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REVIEW ARTICLE

The role of invariant natural killer T cells in microbial immunity

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Abstract Invariant natural killer T cells (*i*NKT cells) are unique lymphocytes with characteristic features, such as expression of an invariant T-cell antigen receptor (TCR) α-chain, recognition of glycolipid antigens presented by CD1d molecules, and ability to rapidly produce large amounts of cytokines, including interferon-γ (IFN-γ) and interleukin 4 (IL-4) upon TCR stimulation. Many studies have demonstrated that *i*NKT cells participate in immune response against diverse microbes, including bacteria, fungi, protozoan parasites, and viruses. Generally, these cells play protective roles in host defense against infections. However, in some contexts they play pathogenic roles, by inducing or augmenting inflammation. Recent reports show that *i*NKT cells recognize glycolipid antigens from pathogenic bacteria including *Streptococcus pneumoniae*, and they contribute to host defense against infection. *i*NKT cell responses to these microbial glycolipid antigens are highly conserved between rodents and humans, suggesting that *i*NKT cells are evolutionally conserved because their invariant TCR is useful in detecting certain pathogens. Furthermore, glycolipid-mediated *i*NKT cell activation during immunization has adjuvant activity, enhancing humoral and cell-mediated responses. Therefore, *i*NKT cell activation is an attractive target for developing new vaccines for infectious diseases.

Keywords NKT cell · Glycolipid · CD1d · Invariant · Bacteria

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Introduction

Natural killer T (NKT) cells are innate-like lymphocytes that co-express both a T-cell antigen receptor (TCR) and NK receptors [1–5]. In contrast to conventional T cells that recognize peptide antigens presented by major histocompatibility complex (MHC) class I and II molecules, NKT cells recognize glycolipid antigens presented by CD1d, an MHC class I-like antigen-presenting molecule. Crystal structure analyses show that the CD1d molecule possesses two antigen-binding grooves, called the A′ and F′ pockets [5, 6]. These pockets are deep, narrow, and hydrophobic, making them suitable for binding the lipid tails of glycolipid antigens [5, 6].

A major subset of NKT cells expresses an invariant TCR that uses the Vα14-Jα18 gene segments in mice and the homologous Vα24-Jα18 in humans. These cells are referred to type I [7] or as invariant NKT (*i*NKT) cells [1–5]. α-Galactosylceramide (α-GalCer), a synthetic homologue of marine sponge-derived glycolipid, was the first *i*NKT cell antigen discovered, and this highly potent glycolipid antigen has been critical for understanding the functions of these cells [8]. Upon antigen recognition, *i*NKT cells rapidly produce large quantities of cytokines, including both interferon-γ (IFN-γ) and interleukin 4 (IL-4), in addition to others, and they express costimulatory molecules such as CD40 ligand (CD40L) [1–4]. Activated *i*NKT cells can stimulate dendritic cells (DCs), NK cells, and other

immune cells through cytokines such as IFN-γ, and expression of CD40L, leading to enhancement of the innate immune response, which ultimately contributes to augmenting acquired immunity. With these unique features, *i*NKT cells participate in mouse models of various immune and inflammatory responses including cancer immunity, microbial immunity, autoimmunity, atherosclerosis, and asthma.

The *i*NKT cell response to glycolipid antigens is highly conserved between human and mouse. Mouse *i*NKT cells can recognize glycolipid antigens presented by human CD1d, whereas human *i*NKT cells can recognize the same antigen presented by mouse CD1d molecules [9]. This evolutionary conservation suggests that the *i*NKT cell response also is an important immune mechanism for humans. Therefore, findings obtained from mouse studies may provide useful information for understanding the possible roles of human *i*NKT cells in immune responses.

Another population of CD1d-reactive NKT cells exists that expresses a more diverse TCR repertoire; these are sometimes called type II NKT cells. Sulfatide has been shown to activate some of these type II cells, and there is evidence they exert suppressive roles in experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis [10]. In contrast to the protective role of *i*NKT cells in tumor immunity, sulfatide-reactive NKT cells were shown to inhibit antitumor responses [11]. Although new antigens for type II NKT cells are continually being discovered, the function of these cells in microbial immunity remains to be elucidated [12, 13].

In this review, we focus on *i*NKT cells and summarize the roles of these cells in response to diverse microbes, concentrating on selected examples of infections by bacteria, fungi, protozoan parasites, and viruses. We describe how intestinal microbes affect *i*NKT cell function and regulate inflammation. We also review recent discoveries of bacterial glycolipid antigens recognized by *i*NKT cells and their importance in microbial immunity.

Role of *i*NKT cells in host defense against infections

Bacterial infections

It has been shown that *i*NKT cells contribute toward host defense against a number of bacterial infections; a few examples are summarized here. *Streptococcus pneumoniae* is a leading cause of community-acquired pneumonia and secondary bacterial pneumonia post influenza virus infection. Following pulmonary infection with *S. pneumoniae*, Jα18-deficient (Jα18KO mice that lack *i*NKT cells [14] had significantly higher bacterial burden in the lung and a lower survival rate compared to wild-type (WT) mice [15]. Neutrophil recruitment to the lung was impaired in Jα18KO mice in accordance with lower production of neutrophil-recruiting cytokines, including tumor necrosis factor (TNF), as well as reduced macrophage inflammatory protein 2 (MIP-2). Furthermore, transfer of liver mononuclear cells (20–40 % *i*NKT cells) from WT mice into Jα18KO mice restored neutrophil accumulation through increased production of TNF and MIP-2, resulting in bacterial clearance [16]. However, transfer of liver mononuclear cells from Jα18KO or IFNγKO mice into Jα18KO mice did not lead to recovery of neutrophil accumulation and a normal level of bacterial clearance, suggesting that IFN-γ, possibly from *i*NKT cells, plays an important role in host defense against *S. pneumoniae* infection.

It should be noted that Jα18KO mice have recently been reported to have deficits in rearrangements of approximately 60 % of their Jα segments, all of those upstream from Jα18, likely the result of insertion of the gene encoding neomycin resistance during construction of the gene-deficient mouse strain [17]. This finding raises an important caveat to assigning any defect in Jα18KO mice solely to the *i*NKT cell defect. In *S. pneumoniae* infection, however, the evidence for the involvement of *i*NKT cells is compelling. *i*NKT cells were strongly activated in the lung to produce cytokines such as IFN-γ and IL-17A within hours of infection [18], and they act to stimulate the innate immune response to protect the host within days. This rapid action is not consistent with the kinetics of a typical adaptive immune response that would be affected by altering the Jα repertoire.

*i*NKT cells also play a role in host defense against bacterial infection through activation of DCs and induction of IFN-γ production by $CD4^+$ and $CD8^+$ T cells. In *Chlamydophila* (formerly called *Chlamydia*) *pneumoniae* infection, *i*NKT cells accumulated in the lungs in the early phase of infection and then expressed intracellular IFN-γ [19]. In Jα18KO mice, expression of CD40 and intracellular IL-12 in $CD8\alpha^+$ DCs and IFN-γ production by $CD4^+$ and $CD8^+$ T cells were reduced compared to WT mice [20]. IL-12 production by $CD8\alpha^+$ DCs is known to be dependent on IFN-γ and the CD40–CD40L interaction, suggesting that *i*NKT cells may promote bacterial clearance by enhancing the Th1 response through stimulation of DCs with IFN-γ and costimulatory molecules during *C. pneumoniae* infection. *Chlamydia muridarum* is the mouse model for *Chlamydia trachomatis* infection, a sexually transmitted infection that can cause serious damage to the female reproductive tract. The results from two recent studies indicate this organism has an antigen for *i*NKT cells [21, 22], although its structure is unknown. Also, infected epithelial cells degrade CD1d, suggestive of an immune evasion mechanism [23]. Despite this, there is no consensus on the role of *i*NKT cells in the host response to