development of active form of the disease. The findings of the previously published studies implicate that type I IFN is a down regulator of protective immune response in both mouse and human *M. tuberculosis* infection [33,61].

Later, it has been found that successful treatment of active tuberculosis leads to the disappearance of type I IFN signature, and these data confirm that IFN type I correlate with

lacking of *M. tuberculosis* infection control [63]. Since it is well known that IFN- γ driven Th1 responses are crucial for the immune response in *M. tuberculosis* infection, one of the suggested mechanisms for type I IFN mediated loss of *M. tuberculosis* infection control is the blockade of Th1 responses [57]. Recently it was shown that type I IFNs induce the immunosuppressive cytokine IL-10 and therefore reduced immune response due to type I IFN-induced IL-10 contribute to loss of *M. tuberculosis* infection control [58].

Novikov and coworkers recently have shown that IL-1 β , induced by virulent *M. tuberculosis*, is negatively regulated by type I IFN. In this study, it has been demonstrated that there is a functional intersection between type I IFN and IL-1 β signaling [5].

IL-1 β is useful in the protection against *M. tuberculosis* and it can be suppressed by type I IFNs [5,64]. Recently it has been demonstrated that there is a reciprocal control of type I IFNs by IL-1 β through a prostaglandin E2 mediated mechanism and that treatment with prostaglandin E2 reduce type I IFN levels and increases the protection in infection with *M. tuberculosis* [65].

In conclusion, in several bacterial infections requiring strong IFN- γ responses for protection, type I IFN appears to be detrimental through 3 separate, but overlapping, type I IFN-mediated mechanisms. These mechanisms are: induction of excessive apoptosis, specific suppression of Th1 and IFN- γ responses, and dampening of the immune response by strong IL-10 induction [66].

IFN TYPE II IN *M. TUBERCULOSIS* INFECTION CONTROL

IFN- γ is classified as a Th1 type cytokine that stimulates cell-mediated immune responses that are critical for the development of host protection against pathogenic intracellular microorganisms such as *M. tuberculosis* [67]. It is important to note that type I and type II IFNs often work together to activate the mechanisms of innate and adaptive immune responses that result in the induction of effective immunity [6].

It has well established that IFN- γ has protective function against *M. tuberculosis* and the other mycobacteria and that the primary source of this cytokine are CD4+ and CD8+ T cells. Recently it has been shown that also the innate lymphocytes, $\gamma\delta$ T cells, Natural Killer (**NK**) T cells and NK cells that possess restricted or invariant receptor repertoires, can also be the source of IFN- γ in response to *M. tuberculosis* infection. These cells can be the secondary sources of IFN- γ that prevent the adaptive immune system from being overwhelmed due to heavy or the exposure with the hyper virulent *M. tubersulosis* strains [2]. There is a possibility that these innate sources have the important protective role in humans, particularly in the case of T cell immunodeficiency as seen in individuals infected with HIV who were infected with *M. tuberculosis* [15].

It has been hypothesized that defects in multiple TLRs plays a role in *M. tuberculosis* infection control [17]. For instance, TLR2 and TLR9 double knockout mice display greater defects of IL-12 and IFN- γ production in comparison with single TLR knockout mice. That leads to earlier manifestation of the infection even with the low inoculums of *M. tuberculosis* [68].

It is well known that IFN- γ promotes cellular proliferation, cell adhesion and apoptosis. IFN- γ in the infected macrophages induces respiratory burst contributing to the production of RNI and ROIs [69]. After this induction, activated macrophages produce immunomodulatory and chemotactic molecules that promote up regulation of receptors for TNF- α and NRAMP-1 molecules. One of the most important effects of IFN- γ in *M. tuberculosis* infection control is the production of large amounts of ROIs and NO by the cells of innate immune response [69]. In the mice model of infection with *M. tuberculosis*, it has been shown that NO is essential in the killing of bacilli by mononuclear phagocytes [70]. The marked defects in the IFN- γ -signaling pathways in the IFN- γ deficient mice lead to high susceptibility to mycobacterial infection. These animals also fail to develop granulomas following inhalation of *M. tuberculosis*, with significant defects of macrophages activation [70].

Today it is known that the innate lymphocytes, $\gamma\delta$ T cells, natural killer T cells and NK cells that possess restricted or invariant receptor repertoires can also produce IFN- γ in response to mycobacterial stimulation and display protective effects against *M. tuberculosis* both *in vitro* and *in vivo* [15].

Till today is still unclear if these cells contribute to the IFN- γ dependent control of infection when an adaptive immune response occurred. Feng and coworkers demonstrated that NK cells producing IFN- γ are responsible for partial resistance of rag-/- mice to infection with *M. tuberculosis*, but similar depletion of NK cells in T cell sufficient WT mice seems to have no influence on control of infection [71].

Interestingly, a recently published study provided evidence that CD4+ cells can induce *M. tuberculosis* growth arrest without to secrete IFN- γ , TNF or both cytokines [72]. In that study it has been shown that the growth arrest over the first 21 days after aerosol challenge occurred even when T cells were unable to express Th1 promoting transcription factor or to secrete IFN- γ , TNF or both cytokines [72]. They found that there was no need for host IFN- γ , TNF, the Inducible Nitric Oxide Synthase (**iNOS**) gene, or superoxide-generating machinery to mediate this control.

In another study it has been published that Th2 differentiated cells could not induce the growth arrest of *M. tuberculosis* as efficiently as Th1 or even Th17 differentiated cells [3]. However, recently published data have shown that *in vitro* generated memory CD4+ T cell enhance protection by induction of multiple innate cytokines and chemokines in the lung in a antigen-dependent, but IFN- γ and TNF independent manner [73]. It is important to note that the regulatory T cell are induced very early and can regulate the effectors function in the aerosol *M. tuberculosis* infection [74].

Recently it has been shown that IFN- γ production in the activated CD4+ T cells is suboptimal in the lungs of the infected animals and that contributes to the inability of the host to eliminate *M. tuberculosis*. The low frequency of activated T cells in infected animals enables persistence of *M. tuberculosis in vivo* [75]. In this study it has been shown that the frequency of activated CD4+ T

cells that secrete IFN- γ in infected animals is low even the peak of the response and it decreases during the chronic phase [75]. This findings support previous work that suggested this pattern [76].

Regarding the induction of immune response it has been shown that the frequency of IFN- γ producing cells correlates with the availability of cognate antigen. Also it has been noted that the delivery of the antigen peptide results in significantly increased frequency of cytokine expression [75]. In this study, it has been found that inside the granuloma there was small number of cells expressing INF- γ [75]. These findings were supported in another study, where it has been shown that the delivery of exogenous peptide antigen was failed to signal both migration arrest and cytokine production by the effectors cells [77]. An important observation of this study was that the cells that exhibit migration arrest could produce the cytokines to a closely adjacent infected cell. This finding suggests that inside the granuloma, T cell derived IFN- γ mediation of infected phagocyte activation is an important protective mechanism [77].

In an interesting study published in 2011, biomarkers distinguish active tuberculosis from latent infection by gene expression profile of peripheral blood mononuclear cells have been investigated. It has been found that IFN- γ , CXCL10, IL-2 and CXCL11 exhibit statistically significant difference in the comparison with latent and healthy group [78]. In another study by Berry et al., a neutrophil-driven IFN (both IFN- γ and type I IFNs)-inducible gene profile was identified to have a specific signature upon *M. tuberculosis* infection [4].

In a study taken from Harari and contributors, the cytokine profile (IFN- γ , TNF- α and IL-2) for *M. tuberculosis* specific CD4+ T cell responses in subjects with latent *M. tuberculosis* infection and active tuberculosis disease have been investigated. The measurement of the *M. tuberculosis* specific T cells was made by polychromatic flow cytometry. The results of this study have showed substantial increase in the proportion of single-positive TNF- α (+) specific CD4+ T cells in subjects with active disease, and this parameter was the strongest predictor of diagnosis of active disease versus latent infection [79].

The cytokine profile of *M. tuberculosis* specific CD4+ T cells showed that persons with latent tuberculosis infection had a higher frequency of double-positive IFN- γ (+) IL-2(+) CD4+ T cells and triple-positive IFN- γ (+) IL-2(+) TNF(+) CD4+ T cells compared to those without latent infection. It seems that patients with and without latent tuberculosis are characterized by different intracellular cytokine profiles [80].

The data shown in study published by Lu and coworkers indicate that signaling pathway of IFN- γ displayed significant difference in the comparison between active tuberculosis and latent tuberculosis group, implicating its key role in immune response to *M. tuberculosis* [78].

In an interesting opinion article published by Behar' group, the central dogma of the immunity to *M. tuberculosis* was reassessed. Previously it was widely accepted that the production of IFN- γ by CD4+ T cells is the major driver of immunity to *M. tuberculosis* [81].

These findings were based mostly on the studies on knockout mice (lacking IFN- γ) and the findings that patients infected with HIV, when develop AIDS, often develop infection caused by mycobacteria.

A central role of IFN- γ , a cytokine that is involved in the response against viruses and intracellular bacteria, in antimycobacterial immunity is based on the extreme susceptibility of mice that lack IFN- γ [56]. Also, it has been found that some families are more susceptible to mycobacterial infections due to impairment of STAT 1 signaling [82]. Current studies of association between genetic polymorphisms in the innate immune system and the risk of *M. tuberculosis* infection have actually shown that there is a wide variety of innate mechanisms that have protective roles in early infection [39].

However, in reassessing the central dogma, it is worth to note that the risk of active tuberculosis significantly increases during the first year after HIV infection, despite normal CD4+ T cell counts [83]. Also, the progression to AIDS, which is characterized by a substantial loss of CD4+ T cells, does not correlate with the development of active tuberculosis [84]. It is possible that alterations in CD4+ T cell function secondary to HIV infection increase susceptibility to tuberculosis even before CD4+ T cell numbers decrease. It is interesting that most people who develop active tuberculosis have no obvious defects in their T cell compartment and generate specific IFN- γ response to *M. tuberculosis*.

Today is known that T cells producing IFN- γ are not sufficient to prevent active disease. In fact, it has been shown that patients whose T cells produce greater amounts of IFN- γ are more likely develop progress to active disease than patients with weaker responses [85].

Several studies have shown that CD4+ T cells protect mice against *M. tuberculosis* independently of IFN- γ [72]. The most important is that the balance between IFN- γ and different cytokines such as IL-10 and other Th2 cell cytokines actually influence disease outcome [86].

Although IFN- γ is a proinflammatory cytokine, it can also limits inflammation, at least in part by the direct and indirect inhibition of neutrophils. IFN- γ can have antiproliferative effects on T cells and modulate their function, including inhibition of IL-17 production. IL-17 is a cytokine that drives inflammation caused by neutrophils [15].

T cells have the role in influencing the balance of pro- and anti- inflammatory signals. IFN- γ functions as a key negative regulator of innate immunity, including neutrophils and IL-1 β , both of which might be beneficial in early stages of infection, but have detrimental effects if they persist in chronic phase. The anti-inflammatory effect of T cells may prevent extensive protective response that causes harmful effect and tissue damage during chronic infection [38].

THE POTENTIAL ROLE OF IFN TYPE III IN *M. TUBERCULOSIS* INFECTION

Type III IFNs are cytokines exhibiting antiviral and anti-proliferative capacity together with other immunological responses. They display structural features analogous to IL-10-related

cytokines. The role of IFN- λ s in viral infection has been demonstrated in lot of publications, especially considering HCV infection [87,88].

Today it is known that type I and type III IFNs have different biological properties from the type II IFN. IFN- α/β and IFN- λ appear to have potent antiviral and antitumor activities, whereas IFN- γ has antibacterial, antiparasitic and antifungal properties, along with the very important role in immune mediation [6,89].

In the last decade, it has been shown that receptor for IFN- λ s is heterodimeric and it consists from a specific ligand-binding IFN- λ R1 (IL-28R α) chain and the accessory receptor chain, IL-10R2, which is also part of the receptors for IL-10, IL-22 and IL-26 [8,9,90].

Binding of the IFN- λ ligand to its receptor initiates signaling path similar to the type I IFNs. Ligation of STAT2 leads to phosphorylation, activation of ISGF3 and induction of OAS and MxA [8]. Later, it has been observed that IFN- λ ligands also induce the phosphorylation of STAT1, STAT3, STAT5 and STAT4 [91]. These findings suggests more complex properties for the IFN- λ family, since STAT 3 is the signaling mechanism used by members of the IL-10 family (IL-10, IL-19, IL-20) [91], whereas STAT5 is used by IL-2, IL-3 and Granulocyte-Macrophage Colony Stimulating Factor (**GM-CSF**) [92].

Lot of studies has shown that type III IFN has similar biological properties as IFN type I, such as antiviral and antitumor activities [93,94,95]. However, their physiological action can be diverse due to the distribution of their receptor in tissues [96,97]. It has been noted that, unlike for ubiquitously distributed IFNAR, IFN- λ receptor is primary expressed on epithelial cells and specific subsets of immune cells, acting predominately on the mucosa surface [97,98]. Similar as the activation process of the type I IFN, the IFN- λ s receptor activation induce JAK/STAT signaling pathway, leading to transcriptional activation of similar sets of IFN-responsive genes [98], but with different kinetics [99].

Today it is known that IFN genes are expressed following recognition of Microbial Associated Molecular Patterns (**MAMP**) by PRR, acting as sensors for microbial products at the cell surface and endosomal and cytosolic compartments [100,101].

Bacterial infection, including *M. tuberculosis* infection, trigger the expression of interferon I and II genes, but till today little is known about their effect on IFN III genes, whose products plays an important role in epithelial immunity [102]. IFN III genes are activated in Monocyte-Derived Dendritic Cells (**MDDC**), following stimulation with bacterial components, such is lipopolysaccharide and other TLR4 and TLR9 agonists [90,103,104]. Recently it has been found that *M. tuberculosis* infection in A549 lung epithelial cells stimulate up-regulation of IFN- λ genes *in vitro* [102]. In that study 120% fold increasing of IFN- λ 2 gene induction, after *M. tuberculosis* infection was shown.

In a recent study taken from our research group, IFN- λ 2 levels in sputum of the patients with

pulmonary tuberculosis were measured. In this study, statistically important difference between IFN- λ 2 levels in sputum of patients with *M. tuberculosis* active pulmonary infection comparing levels in sputum of healthy persons and persons with latent tuberculosis have been observed.

Also the significant correlation between Bartlett score and IFN- λ 2 level has been observed only in the group of patients with active tuberculosis, suggesting that only the cells that have been stimulated by *M. tuberculosis* components can be source of IFN- λ 2 [105]. The findings of this study suggest that only live bacteria can lead to stimulation of the PRR and to cause induction of IFN- λ 2 synthesis.

Regarding the potential mechanisms in *M. tuberculosis* infection control, it is interesting to note that type III IFNs has recently been described as modulator of the Th2 response, with inhibitory effect on Th2 mediated inflammation [106]. IFN- λ also can have a role in modulation of Th1/Th2 response [107]. Recently it has been described that IL-29 inhibits Th2 cytokine production, especially IL-13 in human cell culture systems [108]. Previously it has been shown that IL-28A increase the induction of Th1 response [109]. These mechanisms are not essential for *M. tuberculosis* infection control, but can give some contribution in immune response to this pathogen.

References

1. World Health Organization. Global tuberculosis report 2014. WHO/HTM/TB/2014.08. Geneva, World Health Organization. 2014.
2. Cooper AM, Khader SA. The role of cytokines in the initiation, expansion, and control of cellular immunity to tuberculosis. *Immunol Rev.* 2008; 226: 191-204.
3. Cooper AM. Cell-mediated immune responses in tuberculosis. *Annu Rev Immunol.* 2009; 27: 393-422.
4. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SAA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature.* 2010; 466: 973-977.
5. Novikov A, Cardone M, Thompson R, Shenderov K, Kirschman KD, Mayer-Barber KD, et al. Mycobacterium tuberculosis triggers host type I IFN signaling to regulate IL-1 β production in human macrophages. *J Immunol.* 2011; 187: 2540-2547.
6. Pestka S, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. *Immunol Rev.* 2004; 202: 8-32.
7. Uzé G, Schreiber G, Piehler J, Pellegrini S. The receptor of the type I interferon family. *Curr Top Microbiol Immunol.* 2007; 316: 71-95.
8. Kolenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, et al. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat Immunol.* 2003; 4: 69-77.
9. Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat Immunol.* 2003; 4: 63-68.
10. Renauld JC. Class II cytokine receptors and their ligands: key antiviral and inflammatory modulators. *Nat Rev Immunol.* 2003; 3: 667-676.
11. Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet.* 2013; 45: 164-171.
12. Cooper AM, Mayer-Barber KD, Sher A. Role of innate cytokines in mycobacterial infection. *Mucosal Immunol.* 2011; 4: 252-260.
13. Korbel DS, Schneider BE, Schaible UE. Innate immunity in tuberculosis: myths and truth. *Microbes Infect.* 2008; 10: 995-1004.
14. Ahmad S. Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. *Clin Dev Immunol.* 2011; 2011: 814943.
15. Zuñiga J, Torres-García D, Santos-Mendoza T, Rodríguez-Reyna TS, Granados J, Yunis EJ. Cellular and humoral mechanisms involved in the control of tuberculosis. *Clin Dev Immunol.* 2012; 2012: 193923.

16. Dheda K, Schwander SK, Zhu B, van Zyl-Smit RN, Zhang Y. The immunology of tuberculosis: from bench to bedside. *Respirology*. 2010; 15: 433-450.
17. Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R. Innate immune recognition of *Mycobacterium tuberculosis*. *Clin Dev Immunol*. 2011; 2011: 405310.
18. Saiga H, Shimada Y, Takeda K. Innate immune effectors in mycobacterial infection. *Clin Dev Immunol*. 2011; 2011: 347594.
19. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol*. 2004; 5: 987-995.
20. Schwander S, Dheda K. Human lung immunity against *Mycobacterium tuberculosis*: insights into pathogenesis and protection. *Am J Respir Crit Care Med*. 2011; 183: 696-707.
21. Bousso P. T-cell activation by dendritic cells in the lymph node: lessons from the movies. *Nat Rev Immunol*. 2008; 8: 675-684.
22. Behar SM, Martin CJ, Nunes-Alves C, Divangahi M, Remold HG. Lipids, apoptosis, and cross-presentation: links in the chain of host defense against *Mycobacterium tuberculosis*. *Microbes Infect*. 2011; 8-9: 749-756.
23. Bodnar KA, Serbina NV, Flynn JL. Fate of *Mycobacterium tuberculosis* within murine dendritic cells. *Infect Immun*. 2001; 69: 800-809.
24. van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, Pierson J, et al. *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell*. 2007; 129: 1287-1298.
25. Grotzke JE, Siler AC, Lewinsohn DA, Lewinsohn DM. Secreted immunodominant *Mycobacterium tuberculosis* antigens are processed by the cytosolic pathway. *J Immunol*. 2010; 185: 4336-4343.
26. Woodworth JS, Fortune SM, Behar SM. Bacterial protein secretion is required for priming of CD8⁺ T cells specific for the *Mycobacterium tuberculosis* antigen CFP10. *Infect Immun*. 2008; 76: 4199-4205.
27. Woodworth JS, Behar SM. *Mycobacterium tuberculosis*-specific CD8⁺ T cells and their role in immunity. *Crit Rev Immunol*. 2006; 26: 317-352.
28. Hanekom WA, Mendillo M, Manca C, Haslett PA, Siddiqui MR, Barry C 3rd, et al. *Mycobacterium tuberculosis* inhibits maturation of human monocyte-derived dendritic cells in vitro. *J Infect Dis*. 2003; 188: 257-266.
29. Samstein M, Schreiber HA, Leiner IM, Susac B, Glickman MS, Pamer EG. Essential yet limited role for CCR2⁺ inflammatory monocytes during *Mycobacterium tuberculosis*-specific T cell priming. *Elife*. 2013; 2: e01086.
30. Bhatt K, Hickman SP, Salgame P. Cutting edge: a new approach to modeling early lung immunity in murine tuberculosis. *J Immunol*. 2004; 172: 2748-2751.
31. Chackerian A, Alt J, Perera V, Behar SM. Activation of NKT cells protects mice from tuberculosis. *Infect Immun*. 2002; 70: 6302-6309.
32. Wolf AJ, Desvignes L, Linas B, Banaiee N, Tamura T, Takatsu K, et al. Initiation of the adaptive immune response to *Mycobacterium tuberculosis* depends on antigen production in the local lymph node, not the lungs. *J Exp Med*. 2008; 205: 105-115.
33. Pandey AK, Yang Y, Jiang Z, Fortune SM, Coulombe F, Behr MA, et al. NOD2, RIP2 and IRF5 play a critical role in the type I interferon response to *Mycobacterium tuberculosis*. *PLoS Pathog*. 2009; 5: e1000500.
34. Stanley SA, Johndrow JE, Manzanillo P, Cox JS. The Type I IFN response to infection with *Mycobacterium tuberculosis* requires ESX-1-mediated secretion and contributes to pathogenesis. *J Immunol*. 2007; 178: 3143-3152.
35. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwiertman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med*. 2001; 345: 1098-1104.
36. Yang CT, Cambier CJ, Davis JM, Hall CJ, Crosier PS, Ramakrishnan L. Neutrophils exert protection in the early tuberculous granuloma by oxidative killing of mycobacteria phagocytosed from infected macrophages. *Cell Host Microbe*. 2012; 12: 301-332.
37. Kamath AB, Alt J, Debbabi H, Behar SM. The major histocompatibility complex haplotype affects T-cell recognition of mycobacterial antigens but not resistance to *Mycobacterium tuberculosis* in C3H mice. *Infect Immun*. 2004; 72: 6790-6798.
38. Nandi B, Behar SM. Regulation of neutrophils by interferon- γ limits lung inflammation during tuberculosis infection. *J Exp Med*. 2011; 208: 2251-2262.
39. Orme IM, Robinson RT, Cooper AM. The balance between protective and pathogenic immune responses in the TB-infected lung. *Nat Immunol*. 2015; 16: 57-63.
40. Divangahi M, Behar SM, Remold H. Dying to live: how the death modality of the infected macrophage affects immunity to tuberculosis. *Adv Exp Med Biol*. 2013; 783: 103-120.
41. Taniguchi T, Takaoka A. The interferon-alpha/beta system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr Opin Immunol*. 2002; 14: 111-116.

42. Tomasello E, Pollet E, Vu Manh TP, Uzé G, Dalod M. Harnessing Mechanistic Knowledge on Beneficial Versus Deleterious IFN- γ Effects to Design Innovative Immunotherapies Targeting Cytokine Activity to Specific Cell Types. *Front Immunol.* 2014; 5: 526.
43. Theofilopoulos AN, Baccala R, Beutler B, Kono DH. Type I interferons (alpha/beta) in immunity and autoimmunity. *Annu Rev Immunol.* 2005; 23: 307-336.
44. Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, et al. LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. *J Exp Med.* 2003; 198: 1043-1055.
45. McWhirter SM, Fitzgerald KA, Rosains J, Rowe DC, Golenbock DT, Maniatis T. IFN-regulatory factor 3-dependent gene expression is defective in Tbk1-deficient mouse embryonic fibroblasts. *Proc Natl Acad Sci U S A.* 2004; 101: 233-238.
46. Malmgaard L. Induction and regulation of IFNs during viral infections. *J Interferon Cytokine Res.* 2004; 24: 439-454.
47. Beutler B. Inferences, questions and possibilities in Toll-like receptor signalling. *Nature.* 2004; 430: 257-263.
48. Akira S, Takeda K. Functions of toll-like receptors: lessons from KO mice. *C R Biol.* 2004; 327: 581-589.
49. Doyle SL, Husebye H, Connolly DJ, Espevik T, O'Neill LA, McGettrick AF. The GOLD domain-containing protein TMED7 inhibits TLR4 signalling from the endosome upon LPS stimulation. *Nat Commun.* 2012; 3: 707.
50. Rothfuchs AG, Bafica A, Feng CG, Egen JG, Williams DL, Brown GD, et al. Dectin-1 interaction with *Mycobacterium tuberculosis* leads to enhanced IL-12p40 production by splenic dendritic cells. *J Immunol.* 2007; 179: 3463-3471.
51. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol.* 2005; 23: 275-306.
52. Geijtenbeek TB, Van Vliet SJ, Koppel EA, Sanchez-Hernandez M, Vandenbroucke-Grauls CM, Appelmek B, et al. *Mycobacteria* target DC-SIGN to suppress dendritic cell function. *J Exp Med.* 2003; 197: 7-17.
53. Gringhuis SI, den Dunnen J, Litjens M, van der Vliet M, Geijtenbeek TB. Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to *Mycobacterium tuberculosis*, HIV-1 and *Helicobacter pylori*. *Nat Immunol.* 2009; 10: 1081-1088.
54. Yadav M, Schorey JS. The beta-glucan receptor dectin-1 functions together with TLR2 to mediate macrophage activation by mycobacteria. *Blood.* 2006; 108: 3168-3175.
55. Leber JH, Crimmins GT, Raghavan S, Meyer-Morse NP, Cox JS, Portnoy DA. Distinct TLR- and NLR-mediated transcriptional responses to an intracellular pathogen. *PLoS Pathog.* 2008; 4: e6.
56. MacMicking JD. Interferon-inducible effector mechanisms in cell-autonomous immunity. *Nat Rev Immunol.* 2012; 12: 367-382.
57. Manca C, Tsenova L, Bergtold A, Freeman S, Tovey M, Musser JM, et al. Virulence of a *Mycobacterium tuberculosis* clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN-alpha /beta. *Proc Natl Acad Sci U S A.* 2001; 98: 5752-5757.
58. McNab FW, Ewbank J, Rajsbaum R, Stavropoulos E, Martirosyan A, Redford PS, et al. TPL-2-ERK1/2 signaling promotes host resistance against intracellular bacterial infection by negative regulation of type I IFN production. *J Immunol.* 2013; 191: 1732-1743.
59. Flynn JL, Chan J. What's good for the host is good for the bug. *Trends Microbiol.* 2005; 13: 98-102.
60. Antonelli LRV, Rothfuchs AG, Gonçalves R, Roffe E, Cheever AW, Bafica A, et al. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. *Clin Invest.* 2010; 120: 1674-1682.
61. Remoli ME, Giacomini E, Lutfalla G, Dondi E, Orefici G, Battistini A, et al. Selective expression of type I IFN genes in human dendritic cells infected with *Mycobacterium tuberculosis*. *J Immunol.* 2002; 169: 366-374.
62. Giacomini E, Remoli ME, Scandurra M, Gafa V, Pardini M, Fattorini L, et al. Expression of Proinflammatory and Regulatory Cytokines via NF- κ B and MAPK-Dependent and IFN Regulatory Factor-3-Independent Mechanisms in Human Primary Monocytes Infected by *Mycobacterium tuberculosis*. *Clin Dev Immunol.* 2011: 841346.
63. Bloom CI, Graham CM, Berry MP, Wilkinson KA, Oni T, Rozakeas F, et al. Detectable changes in the blood transcriptome are present after two weeks of antituberculosis therapy. *PLoS One.* 2012; 7: e46191.
64. Mayer-Barber KD, Andrade BB, Barber DL, Hieny S, Feng CG, Caspar P, et al. Innate and adaptive interferons suppress IL-1 α and IL-1 β production by distinct pulmonary myeloid subsets during *Mycobacterium tuberculosis* infection. *Immunity.* 2011; 35: 1023-1034.
65. Mayer-Barber KD, Andrade BB, Oland SD, Amaral EP, Barber DL, Gonzales J, et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. *Nature.* 2014; 511: 99-103.
66. Davidson S, Maini MK, Wack A. Disease-promoting effects of type I interferons in viral, bacterial, and coinfections. *J Interferon Cytokine Res.* 2015; 35: 252-264.

67. Bach EA, Aguet M, Schreiber RD. The IFN gamma receptor: a paradigm for cytokine receptor signaling. *Annu Rev Immunol.* 1997; 15: 563-591.
68. Hölscher C, Reiling N, Schaible UE, Hölscher A, Bathmann C, Korbel D, et al. Containment of aerogenic *Mycobacterium tuberculosis* infection in mice does not require MyD88 adaptor function for TLR2, -4 and -9. *Eur J Immunol.* 2008; 38: 680-694.
69. Cooper AM, Adams LB, Dalton DK, Appelberg R, Ehlers S. IFN-gamma and NO in mycobacterial disease: new jobs for old hands. *Trends Microbiol.* 2002; 10: 221-226.
70. Cooper AM, Pearl JE, Brooks JV, Ehlers S, Orme IM. Expression of the nitric oxide synthase 2 gene is not essential for early control of *Mycobacterium tuberculosis* in the murine lung. *Infect Immun.* 2000; 68: 6879-6882.
71. Feng CG, Kaviratne M, Rothfuchs AG, Cheever A, Hieny S, Young HA, et al. NK cell-derived IFN-gamma differentially regulates innate resistance and neutrophil response in T cell-deficient hosts infected with *Mycobacterium tuberculosis*. *J Immunol.* 2006; 177: 7086-7093.
72. Gallegos AM, van Heijst JW, Samstein M, Su X, Pamer EG, Glickman MS. A gamma interferon independent mechanism of CD4 T cell mediated control of *M. tuberculosis* infection *in vivo*. *PLoS Pathog.* 2011; 7: e1002052.
73. Strutt TM, McKinstry KK, Dibble JP, Winchell C, Kuang Y, Curtis JD, et al. Memory CD4⁺ T cells induce innate responses independently of pathogen. *Nat Med.* 2010; 16: 558-564, 1p following 564.
74. Shafiani S, Tucker-Heard G, Kariyone A, Takatsu K, Urdahl KB. Pathogen-specific regulatory T cells delay the arrival of effector T cells in the lung during early tuberculosis. *J Exp Med.* 2010; 207: 1409-1420.
75. Bold TD, Banaei N, Wolf AJ, Ernst JD. Suboptimal activation of antigen-specific CD4⁺ effector cells enables persistence of *M. tuberculosis in vivo*. *PLoS Pathog.* 2011; 7: e1002063.
76. Winslow GM, Roberts AD, Blackman MA, Woodland DL. Persistence and turnover of antigen-specific CD4 T cells during chronic tuberculosis infection in the mouse. *J Immunol.* 2003; 170: 2046-2052.
77. Egen JG, Rothfuchs AG, Feng CG, Horwitz MA, Sher A, Germain RN. Intravital imaging reveals limited antigen presentation and T cell effector function in mycobacterial granulomas. *Immunity.* 2011; 34: 807-819.
78. Lu C, Wu J, Wang H, Wang S, Diao N, Wang F, et al. Novel biomarkers distinguishing active tuberculosis from latent infection identified by gene expression profile of peripheral blood mononuclear cells. *PLoS One.* 2011; 6: e24290.
79. Harari A, Rozot V, Bellutti Enders F, Perreau M, Stalder JM, Nicod LP, et al. Dominant TNF- α *Mycobacterium tuberculosis*-specific CD4⁺ T cell responses discriminate between latent infection and active disease. *Nat Med.* 2011; 17: 372-376.
80. Sauzullo I, Scrivo R, Mengoni F, Ermocida A, Coppola M, Valesini G, et al. Multi-functional flow cytometry analysis of CD4⁺ T cells as an immune biomarker for latent tuberculosis status in patients treated with tumour necrosis factor (TNF) antagonists. *Clin Exp Immunol.* 2014; 176: 410-417.
81. Nunes-Alves C, Booty MG, Carpenter SM, Jayaraman P, Rothchild AC, Behar SM. In search of a new paradigm for protective immunity to TB. *Nat Rev Microbiol.* 2014; 12: 289-299.
82. Casanova JL, Abel L. Genetic dissection of immunity to mycobacteria: the human model. *Annu Rev Immunol.* 2002; 20: 581-620.
83. Sonnenberg P, Glynn JR, Fielding K, Murray J, Godfrey-Faussett P, Shearer S. How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. *J Infect Dis.* 2005; 191: 150-158.
84. Murray J, Sonnenberg P, Nelson G, Bester A, Shearer S, Glynn JR. Cause of death and presence of respiratory disease at autopsy in an HIV-1 seroconversion cohort of southern African gold miners. *AIDS.* 2007; 21 Suppl 6: S97-97S104.
85. Diel R, Loddenkemper R, Niemann S, Meywald-Walter K, Nienhaus A. Negative and positive predictive value of a whole-blood interferon- γ release assay for developing active tuberculosis: an update. *Am J Respir Crit Care Med.* 2011; 183: 88-95.
86. Pitt JM, Stavropoulos E, Redford PS, Beebe AM, Bancroft GJ, Young DB, et al. Blockade of IL-10 signaling during bacillus Calmette-Guérin vaccination enhances and sustains Th1, Th17, and innate lymphoid IFN- γ and IL-17 responses and increases protection to *Mycobacterium tuberculosis* infection. *J Immunol.* 2012; 189: 4079-4087.
87. Zhu H, Butera M, Nelson DR, Liu C. Novel type I interferon IL-28A suppresses hepatitis C viral RNA replication. *Virol J.* 2005; 2: 80.
88. Marcello T, Grakoui A, Barba-Spaeth G, Machlin ES, Kotenko SV, MacDonald MR, et al. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. *Gastroenterology.* 2006; 131: 1887-1898.
89. Zhang SY, Boisson-Dupuis S, Chappier A, Yang K, Bustamante J, Puel A, et al. Inborn errors of interferon (IFN)-mediated immunity in humans: insights into the respective roles of IFN-alpha/beta, IFN-gamma, and IFN-lambda in host defense. *Immunol Rev.* 2008; 226: 29-40.
90. Coccia EM, Severa M, Giacomini E, Monneron D, Remoli ME, Julkunen I, et al. Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells. *Eur*

91. Dumoutier L, Tounsi A, Michiels T, Sommereyns C, Kotenko SV, Renauld JC. Role of the interleukin (IL)-28 receptor tyrosine residues for antiviral and antiproliferative activity of IL-29/interferon-lambda 1: similarities with type I interferon signaling. *J Biol Chem.* 2004; 279: 32269-32274.
92. Hou J, Schindler U, Henzel WJ, Wong SC, McKnight SL. Identification and purification of human Stat proteins activated in response to interleukin-2. *Immunity.* 1995; 2: 321-329.
93. Iversen MB, Paludan SR. Mechanisms of type III interferon expression. *J Interferon Cytokine Res.* 2010; 30: 573-578.
94. Ank N, West H, Bartholdy C, Eriksson K, Thomsen AR, Paludan SR. Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections *in vivo*. *J Virol.* 2006; 80: 4501-4509.
95. Tezuka Y, Endo S, Matsui A, Sato A, Saito K, Semba K, et al. Potential anti-tumor effect of IFN-λ2 (IL-28A) against human lung cancer cells. *Lung Cancer.* 2012; 78: 185-192.
96. Zhou Z, Hamming OJ, Ank N, Paludan SR, Nielsen AL, Hartmann R, et al. Type III interferon (IFN) induces a type I IFN-like response in a restricted subset of cells through signaling pathways involving both the Jak-STAT pathway and the mitogen-activated protein kinases. *J Virol.* 2007; 81: 7749-7758.
97. Sommereyns C, Paul S, Staeheli P, Michiels T. IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells *in vivo*. *PLoS Pathog.* 2008; 4: e1000017.
98. Kotenko SV. IFN-λs. *Curr Opin Immunol.* 2011; 23: 583-590.
99. Maher SG, Sheikh F, Scarzello AJ, Romero-Weaver AL, Baker DP, Donnelly RP, et al. IFNα and IFNλ differ in their antiproliferative effects and duration of JAK/STAT signaling activity. *Cancer Biol Ther.* 2008; 7: 1109-1115.
100. Onoguchi K, Yoneyama M, Takemura A, Akira S, Taniguchi T, Namiki H, et al. Viral infections activate types I and III interferon genes through a common mechanism. *J Biol Chem.* 2007; 282: 7576-7581.
101. Nagarajan U. Induction and function of IFNλ during viral and bacterial infection. *Crit Rev Immunol.* 2011; 31: 459-474.
102. Bierre H, Travier L, Mahlaköiv T, Tailleux L, Subtil A, Lebreton A, et al. Activation of type III interferon genes by pathogenic bacteria in infected epithelial cells and mouse placenta. *PLoS One.* 2012; 7: e39080.
103. Pietilä TE, Latvala S, Osterlund P, Julkunen I. Inhibition of dynamin-dependent endocytosis interferes with type III IFN expression in bacteria-infected human monocyte-derived DCs. *J Leukoc Biol.* 2010; 88: 665-674.
104. Witte K, Witte E, Sabat R, Wolk K. IL-28A, IL-28B, and IL-29: promising cytokines with type I interferon-like properties. *Cytokine Growth Factor Rev.* 2010; 21: 237-251.
105. Travar M, Vucic M, Petkovic M. Interferon lambda-2 levels in sputum of patients with pulmonary *Mycobacterium tuberculosis* infection. *Scand J Immunol.* 2014; 80: 43-49.
106. Gallagher G, Megjugorac NJ, Yu RY, Eskdale J, Gallagher GE, Siegel R, et al. The lambda interferons: guardians of the immune-epithelial interface and the T-helper 2 response. *J Interferon Cytokine Res.* 2010; 30: 603-615.
107. Li M, Liu X, Zhou Y, Su SB. Interferon-lambdas: the modulators of antiviral, antitumor, and immune responses. *J Leukoc Biol.* 2009; 86: 23-32.
108. Jordan WJ, Eskdale J, Srinivas S, Pekarek V, Kelner D, Rodia M, et al. Human interferon lambda-1 (IFN-lambda1/IL-29) modulates the Th1/Th2 response. *Genes Immun.* 2007; 8: 254-261.
109. Siebler J, Wirtz S, Weigmann B, Atreya I, Schmitt E, Kreft A, et al. IL-28A is a key regulator of T-cell-mediated liver injury via the T-box transcription factor T-bet. *Gastroenterology.* 2007; 132: 358-371.