

# Invariant Natural Killer T Cells: Boon or Bane in Immunity to Intracellular Bacterial Infections?

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## Key Words

Natural killer T cells · Immune response · T helper cells · Intracellular bacteria

## Abstract

Invariant natural killer T (iNKT) cells represent a specialized subset of innate lymphocytes that recognize lipid and glycolipid antigens presented to them by nonclassical MHC-I CD1d molecules and are able to rapidly secrete copious amounts of a variety of cytokines. iNKT cells possess the ability to modulate innate as well as adaptive immune responses against various pathogens. Intracellular bacteria are one of the most clinically significant human pathogens that effectively evade the immune system and cause a myriad of diseases of public health concern globally. Emerging evidence suggests that iNKT cells can confer immunity to intracellular bacteria but also inflict pathology in certain cases. We summarize the current knowledge on the contribution of iNKT cells in the host defense against intracellular bacterial infections, with a focus on the underlying mechanisms by which these cells induce protective or pathogenic reactions including the pathways of direct action (acting on in-

fecting cells) and indirect action (modulating dendritic, NK and T cells). The rational exploitation of iNKT cells for prophylactic and therapeutic purposes awaits a profound understanding of their functional biology.

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## Introduction

Natural killer T (NKT) cells are an unconventional T lymphocyte population that belongs to the family of innate lymphocytes. The term 'NKT cells' is nowadays used for CD1d-restricted cells. These cells recognize lipids and glycolipids presented by the nonclassical MHC-I CD1d molecules and share some markers typical of NK cells (such as NK1.1 in mice and CD161 in humans). However, it should be noted that not all NKT cells express NK cell markers, and that not all T cells with NK cell markers are CD1d-restricted NKT cells [1]. NKT cells consist of 2 types: type 1 or classical/invariant NKT (iNKT) and type 2 or nonclassical NKT cells. The iNKT cells are the most widely studied; they express a semi-invariant  $\alpha\beta$  T cell receptor (TCR) with the use of chain V $\alpha$ 14-J $\alpha$ 18 in mice

and Va24-Ja18 in humans. This unique conformation of  $\alpha\beta$  TCR chains enables them to specifically recognize lipid and glycolipid antigens presented to them by nonclassical major histocompatibility complex I CD1d molecules [1, 2]. A glycosphingolipid,  $\alpha$ -galactosylceramide ( $\alpha$ -GalCer), which was originally extracted from a marine poriferan, *Agelas mauritianus*, is considered to be the prototypic antigen for iNKT cells and has been used to track and target iNKT cells in mice and humans [3–7]. Recently, some microbial antigens recognized by iNKT cells have been identified, most notably the sphingolipids of *Sphingomonas* [8–12]. A hallmark of iNKT cells is the impromptu production of multiple T helper ( $T_H$ )1,  $T_H$ 2 and  $T_H$ 17 cytokines following their activation [1, 13, 14]. The possession of this characteristic endows iNKT cells with the ability to play a role in diverse conditions, such as tumor rejection, regulation of autoimmune diseases and defense against various pathogens [15, 16]. In contrast to iNKT cells, type 2 NKT cells, although being CD1d-restricted, lack the invariant TCR and do not react with  $\alpha$ -GalCer. They express a more diverse  $\alpha\beta$  TCR repertoire and recognize hydrophobic antigens like sulfatides. Type 2 NKT cells appear to be a heterogeneous population of cells, but their immunobiology is not well understood [16].

Intracellular bacteria possess the ability to gain access to and replicate within the host cells, which allows them to efficiently evade the immune system as well as to successfully continue their life/developmental cycle [17]. Some of these bacteria including *Mycobacterium* and *Chlamydia* afflict humans with a wide spectrum of diseases, thus posing a menace to public health across the globe. Accumulating evidence suggests that  $T_H$ 1 responses, characterized by enhanced IFN- $\gamma$  production, are critical for protective immunity to intracellular bacteria, while  $T_H$ 2 responses may culminate in susceptibility and/or immunopathology [18]. Significant data have been generated from mouse models and human studies to support an important role for iNKT cells in intracellular bacterial infections. While many studies show that iNKT cells elicit protection against bacteria, some have also highlighted a pathogenic effect of these cells on the outcome of infection. Growing evidence further points out that iNKT cells can directly act on infected cells to kill them and/or indirectly impact the quality and quantity of host immune responses via modulation of the function of other immune cells like dendritic cells (DC) [8, 19–25]. In this article, we review the literature on the role that iNKT cells play in inducing protective immunity and immunopathology against intracellular bacterial infections

and the underlying mechanisms that mediate their effector functions. Understanding the functional dynamics of iNKT cells may provide new modes of intervention for prophylactic and therapeutic purposes.

### **iNKT Cells in Immunity to Intracellular Bacterial Infections in Mouse Models**

A great deal of evidence on the role of iNKT cells in intracellular bacterial infections comes from studies using knockout (KO) mice,  $\alpha$ -GalCer stimulation and adoptive transfer approaches (table 1). Treatment with  $\alpha$ -GalCer has been found to reduce bacterial load and pathology as well as prolong the survival of susceptible mice following *Mycobacterium tuberculosis* infection, suggesting that the activation of iNKT cells by  $\alpha$ -GalCer promotes protective immunity [26]. In combination with isoniazid, an antituberculosis drug,  $\alpha$ -GalCer shows a strong synergistic effect in controlling murine pulmonary tuberculosis [27]. Whether the pharmacological ability of  $\alpha$ -GalCer to induce protection represents the physiological role played by iNKT cells in vivo is an important question to be addressed. In this context, it is noteworthy that, depending on the administration,  $\alpha$ -GalCer can cause anergy or unresponsiveness and a loss of iNKT cells [28, 29]. Following infection with *M. tuberculosis*, CD1d-KO mice, which lack both iNKT and type 2 NKT cells, showed no significant difference from wild-type (WT) mice in terms of pathology and survival [30–33]. In line with these findings, studies using Ja18/Ja281-KO mice, which are deficient in iNKT cells, found that there were no appreciable differences in the pulmonary pathology and bacterial burden between KO and WT mice after infection challenge [33, 34]. This indicates that the absence of iNKT cells does not change the outcome of mycobacterial infection, implying that these cells exhibit a state of redundancy and are dispensable for optimal immunity. However, adoptive transfer experiments by Sada-Ovalle et al. [20] recently demonstrated that iNKT cells play a protective role in immune responses against *M. tuberculosis* infection. Following the adoptive transfer of iNKT cells from naïve mice into irradiated mice infected with *M. tuberculosis*, the recipient mice showed a reduced bacterial burden in both the lung and spleen [20]. These findings contrast with those from previous studies. The discrepancy could be attributed to the low number of lung-resident iNKT cells that were insufficient to effectively control infection (in the early studies) versus the large number of adoptively transferred iNKT cells (in the study by Sada-

**Table 1.** Overview of the role of iNKT cells in murine intracellular bacterial infections

Bacterial species	Route of infection	Primary site of infection	Experimental model/ approach for analysis	Effect of iNKT cells on outcome of infection	References
<i>M. tuberculosis</i>	intranasal	lungs	$\alpha$ -GalCer, CD1d-KO, Ja18-KO, adoptive transfer	protection, no effect, no effect, protection	[20, 26, 27, 30–34]
<i>C. trachomatis</i>	intra-articular	knee joint	$\alpha$ -GalCer, CD1d-KO	protection, protection	[36]
<i>C. muridarum</i>	intranasal	lungs	$\alpha$ -GalCer, CD1d-KO, Ja18-KO	pathology, pathology, pathology	[19, 35, 37, 38]
	intravaginal	genital tract	$\alpha$ -GalCer, CD1d-KO, Ja18-KO	protection, pathology, pathology	
<i>C. pneumoniae</i>	intranasal	lungs	$\alpha$ -GalCer, Ja18-KO, adoptive transfer	protection, protection, protection	[19]
<i>L. pneumophila</i>	intranasal	lungs	$\alpha$ -GalCer, Ja18-KO	no effect, pathology	[39]
<i>S. typhimurium</i>	oral	spleen and liver	CD1d-KO	no effect	[41]
<i>S. choleraesuis</i>	intraperitoneal	spleen and liver	Ja18-KO	pathology	[40]
<i>L. monocytogenes</i>	oral	liver	$\alpha$ -GalCer, Ja18-KO, adoptive transfer	protection, pathology, protection	[22, 23]
<i>E. muris</i>	intraperitoneal	liver	CD1d-KO	protection against mild infection/ pathology against lethal infection	[10, 42]

Ovalle et al. [20]) that maybe amplified the protective effect. It may be speculated that, in natural infection due to *M. tuberculosis*, iNKT cells are not activated optimally whereas in a state of enhanced stimulation as observed in the  $\alpha$ -GalCer activation model [26], they can exert an active protective response. More studies are needed to address the functional role of iNKT cells and the mechanisms in host immune responses against *M. tuberculosis* infection.

Using different chlamydial infection models, we and others have extensively investigated the contribution of iNKT cells in the host defense against intracellular bacteria. To delineate the role of iNKT cells, investigators have used various experimental approaches, KO mice (including the CD1d-KO and Ja18-KO strains), and  $\alpha$ -GalCer-induced iNKT stimulation. In vivo stimulation with

$\alpha$ -GalCer ameliorated the genital-tract *Chlamydia muridarum* infection, a mouse biovar of *Chlamydia trachomatis* [35]. Bharhani et al. [36] also reported that the activation of iNKT cells by  $\alpha$ -GalCer induced both a protective and a regulatory role in *C. trachomatis*-induced arthritis. These results were further confirmed by using CD1d-KO mice [36]. In a lung infection model,  $\alpha$ -GalCer induced protection against *Chlamydia pneumoniae* [19]. Consistent with the finding obtained using the  $\alpha$ -GalCer treatment model, when we used Ja18-KO mice, we found that iNKT cells play a critical role in enhancing protective immunity to *C. pneumoniae* infection through skewing the adaptive T cell responses towards the development of protective type 1 immune responses [19]. Rather surprisingly, we found an increased resistance to *C. muridarum* infection in CD1d-KO and Ja18-KO mice, which sug-

gested that NKT cells exacerbate the infection [19, 37]. These KO mice exhibited a significantly reduced weight loss, chlamydial in vivo growth and lung pathology compared with WT mice following *C. muridarum* infection. Further analysis of immune response in the KO mice showed reduced  $T_H2$  responses [19, 37]. Consistently, when subjected to a *C. muridarum* infection of the genital tract, CD1d-KO mice showed reduced pathological changes in the oviduct [38]. The comparison of the iNKT cells in *C. pneumoniae* and *C. muridarum* infections has revealed distinct cytokine patterns, i.e. the predominant secretion of IFN- $\gamma$  in the former and of IL-4 in the latter, which suggests that functionally different iNKT cell subsets (e.g. iNKT-like or type 2-like cells) were generated. The type of iNKT cell response matched the cytokine patterns of CD4 and CD8 T cells and so the type of adaptive immune responses resulted in either a protective or a pathological outcome in these two different infections, although the species are related [19]. The mechanism underlying the generation of differential iNKT cell responses in these two *Chlamydia* infections remains unclear. It is likely that iNKT cells are activated by certain antigens/epitopes specific for the bacterial species and that the antigenic variability between the *C. muridarum* and *C. pneumoniae* species may account for the differential functional roles of these cells. In a broad sense, the above studies suggest that the effect of NKT cell activation or the activation itself is pathogen-specific or even strain-specific and that this may account for the immunological and infectious outcomes. More in-depth studies on the characterization of glycolipid antigens derived from different chlamydial species and the antigenic effects on iNKT cells may provide further insight into the mechanisms underlying the immunological activity exerted by these cells.

During lung infection with *Legionella pneumophila*, no significant changes in bacterial burden and pathology in the lungs of mice were observed, despite  $\alpha$ -GalCer administration; however, following infection, Ja18-KO mice exhibited greater resistance and survival with reduced bacterial loads and less severe lung injury than control mice [39]. Similarly, Ja18-KO mice showed more resistance than the control mice against *Listeria monocytogenes* infection [23]. However, studies with  $\alpha$ -GalCer stimulation and the adoptive transfer of iNKT cells into alymphoid Rag<sup>-/-</sup>  $\gamma$ <sup>-/-</sup> mice led to protection against *L. monocytogenes* infection, with a reduced bacterial burden and systemic production of IFN- $\gamma$  [22, 23]. Together, these findings show that iNKT cells exert a pathogenic effect in the natural response against *L. monocytogenes*, but induce protection if they are activated by  $\alpha$ -GalCer or

adoptively transferred to immune-deficient mice. A distinction in the two subsets, NK1.1+ and NK1.1- iNKT cells, in terms of kinetics and function has been observed in murine listeriosis. Interestingly, the NK1.1+ iNKT cells produce large quantities of IL-4 and exacerbate the disease whereas NK1.1- iNKT cells secreting IFN- $\gamma$  exert a protective effect [23]. It is becoming increasingly clear that different subsets of iNKT cells could play different roles during microbial infections. In *Salmonella choleraesuis* infection, reduced hepatic pathology and serum alanine transaminase levels in Ja18-KO mice have suggested a pathogenic role for iNKT cells in the causation of hepatic injury [40]. In another study, although *Salmonella typhimurium* infection induced rapid activation of iNKT cells, as reflected in their increased numbers, the upregulation of CD69 and the production of IFN- $\gamma$ , there was no significant difference observed in the bacterial load between the CD1d-KO and control mice [41].

When subjected to a mildly virulent *Ehrlichia muris* infection, CD1d-KO mice were unable to clear bacteria compared to the WT mice, suggesting that NKT cells play a crucial role in protection against this infection [10]. However, in a lethal model of ehrlichial infection, although NKT cells induced early protection against infection, they promoted *Ehrlichia*-mediated toxic shock-like syndrome and liver injury [42]. Thus, NKT cells exert both protective as well as pathogenic effects in ehrlichial infection, depending upon the virulence of the bacterial strain used.

Discrepancies in findings from studies using different infection models and experimental approaches regarding the role of iNKT cells in various intracellular bacterial infections underscore careful scrutiny. It is quite possible that the effect of iNKT cell activation on the outcome of infection is influenced by multiple factors, such as type of pathogen/nature of antigens, infective dose and disease stage. Since the host genetic background is a key factor influencing the immune response and outcome of infections, it is of importance to test the effect of iNKT cells in mice with different genetic backgrounds in mouse models of infection. It is also important to use control mice (heterozygous/homozygous WT mice) that are littermates to KO animals. Evidence points out that iNKT cells can play diverse roles in immune responses even in infections by closely related bacterial species, and also in the case of infections due by the same species but at different sites.



## iNKT Cells in Immunity to Intracellular Bacterial Infections in Humans

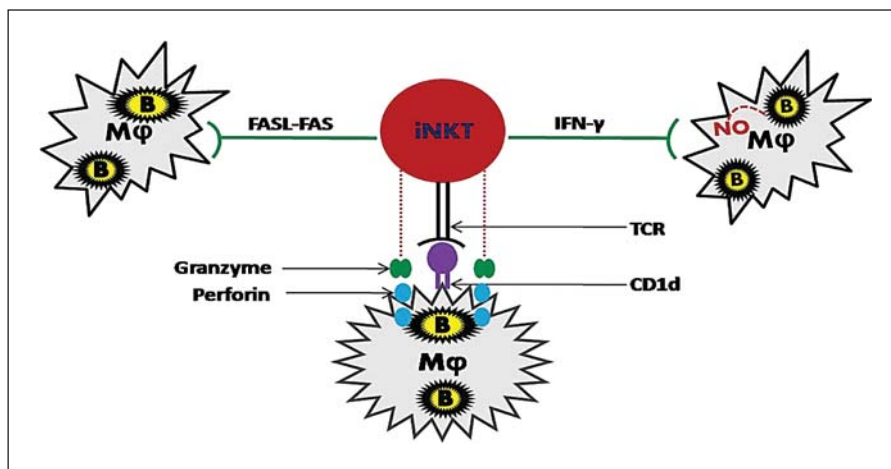
iNKT cell biology in humans with intracellular bacterial infections is still obscure due to the low frequencies of these cells. Emerging evidence in this area is mainly derived from tuberculosis patients. Alteration in the frequencies of iNKT cells has been reported in pulmonary tuberculosis patients. Using antibodies mainly against CD3 and the V $\alpha$ 24 and V $\beta$ 11 chains of iNKT cell TCR to identify iNKT cells, some studies have shown a numerical deficiency of iNKT cells in tuberculosis patients [43–47]. However, other studies contradict these findings and report a steady incline in the number of these cells in the peripheral blood [48–51]. For example, a flow cytometric analysis of peripheral blood T lymphocytes in patients with pulmonary tuberculosis showed that the number of iNKT cells was much higher in patients than in controls [48], and that these cells secreted enhanced levels of IFN- $\gamma$  and IL-4 [50]. Another study examined the frequency of iNKT cells in tuberculosis patients with or without diabetes mellitus, showing that these cells are significantly increased in the peripheral blood and bronchoalveolar lavage of the diabetic compared to in the nondiabetic patients [49]. This increase in iNKT cell number may have been due to the higher bacterial burden in the diabetic tuberculosis patients. In humans, iNKT cells can be identified using  $\alpha$ -GalCer-loaded CD1d tetramers and T cell markers [52, 53]. By combining the use of CD1d- $\alpha$ GalCer tetramer and anti-V $\alpha$ 24 antibodies, Lee et al. [53] showed that iNKT cells could be stained with more specificity, even at very low frequencies in the blood. iNKT cells can be divided into distinct subsets on the basis of the presence or absence of CD4 and CD8 expression, i.e. CD4+CD8-, CD8+CD4-, CD4+CD8+ and CD4-CD8- iNKT cells. CD4+CD8- iNKT cells have been shown to secrete both T<sub>H</sub>1 and T<sub>H</sub>2 cytokines, but CD8+CD4- iNKT cells predominantly produce T<sub>H</sub>1 cytokines [52, 53]. In active tuberculosis patients, CD4-CD8- iNKT cells are significantly reduced in the blood, while CD4+CD8- iNKT cells increase [54]. In the case of *Bruccella suis* infection, CD4+CD8- iNKT cells have been found to effectively inhibit bacterial growth and/or kill the bacteria through the production of IFN- $\gamma$  and cytotoxic activities [55]. Recently, Kee et al. [46] conducted a detailed analysis of iNKT cells in patients with active *M. tuberculosis* infection. They found that the number of these cells was lower in the peripheral blood of both pulmonary and extrapulmonary tuberculosis patients when compared to patients with latent tuberculosis and to

healthy controls. The reduction of iNKT cells in the infected patients was significantly correlated to C-reactive protein levels. Furthermore, *M. tuberculosis*-infected patients show a reduced response to  $\alpha$ -GalCer, found to be due to increased iNKT cell apoptosis and reduced CD1d expression. In addition, iNKT cells from tuberculosis patients show higher levels of the inhibitory programmed death-1 (PD-1) receptor, and the blocking of PD-1 signaling improves the response to  $\alpha$ -GalCer [46]. Thus, iNKT cell levels and functions appear to be diminished in *M. tuberculosis* patients and these deficiencies reflect the presence of active tuberculosis. On the other hand, it has been shown that  $\alpha$ GalCer-activated human iNKT cells restrict the growth of intracellular *M. tuberculosis* in vitro in a CD1d-dependent manner [56]. Taken together, these findings suggest a possible role for iNKT cells in host antibacterial resistance to *M. tuberculosis* infection. From a clinical standpoint, the determination of iNKT cell alterations in the peripheral blood has an important implication for human diseases such as tuberculosis, as analysis of these cells could serve as an immunological adjunct marker for assessing disease activity, albeit with extreme caution. Future studies are needed to examine the biology of these cells in other intracellular bacterial infections. Of note, the method used to analyze iNKT cells appears to be critical for accuracy in flow cytometric analysis because the activated iNKT cells may show downregulation/internalization of some of the surface markers, such as NK1.1 molecules [57]. The use of  $\alpha$ -GalCer or its analogue-loaded CD1d tetramers is more reliable for iNKT cell analysis.

### Mechanisms of iNKT Cell-Induced Immunity to Intracellular Bacterial Infections

The activation of iNKT cells is an initial but crucial step for their function in immunity, which can be CD1d-dependent and/or CD1d-independent. CD1d-dependent activation of iNKT cells involves the interaction between iNKT TCR and its cognate ligand, whereas in CD1d-independent activation, local cytokines and other immune cells can lead to iNKT-cell activation [24]. Much of our understanding of how iNKT cell activation occurs via iNKT TCR is based on in vitro and in vivo studies using  $\alpha$ -GalCer and its analogues, which specifically activate iNKT cells in a CD1d-dependent fashion [3–7]. While many studies have documented a function for iNKT cells during infection, if microbial antigens are indeed the major activator of iNKT cells has been a contentious issue. Emerging data illustrate that iNKT cells possess antigen

**Fig. 1.** Pathway of direct action: direct action of iNKT cells upon bacteria-infected macrophages to inhibit bacterial growth. IFN- $\gamma$  production by iNKT cells stimulates infected macrophages to secrete NO that subsequently inhibits bacterial growth. It can also inhibit the growth of bacteria through enhanced cytotoxic activities, such as the induction of Fas-FasL signalling and the release of perforin and granzyme, which lead to apoptosis of the infected cells. B = Intracellular bacterium; M $\phi$  = macrophage.



sensitivity against glycolipids and lipids derived from some bacterial pathogens, both extracellular and intracellular. The first natural iNKT cell microbial antigen, a tetramannosylated form of phosphatidylinositol, was extracted from *Mycobacterium bovis* BCG, which activated the iNKT cells, as measured by their IFN- $\gamma$  production and cytotoxic activities [12]. Recently, we identified the ability of a glycolipid exoantigen purified from *C. muridarum* (GLXA) to specifically activate iNKT cells. The GLXA activated the cells to express higher levels of the activation marker CD69, and enhanced the production of IFN- $\gamma$  and IL-4 in vivo [9]. In accordance with these findings, Jiang et al. [38] showed that sonicated *C. muridarum* activated iNKT and type 2 NKT cells in an antigen-presenting cell-free culture system coated with CD1d molecules and with the use of iNKT or type 2 NKT cell hybridomas. Since the ligands of type 2 NKT cells and iNKT cells are different [58, 59], it is likely that *C. muridarum* contains different kinds of lipid antigens for these NKT cell types. The nature of antigens specific for activating type 2 NKT cells is not yet clear. Nevertheless, the above findings suggest that the conserved TCR of iNKT cells may be directly involved in mounting the immune responses against intracellular bacterial infections.

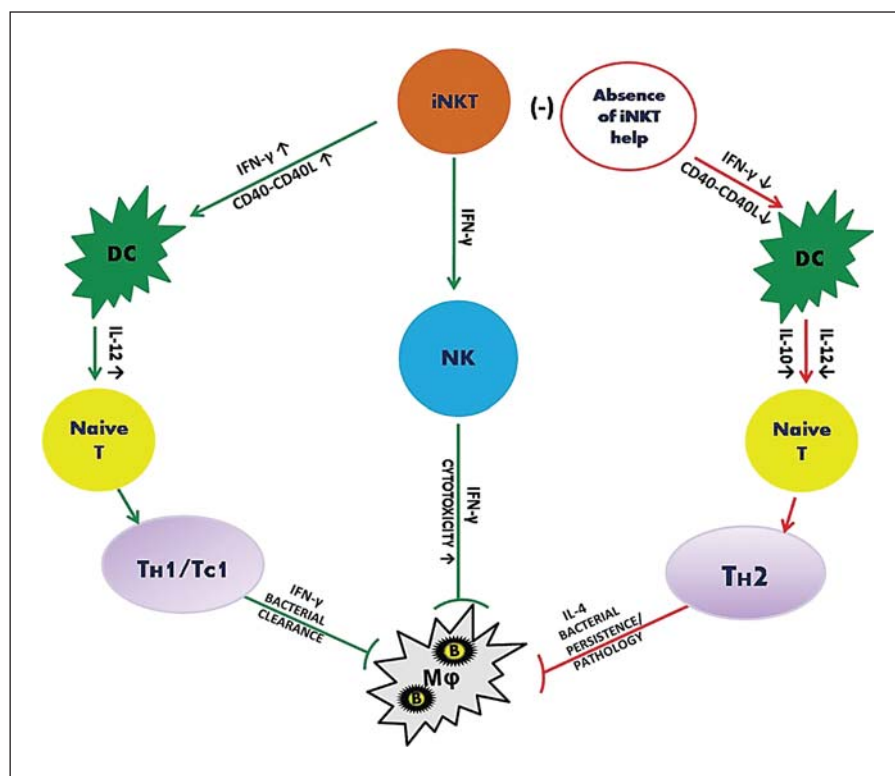
It is important to consider that infection induces a very dynamic environment inside the host with changes occurring in the responses by immune cells, their interactions and the release of soluble mediators, such as cytokines and chemokines. Besides antigen-specific activation by direct recognition of microbial glycolipid antigens via their TCR, the activation of iNKT cells can occur through innate or inflammatory stimuli, in conjunction with self-glycolipid antigen recognition [10, 60]. Recent-

ly, a study by Brigl et al. [61] investigated the mechanism of iNKT cell activation in response to microbial infection by using a large panel of diverse bacteria, including some intracellular bacterial pathogens like *L. monocytogenes* and *M. tuberculosis*. Their findings suggest that innate cytokine-driven activation is the dominant pathway for iNKT cell activation in response to infectious agents that induce the production of IL-12 by antigen-presenting cells like DC after Toll-like receptor-mediated activation. For the function of iNKT cells in the host defense against intracellular bacterial infections, a combination of direct and indirect mechanisms may operate in which the polarization of either mechanism may depend on the type of pathogen, the dose/stage of infection and the genetic background. Once activated, iNKT cells can lead to the generation of immune responses against infection via pathways of direct and/or indirect actions.

#### Pathway of Direct Action

In this pathway, iNKT cells can directly act upon bacteria-infected macrophages and suppress their growth and/or kill them without the assistance of other cell types (fig. 1). Recognition of infected macrophages by iNKT cells is CD1d-dependent and is critical for the inhibition of bacterial replication [20, 21]. Coculture of splenocytes and macrophages infected with *M. tuberculosis* has shown that IFN- $\gamma$  production by iNKT cells leads to the production of nitric oxide (NO), which subsequently suppresses the bacterial replication, suggesting that IFN- $\gamma$  plays a key role in containing bacterial infection [20]. In contrast, although Ja18-KO mice showed increased resistance to *L. pneumophila* infection compared to WT mice, the level of IFN- $\gamma$  was not different between the KO and WT mice

**Fig. 2.** Pathway of indirect action: modulation of DC, NK and T cells by iNKT cells in order to influence the outcome of infection. iNKT cells induce IL-12 secretion by DC, which influences naïve T cells to get transformed into  $T_H1/T_C1$  (cytotoxic T cells) cells and confer protective CD4/CD8 T cell responses against infection. iNKT cell-mediated modulation of DC function is largely dependent upon CD40-CD40L interaction and IFN- $\gamma$  production. In certain bacterial infections, in the absence of assistance from iNKT cells, DC function is modulated and the DC direct T cells towards a  $T_H2$  response, with increased IL-4 secretion, resulting in susceptibility and/or immunopathology. In addition, iNKT cells can also influence NK cells to modulate the immune response. iNKT cells secrete IFN- $\gamma$  that induces NK cells to enhance their cytotoxic activity and kill the bacteria inside the macrophages. B = Intracellular bacterium; CD40L = CD40 ligand; M $\phi$  = macrophage; (-) = absence;  $\uparrow$  = higher;  $\downarrow$  = lower.



[39]. Moreover, Selvanantham et al. [62] recently demonstrated that cytosolic peptidoglycan-sensing receptors Nod1 and Nod2 are necessary for optimal IFN- $\gamma$  production by iNKT cells during *S. typhimurium* and *L. monocytogenes* infections. The inclusion of neutralizing anti-IL-12p40 or IL-18 antibodies in the coculture abrogated the production of IFN- $\gamma$  and NO, resulting in altered control of bacterial growth. This suggests that an iNKT cell-mediated bactericidal effect is dependent on IL-12p40 and IL-18 [20]. Another mechanism used by iNKT cells is Fas-FasL (Fas ligand) signalling, in which the FasL on the iNKT cells interacts with the Fas molecules on the infected macrophages and triggers the death of infected cells. The bactericidal effect of the iNKT cells was severely impaired upon blocking Fas-FasL signalling with anti-Fas antibodies [21]. A recent study revealed that increased FasL expression on iNKT cells is modulated by Toll-like receptor 2 [63]. In addition, iNKT cells have also been shown to release lytic granules such as perforin and granzyme that kill the infected cells [21, 56]. Collectively, an iNKT cell-mediated direct bactericidal effect may result from a single or combination of different mechanisms, such as the production of IFN- $\gamma$  and NO, the discharge of lytic granules and Fas-FasL interactions.

#### Pathway of Indirect Action

The pathway by which iNKT cells can orchestrate the induction of adaptive immunity by modulating the functions of other immune cells, such as DC and NK cells, is referred to as the pathway of indirect action (fig. 2). Considering the small percentage of iNKT cells compared to effector CD4+ and CD8+ T cells and NK cells, this indirect pathway appears to be more broadly involved in the host defense against infections. Earlier studies using  $\alpha$ -GalCer model systems have shown that the activation of iNKT cells induced rapid maturation of DC in vivo and subsequently enhanced T cell immune responses against antigenic challenge [64–67]. Subsequently, the functional cooperation of iNKT cells with DC in immunity to viral and bacterial infections was also reported [8, 61, 68–71]. Infection with influenza A virus led to reduced accumulation and maturation of DC in  $\text{J}\alpha 18$ -KO mice compared to WT mice, but iNKT cells promoted a type 1 IFN response against lymphocytic choriomeningitis virus by plasmacytoid DC interaction, suggesting that iNKT cells can exercise their function through DC [68, 69]. In order to unravel the iNKT cell-mediated mechanisms of protective immunity to intracellular bacterial infections, we recently examined the effect of iNKT cell activation in influenc-

ing DC function using Ja18-KO mice, DC-adoptive transfer approaches and a *C. pneumoniae* lung infection model. Upon adoptive transfer, splenic DC from infected WT mice induced strong T<sub>H1</sub> responses whereas those from KO mice induced T<sub>H2</sub> responses and increased disease severity following infection challenge, suggesting that iNKT cells modulate DC function to induce the protective T cell responses [70]. Furthermore, splenic DC consist of two subsets, CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>-</sup> DC, that have different physiological roles. It has been shown that CD8 $\alpha$ <sup>+</sup> DC induce T<sub>H1</sub> dominant responses whereas CD8 $\alpha$ <sup>-</sup> DC induce T<sub>H2</sub> responses [72, 73]. In *C. pneumoniae* infection, iNKT cells preferentially modulated the function of CD8 $\alpha$ <sup>+</sup> DC in inducing protective T<sub>H1</sub> immunity [71]. Similar to splenic DC, iNKT cells transformed the function of lung DC, which reside at the primary site of *C. pneumoniae* infection, to impact T cell responses [unpubl. observation]. We further examined the molecular basis of iNKT cell-DC crosstalk in this infection model, and found that DC from KO mice showed reduced CD40 expression and IL-12 production whereas enhancing iNKT cell activation using  $\alpha$ -GalCer increased CD40 expression and IL-12 production. The coculture of DC with iNKT cells induced a higher IL-12 production by DC in a CD40-CD40L<sup>-</sup>, IFN- $\gamma$ - and cell-cell contact-dependent fashion [70]. These findings revealed that the induction of protective type 1 T cell (i.e. both CD4 and CD8 T cells) responses by iNKT cells through DC modulation is largely dependent on IFN- $\gamma$  and CD40-CD40L signalling.

The interaction of iNKT cells with other immune cells is, however, not confined to DC alone. iNKT cells can also communicate with NK cells to modulate the immune responses against chlamydial infection, which is in agreement with the well-documented findings in  $\alpha$ -GalCer-stimulated models. During *C. muridarum* infection, a deficiency of iNKT cells leads to the reduced expansion of NK cells with differential changes in IFN- $\gamma$  production and the degranulation of NK cells. This enhances IFN- $\gamma$  production but, at the same time, inhibits cytolytic function [74].

## Conclusions

The role of iNKT cells in the host defense against intracellular bacteria is in the process of being elucidated. These cells not only induce innate immunity, but also steer the direction and magnitude of subsequent adaptive immunity via various mechanisms, including the modu-

lation of functions of other cell types of the immune system such as DC, NK cells and T cells, thus serving as a bridge between innate and adaptive immunity. Although iNKT cells are mostly involved in protection against these bacterial infections, some studies point towards a pathogenic role of iNKT cells where they exert a deleterious effect on the outcome of infection. Thus, these cells can be either beneficial or detrimental to the host antibacterial defense, depending upon the type of pathogen, site of infection and host background. Since most data concerning the role of iNKT cells in host defense to infections have been generated from studies in mouse models, it still has to be ascertained if the knowledge gained can be extrapolated to humans. How to rationally harness the therapeutic potential of iNKT cells to combat infections is a pertinent question to be addressed in time to come. Caution should be taken, however, while attempting to develop iNKT cell-based therapies, as this may lead to pathological responses in some situations if it is not carefully designed. Future modalities should be developed on the basis of extensive studies and the functional characterization and dynamics of iNKT cell responses with respect to specific intracellular bacterial infections.

## Acknowledgements

This work was supported by grants from the Canadian Institutes of Health Research, the Manitoba Health Research Council (MHRC) and the Manitoba Institute of Child Health (MICH) to X.Y., who was the Canada Research Chair in Infection and Immunity. S.S. is a recipient of an MHRC/MICH graduate scholarship. Ms. Shipra Shekhar deserves special thanks for her help in drawing the figures.

## Disclosure Statement

The authors declare no conflict of interest.

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