Published in final edited form as:
Expert Opin Ther Targets. 2009 February ; 13(2): 153–162. doi:10.1517/14712590802653601.

NKT Cell Immune Responses to Viral Infection

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Abstract

Background—Natural killer T (NKT) cells are a heterogeneous population of innate T cells that have attracted recent interest because of their potential to regulate immune responses to a variety of pathogens. The most widely studied NKT cell subset is the invariant (*i*)NKT cells that recognize glycolipids in the context of the CD1d molecule. The multifaceted methods of activation *i*NKT cells possess and their ability to produce regulatory cytokines has made them a primary target for therapeutic studies.

Objective/Methods—This review gives insight into the roles of *i*NKT cells during infectious diseases, particularly viral infections. We also highlight the different mechanisms leading to *i*NKT cell activation in response to pathogens.

Conclusions—The *i*NKT cell versatility allows them to detect and respond to several viral infections. However, therapeutic approaches to specifically target *i*NKT cells will require additional research. Notably, examination of the roles of non-invariant NKT cells in response to pathogens warrant further investigations.

Keywords

CD1d; Infectious disease; NKT cells; Viruses

1. INTRODUCTION

An optimal immune response results in the containment, elimination and generation of memory against invading pathogens. The adaptive cells of the immune system provide the specificity necessary to recognize unique antigenic determinants while producing long-lived cells possessing the ability to produce a potent secondary response upon subsequent exposure. In contrast, the cells of the innate immune system respond with a specificity that is limited and fixed. However, their “limitations” provide these cells with the critical ability to respond to an extensive variety of different pathogens. In fact their wide distribution in the host and the diverse array of expressed receptors contribute to the recognition of foreign invaders while bystander cells have the ability to participate through their reaction to cytokine cascades. These critical early reactions influence and shape the down-stream adaptive phase. In this review we discuss a unique subset of T cells, the *i*NKT cells that

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express a specific antigen receptor but also resemble innate immune cells through their rapid activation in response to multiple antigens as well as cytokines.

2. *i*NKT CELLS

Natural killer (NK) T cells were first identified by their expression of an αβ T cell receptor (TCR) and the NK cell activating C-type lectin NK1.1 (CD161, NKR-P1). The invariant NKT cells are the best characterized subset of NKT cells and constitute a unique group of cells that have both innate and adaptive cell surface markers. *i*NKT cells express a restricted TCR, the Vα24Jα18 chain in humans pairs with Vβ11, and in mice the Vα14Jα18 chain associated with Vβ8.2, 7 and 2 chains. *i*NKT cells recognize (glyco)lipids presented by the non-classical MHC molecule CD1d [1]. *i*NKT cells have been described as innate immune effector cells because they are capable of rapidly responding to antigen (Ag), releasing cytokines, proliferating and producing cytolytic mediators, analogous to NK cells [2–4].

*i*NKT cells develop in the thymus from common lymphoid progenitors in a developmental pathway similar to that of mainstream T cells. The two lineages eventually diverge and the *i*NKT cells emerge as $CD4^+$ or double negative (DN) T cell populations with an activated effector memory phenotype $CD69^+CD62L^{low}CD44^+$. *i*NKT cells exit from the thymus following a CD1d-dependent proliferation event and gain the full expression of NK1.1 in the periphery (for reviews see [2–5]). *i*NKT cells make up a relatively small population (~1%) of T cells in mice, primarily located in the liver. Interestingly, examination of a panel of inbred mouse strains recently revealed that the number of liver *i*NKT cells is quite variable, spanning over a 100-fold range [6].

Although variable percentages of $CD8^+$ NKT cells exist in humans, they have not been detected in mice [7–11]. In response to pathogens, *i*NKT cells share common effector functions with conventional T cells and NK cells, including Fas-FasL mediated cytotoxicity, perforin release and cytokine production [12,13]. The ability to produce both Th1 and Th2 cytokines, IFN-γ and IL-4, along with several other cytokines such as IL-10, IL-2, IL-13, TNF-α and IL-17, allow *i*NKT cells to act immediately and regulate the down-stream immune response in reaction to pathogens. *i*NKT cells have been implicated in a variety of immune conditions including autoimmunity, anti-tumor activity and defense against pathogens. This review concentrates on the role of *i*NKT cells during infectious diseases, highlighting the diverse mechanisms of *i*NKT cell activation, and ending with a special focus on *i*NKT cell participation during viral infection.

3. *i*NKT CELL ANTIGENS

Conventional T cells are dependent on MHC proteins for Ag recognition. In contrast, *i*NKT cells interact with the non-polymorphic MHC class I like molecule, CD1d. Instead of recognizing proteins presented in the groove of MHC molecules, *i*NKT cells distinguish particular lipid Ags loaded in the hydrophobic domain of CD1d. CD1d is expressed on all lymphocytes, predominantly on APCs such as dendritic cells (DCs), macrophages (Mϕ) and B cells as well as on liver cell populations including Kupffer cells, hepatic sinusoidal endothelial cells and hepatocytes.

CD1d is a highly conserved member of the immunoglobulin superfamily, present in all mammals and the only CD1 member found in rats and mice. Disruption of CD1d trafficking or lipid loading dramatically impacts the development of *i*NKT cells. Until recently, the only known ligand shown to activate *i*NKT cells in the context of CD1d was α-galactosylceramide (α-GalCer). Now, several self and foreign lipids able to stimulate *i*NKT cells have been identified.

3.1 α-Galactosylceramide

α-GalCer was discovered by the Kirin Brewery Corporation pharmaceutical research division in 1993 as an exogenous glycosphingolipid capable of activating *i*NKT cells [14]. It was originally isolated from extracts of Agelas mauritinius, a marine sponge, and was found to be effective at preventing the growth of transplanted tumors in mice. A synthetic analogue, KRN700 was developed for use in research and clinical trials [15]. Upon exposure to this lipid Ag, *i*NKT cells react immediately by dramatically producing both Th1 (IFN-γ) and Th2 (IL-4) cytokines, internalizing some of their cell surface molecules, notably NK1.1 and the TCR whereas some *i*NKT cells undergo activation induced cell death (AICD). The explosive response to α-GalCer is limited to the *i*NKT cell population, but the downstream result is the indirect activation of NK cells, DC, Mϕ and B cells [16–22].

3.2 Isoglobotrihexosylceramide (iGb3)

iGb3 is a lysosomal glycosphigolipid that was first identified following the observation that mice deficient in the beta subunit of β-hexosaminidase, the enzyme that converts iGb4 to iGb3, displayed a specific loss of *i*NKT cells. A chemically synthesized iGb3 molecule was shown to specifically activate both human and mouse *i*NKT cells *in vitro* and was identified in the human thymus [23,24]. These data led the author to suggest that iGb3 was the main endogenous ligand responsible for NKT cell development. However, this claim has recently been challenged [25–27].

3.3 Microbial glycolipids

Microbial glycosylceramides and diacylglycerol Ag, which strongly activate *i*NKT cells, have also been characterized [28–30]. These lipids are structurally similar to α-GalCer and are found in the cell wall of Gram-negative, LPS deficient bacteria such as *Sphingomonas capsulata*, *Ehrlichia muris*, and *Borrelia burgdoferi* (reviewed in [4,31] and see below).

4. *i*NKT CELLS AND NON-VIRAL PATHOGENS

In order to explore the function of *i*NKT cells, many studies have implemented the use of pathogens including bacteria, parasites and viruses. The use of two mouse strains with a deficiency in *i*NKT cells is often employed to examine the role of this subset, the $J\alpha18^{-/-}$ mice, specifically lacking the gene segment required to form the invariant α-chain, (*i*NKT cell deficient) and $CD1d^{-/-}$ mice, which as mentioned previously, fail to select *i*NKT and other CD1d restricted cells in the thymus. Additionally, WT mice have been treated with anti-CD1d antibodies (Abs) in order to block CD1d functions thereby preventing Ag presentation to the invariant TCR or α-GalCer administered to activate the *i*NKT compartment. In this review we will describe the diverse microbial pathogens that involve *i*NKT cell responses with particular attention given to viral infections.

4.1 BACTERIA

Indicative of its ability to recognize lipid Ags in the context of CD1d, the Vα14 TCR may have evolved to recognize microbial lipids expressed on bacterial cell walls. There is current evidence that *i*NKT cells play a role in controlling gram-negative α-proteobacteria infections, such as *Sphingomonas* species, capable of severe infections in immunocompromised hosts, and the tickborn diseases *Ehrlichia muris* and *Rickettsia* [32,33]. The absence of *i*NKT cells results in delayed clearance of these bacteria and increased mortality. Conversely, a high dose challenge with *Sphingomonas* in an immunocompetent host results in lethal septic shock, while *i*NKT cell deficient mice are protected from this consequence [32,33]. *i*NKT cells recognize certain glycosphingolipids from Sphingomonas through CD1d presentation to the invariant TCR of both mouse and human [30,32]. The *Borrelia burgdorferi* glycolipid α-linked mono-galactosyl

diacylglycerol, which has broad structural similarity to α-GalCer, can also activate *i*NKT cells directly through the TCR to stimulate proliferation and cytokine production for both mouse and human *i*NKT cells [31]. *Borrelia burgdorferi* is the causative agent of Lyme disease, manifesting variable symptoms including arthritis. Interestingly, $CD1d^{-/-}$ mice infected with *B. burgdorferi* have increased incidence of joint inflammation, spirochete DNA in the urinary bladder and secrete the IgG2a isotope commonly associate with susceptibility [34]. Resistance is dependent on appropriate B cell contribution and passive immunization can protect susceptible mouse strains.

Research involving *Salmonella* has presented an alternative method for *i*NKT cell activation. Salmonella infection causes a spectrum of diseases by infecting the host through the oral route whereby bacteria can disseminate to other organs. Salmonella activate DCs to produce IL-12 through Toll-like receptor 4 (TLR4) stimulation by LPS. Activated DCs present endogenous Ag via CD1d to *i*NKT cells during infection [33,35]. The co-requirement for IL-12 and CD1d revealed a unique and indirect mechanism for activating *i*NKT cells, where self-ligand(s) such as iGb3 are presented rather than a microbial Ag. However, LPS-induced *i*NKT cell-derived IFN-γ *in vitro* does not require CD1d-mediated Ag presentation, instead exposure to IL-12 and IL-18 is sufficient to activate these cells [36].

*i*NKT cells have also been implicated in *Pseudomonas aeruginosa* infection *in vivo* but these findings have been recently challenged [37]. Similarly, although *i*NKT cells produce IFN-γ during *Listeria monocytogenes* infection, the role for *i*NKT cells and/or CD1d is not clear [38–40].

4.2 PARASITES

Studies on visceral *Leishmania donovani* suggest that lipophosphoglycan, or glycoinositol phospholipids on the surface of *L. donovani* bind to CD1d molecules and can be recognized by *i*NKT cells. Furthermore, infected $CD1d^{-/-}$ mice develop a defective granuloma response and have a higher parasite burden in the liver and spleen during the innate response compared to WT controls [41]. In this study, *i*NKT cells appear to make IFN-γ immediately after parasite inoculation followed by the reduction of detectable *i*NKT cells. Additionally, *i*NKT cells appear to regulate sustained hepatic CXCL10 mRNA expression during the early stages of infection [42]. However, a recent study shows that *i*NKT cells on the C57BL/6 background only play a minor role in the overall protection against *L. donovani*, where no significant differences between *i*NKT deficient mice *vs* WT controls were found in the chronic stages of infection [43].

Cutaneous *L. major* infection also provides evidence for a protective role of *i*NKT cells where parasite numbers increase in NKT cell deficient mice during the early stages of infection [44,45]. The most dramatic differences were seen after intravenous introduction of parasites *vs* subcutaneous infection where there was a 10–50 fold parasite increase seen in the spleens of NKT cell deficient mice as well as decreased NK cell IFN-γ production. It is of interest that many of the discrepancies between publication results may be due to the strain of mouse used, the route of infection and the strain of the parasite.

Using a mouse model of *Trypanosoma cruzi* infection, WT and $CD1d^{-/-}$ mice both develop mild phenotypic symptoms, but the majority of the mice survive [46,47]. However, the same inoculum given to $J\alpha18^{-/-}$ mice, results in a dramatic increase in mortality and morbidity [46]. Additionally, the production of inflammatory cytokines is significantly enhanced in $J\alpha18^{-/-}$ animals. Furthermore, GPI mucins and GIPLs from the surface of *T. cruzi* bind to CD1d molecules and inhibit α-GalCer activation of NKT cell hybridomas, but these ligands alone do not appear to activate *i*NKT cells. These results suggest a striking contrast in

function between *i*NKT cells and other subsets of NKT cells that are present in the Jα18$^{-/-}$ mice, but not in CD1d$^{-/-}$ mice [47].

The role of *i*NKT cells during helminth infection has recently been appreciated [48]. New findings suggest that, although dispensable for host resistance, *i*NKT cells play a part in the development of the acquired immune response and in the control of pathology during murine schistosomiasis. Here too, major differences were observed between Jα18$^{-/-}$ mice and CD1d$^{-/-}$ mice [49]. Of note, schistosome eggs appear to be the only parasitic stage capable of activating *i*NKT cells in a TCR dependent manner, although the mechanisms still remain unresolved [50].

5. *i*NKT CELLS AND VIRUS

While the speculation and search for pathogenic ligands for the Vα14*i* TCR continue in respect of different bacterial, parasitic and fungal pathogens, viral genomes do not generate lipid molecules. Therefore the mechanism of *i*NKT cell activation during viral infection must use either host lipids in the context of CD1d or an entirely TCR-independent mechanism.

5.1 HIV

Human immunodeficiency virus (HIV) targets *i*NKT cells, which are subsequently depleted from the host [7,51]. Viral entry in *i*NKT cells requires the expression of the chemokine receptors CCR5 or CXCR4 in combination with CD4 [52], although the loss of CD4^{-} *i*NKT cells also occurs. The R5-tropic HIV-1 strain replicates in CD4^{+} *i*NKT cells more vigorously than in mainstream T cells, which might account for their rapid selective loss. In fact, high viremia is correlated with lower numbers of circulating NKT cells. Alternatively, it is possible that *i*NKT cell reduction occurs from the direct or indirect activation by APCs leading to AICD. The loss of *i*NKT cells during HIV infection has a number of potentially harmful consequences. Decreased NKT cell surveillance could be linked to the increased incidence of certain AIDS related tumors such as Kaposi's Sarcoma.

Recently it was shown that CD1d expression is decreased, particularly on CD14^{+} monocytes, in HIV-infected individuals compared to highly active antiretroviral therapy (HAART) patients or healthy donors. Akin to loss of *i*NKT cells, CD1d expression is inversely correlated to viral load. The reduction of CD1d is caused by the HIV-1 protein Nef, which physically associates with the cytoplasmic tail of CD1d interfering with its surface expression [53,54]. As predicted, down-regulation of CD1d was shown for both the human and mouse homologues indicating that Nef targets a highly conserved molecular domain [53,54]. Diminished CD1d expression may negatively influence the ability of *i*NKT cells to recognize infected cells that have up-regulated endogenous ligands.

While HAART is known to reduce viral load in HIV infected patients, it also results in the recovery of predominantly CD4^{-} NKT cells along with conventional CD4^{+} T cells [55]. The rapid restoration of circulating *i*NKT cells is speculated to be the result of their redistribution from tissue sequestration. The recovered *i*NKT cells retain their functional capacity and cytokine profiles. Concurrent administration of α-GalCer with low-dose DNA vaccine enhances both cellular and humoral responses to HIV infection [56]. While HIV therapy has improved the outcome for many infected-individuals, results can be variable depending on differences in disease stages, genetic make up and factors that are outside the bounds of experimental control.

5.2 EMCV

Encephalomyocarditic virus (EMCV) is a picornavirus that causes acute diabetes, paralysis and myocarditis. Resistance to EMCV is dependent on the early production of adequate amounts of IL-12 induced production of IFN-γ from NK cells. Studies comparing WT mice with $CD1d^{-/-}$ mice revealed additional details of the innate-adaptive pathway involved in viral clearance. Sensitive BALB/c mice, are protected from EMCV through treatment with α-GalCer [57] whereas $CD1d^{-/-}$ mice on 129 and B6 backgrounds show increased susceptibility to EMCV infection [58]. $CD1d^{-/-}$ mice produce less IFN-α and IL-12, causing decreased IFN-γ in response to ECMV than their WT counterparts. This cytokine axis results in the inverse correlation between viremia and $CD8^{+}$ T cell activation [59]. Administration of IL-12 in the $CD1d^{-/-}$ mice improves immune resistance, bypassing the contributions of *i*NKT cells and acting directly to increase IFN-γ production by NK cells. Furthermore, *in vitro* addition of anti-CD1d mAb increased EMCV replication in WT splenocyte cultures. However, $J\alpha18^{-/-}$ mice do not show enhanced susceptibility suggesting that *i*NKT cells are not required for EMCV resistance, but another subset of CD1d-dependent cells has a protective role.

5.3 LCMV

Lymphocytic Choriomeningitis Virus (LCMV) is a natural mouse pathogen that has been useful in the study of T cell responses to viral infection. The Armstrong strain of LCMV has been shown to induce the loss of hepatic, splenic and peritoneal NKT cells [60]. This loss is recovered within the liver by two weeks post-infection (p.i.), but persists in the spleen for over three months. This depletion is independent of Fas-FasL interactions as well as IFN-γ and IL-12, cytokines typically produced during LCMV infection. While it was shown that *i*NKT cells are actively infected by LCMV, the means of compartmental loss occurs through the immediate production of IFN-α/β, which subsequently causes *i*NKT cell apoptosis.

Interestingly, the employment of $CD1d^{-/-}$ mice demonstrated the regulatory role *i*NKT or/and non-invariant CD1d restricted cells have during LCMV. $CD1d^{-/-}$ mice show elevated levels of IL-2, IL-4 and IFN-γ in response to LCMV and the cytokine production is maintained even after viral clearance has occurred [61]. The cytokine rich environment enhances the proliferation of T cell subsets, further augmenting cytokine potential and the ability to clear virus more quickly. Both DCs and Mϕ exhibit down-regulation of CD1d at day 10 p.i. following a substantial increase seen at day 6, also independent of IFN-γ or IL-12 [62,63]. However, both WT and $CD1d^{-/-}$ mice are capable of eliminating virus.

5.4 INFLUENZA

Influenza A virus (IAV) infection rapidly spreads around the world in seasonal epidemics and imposes a considerable economic burden. Furthermore, pandemics with highly virulent strains or transference of humans with H5N1 (bird flu) viruses represent current health threats. Of particular interest is the recent demonstration that α-GalCer can serve as a potent mucosal adjuvant to trigger protection against IAV infection [64]. Intranasal (i.n.) co-administration of α-GalCer with either hemagglutinin [65] or inactivated IAV [64], induces long-lasting protective mucosal and systemic immune responses against lethal infection with IAV in the mouse system. More recently, Kamijuku et *al*. provided evidence that i.n. vaccination with α-GalCer plus hemagglutinin induces an effective cross-protection against different strains of influenza virus, including H5N1 [66]. This vaccine protocol resulted in the CXCL16/CXCR6 dependent increase of nasal mucosa *i*NKT cells, IL-4 dependent increase of IgA secretion and improved survival from influenza induced lethal pneumonia. Finally, exogenous activation of *i*NKT cells by means of α-GalCer administration during IAV infection was shown to enhance the early innate immune response in the lungs and contribute to antiviral immunity [67]. As a whole, these studies offer new methods for

combating the high mortality rate of IAV infection by targeting *i*NKT cells and encouraging the development of α-GalCer, or other *i*NKT-activating glycolipid adjuvants. Importantly, De Santo and colleagues recently demonstrated that *i*NKT cells play a critical role in controlling IAV PR8 virus infection and that this effect is CD1d dependent [68].

5.5 RSV

Respiratory syncytial virus (RSV), an RNA virus in the family Paramyxoviridae, causes respiratory disease in humans. It is the most common cause of lower respiratory tract infection in infants and children worldwide. The immune response to primary RSV infection in humans and mice is generally characterized by a mixed Th1/Th2 cytokine response [69–71]. Johnson et *al*. examined the role of CD1d expression and Ag presentation in RSV pathogenesis using CD1d-deficient mice and α-GalCer. They found that *i*NKT cells contribute to the efficient induction of $CD8^+$ T cell responses and amplification of antiviral immune responses to respiratory syncytial virus. They proposed that the absence of CD1d restricted T cell (invariant or/and non-invariant) activation leads to a reduction in early IFN-γ production, resulting in diminished RSV specific $CD8^+$ T cell expansion and delayed viral clearance. [72].

5.6 TMEV

Theiler's murine encephalomyelitis virus (TMEV) causes demyelination with inflammation of the central nervous system in mice and is used as an animal model for multiple sclerosis (MS). On one hand NKT cells have been shown to play a protective role against demyelination, while other investigators demonstrated no function or a detrimental role for NKT cells [73–77]. In a recent study, CD1d restricted NKT cells were shown to play a protective role in TMEV induced neurological disease by alteration of the cytokine profile and virus-specific immune responses. The authors demonstrated that the $CD1d^{-/-}$ mice developed demyelinating disease with more neurological deficits and higher IL-4 production compared to the WT mice [78].

5.7 HSV

Investigation of herpesvirus family members has resulted in divergent results depending on the strain used or the amount of virus inoculated. An early study using the virulent herpes simplex virus (HSV)-1 SC16 strain demonstrated that $CD1d^{-/-}$ and $J\alpha18^{-/-}$ mice were impaired in their ability to clear the virus, showing increased skin lesions, greater morbidity, viral persistence and increased spread of HSV to the nervous system [79]. Recently, Grubor-Bauk et *al*. showed the importance of *i*NKT cells in resistance to HSV and their contribution to the level of latency in mice [80]. In contrast, the HSV-1 less virulent Kos strain exemplified no dissimilarity between NKT cell deficient mice *vs* WT controls [81].

Two recent reports indirectly support a potential role for *i*NKT cells in the clearance of the HSV-1 infection. Yuan *et al.* demonstrated that infection with HSV-1 reduces CD1d cell surface expression on APCs. In this case, HSV-1 prevents the reappearance of endocytosed CD1d on the cell surface by redistributing endocytosed CD1d to the lysosome limiting membrane [82]. Raftery *et al* showed that HSV-1 strain F also affects CD1d expression but it is dependent on the dose of administered viruses. Low MOI increases CD1d expression on DCs and causes *i*NKT proliferation *in vitro*, while high MOI decreases CD1d expression [83]. Recently, Kaposi sarcoma-associated herpesvirus (KSHV) was reported to decrease human CD1d as well [84]. The viral protein modulator of immune recognition is an ubiquitin ligase that associates with the cytoplasmic tail of CD1d, causing cell-surface down-regulation. Interestingly, as mentioned with HIV, the protein Nef influences CD1d expression, which may in part, be influential in aiding this opportunistic virus. Altogether,

these results suggest that inhibition of CD1d surface expression may be an important HSV-1 immune evasion strategy.

5.8 MCMV & HCMV

The cytomegaloviruses (CMVs) are ubiquitous species-specific β-herpesviruses. CMV is a double stranded DNA virus containing more than 240kb, and encoding over 200 potential proteins, making it the largest member of the herpesvirus family [85]. Typically, infection is asymptomatic in immunocompetent hosts, but can result in morbidity and mortality in high-risk individuals. The replication cycle of CMV is divided into the immediate, early, and late phases according to the time of gene transcription. Murine CMV (MCMV) is widely used as an experimental model for human CMV and has been successfully used to elucidate the mechanisms involved in virulence, immune evasion and immune detection. Host resistance is dependent on both innate NK cell containment, followed by the specific adaptive $CD8^+$ T cell elimination of the virus. The critical involvement of NK cells has been demonstrated for both HCMV and MCMV. Individuals lacking a functional NK cell compartment are subject to severe infection and increased mortality to HCMV. Studies in different strains of mice have revealed that not only is the presence of functional NK cells necessary to control MCMV, but the presence of specific NK cell surface receptors is key for orchestrating the successful elimination of the virus. NK cell effector functions are initiated by the production of type I IFN and IL-12, which stimulate NK cell production of IFN-γ, proliferation and cytotoxic activity. The initial activation signal appears to be non-specific, acting on the bulk of the NK cell compartment, however, by day 2–3 of the acute phase, a selective proliferation occurs within the $Ly49H^+$ NK cell subset [86,87]. Mice lacking the activating receptor Ly49H, BALB/c and 129 strains, are susceptible to MCMV, while C57BL/6 are resistant due to the presence of the gene *Cmv1*^{r}, encoding this protein [88–90]. Ly49H interacts directly with the virus encoded gene product m157 that becomes expressed on the surface of MCMV infected cells [91,92]. The activation signaling cascade instigated by this association involves the adaptor protein DAP12 and results in the production of traditional NK cell effector functions in the $Ly49H^+$ subset [93]. In other strains of mice, (K haplotype), protection *in vivo* requires the activating receptor Ly49P recognition of D^k carrying virus peptide fragments on infected cells [94]. Additional analysis uncovered that the selective NK cell response is also dependent on IL-18, IL-12 and interactions with $CD8\alpha^+$ DCs [95].

Initially, an attempt to determine a role for activated *i*NKT cells as a potential target for MCMV immunotherapy was considered. α-GalCer was administered at different times of inoculation using the virulent K181-Perth strain of MCMV [96]. The investigators determined that α-GalCer therapy resulted in reduced viral titers in the spleen and liver of both susceptible BALB/c and resistant B6 mice. The therapeutic effects of α-GalCer activated *i*NKT cells was dependent on their influence over NK cells, as mice depleted of NK cells by α-asialo GM1 treatment, did not limit viral replication.

Recently, *i*NKT cell participation during MCMV infection was examined [97,98]. Two studies found that *i*NKT cells dynamically participate in the initial immune response to MCMV infection [97]. Indeed, shortly after inoculation, *i*NKT cells display signs of activation, up-regulation of the high affinity receptor CD25, decrease in cell numbers in the spleen and liver and robust production of IFN-γ [97,98]. Interestingly, *i*NKT cells react to MCMV as innate immune cells rather than having an adaptive role as an interaction between the TCR and CD1d is dispensable for the activation phenotype and cytokine release [97,98]. Both blocking the invariant TCR recognition with anti-CD1d Abs and the use of adoptively transferred *i*NKT cells into $CD1d^{-/-}$ and $J\alpha18^{-/-}$ hosts results in both *i*NKT and NK cell production of IFN-γ. However, while the TCR was not vital to the outcome, IFN-γ production is at least partially dependent on IL-12 and IFNα/β secretion [97]. Tyznik et *al*.

subsequently demonstrated that the mechanism of the *i*NKT response was through indirect activation [98]. MCMV elicits the activation of DCs in a TLR9 dependent fashion, stimulating the production of IL-12, which subsequently activates the *i*NKT cells to produce IFN-γ.

However, Jα18$^{-/-}$ mice do not succumb to high dose MCMV infection more frequently than their WT counterparts [96,97], and hence *i*NKT cells appear to be dispensable for control of MCMV infection at least in the B6 background. Interestingly, approximately 50% of CD1d$^{-/-}$ mice show increased susceptibility to MCMV than control littermates, revealing a potential role for other CD1d dependent cells having a protective role during the later stage of infection [97].

CONCLUDING REMARKS

*i*NKT cells have been implicated, for better or worse, in a number of different microbial infections. This review attempts to represent just a few in order to depict the range of function and multiple mechanisms of activation that *i*NKT cells possess. We have highlighted the diverse ways in which *i*NKT cells are activated by microbes. First, CD1d presentation of endogenous ligands or exogenous pathogen derived ligands by APCs. Additionally, *i*NKT cell activation can be driven by different cytokines directly or indirectly, IL-12, IL-18, type I and II IFNs, or any combination of stimuli. Microorganisms have evolved different mechanisms in order to evade immune detection and interfere with both innate and adaptive effector abilities. Depending on the pathogen, *i*NKT cells have the ability to respond rapidly and regulate different compartments of the immune system both innate and adaptive. These studies demonstrate that therapies aimed at modulating the *i*NKT response may have beneficial or adverse outcomes, but are valuable targets for therapeutics.

EXPERT OPINION

*i*NKT cells play a multifaceted role in a variety of immune responses in part by bridging innate and adaptive immunity. *i*NKT cells have been extensively studied in the context of a wide range of infectious agents. Although the mechanisms by which *i*NKT cells engage in viral immunity need to be fully elucidated, the current studies emphasize the importance of accurately analyzing the functionality of *i*NKT cells in models of viral infection before they can be exploited in therapeutic settings. It is clear that *i*NKT cells have the competency to promote a spectrum of immunoregulatory responses and hold great promise for development of vaccine adjuvant and immunotherapies. For instance, Huang et *al*. illustrated that α-GalCer enhances the immunogenecity of DNA vaccines, which may aid in designing more effective adjuvant and vaccines against HIV-1 [56]. Similarly, new methods for fighting the flu using *i*NKT cell targets could provide effective cross-protective mucosal immunity to multiple influenza strains [66–68].

In spite of this, *i*NKT cells are often considered a “double edged sword”, particularly in the pathogenesis of certain infections causing liver disease. For instance, *i*NKT cells exert both anti- and proinflammatory responses to hepatitis. Despite the presence of abundant hepatic *i*NKT cells, treatment with α-GalCer is necessary to promote an effective antiviral response to hepatitis B virus (HBV) in transgenic mice bearing a HBV genome [99]. Additionally, Ito *et al* showed that activation of *i*NKT cells promotes the breakage of CTL tolerance in the setting of HBV induced hepatitis [100]. In contrast, *i*NKT cells are negatively implicated in the regeneration process of the liver in a HPV partial hepatectomy model. This was speculated to be, in part, a negative effect of IFN-γ on hepatocytes [101].

Importantly, administration of α-GalCer can cause *i*NKT cells to become unresponsive, raising the issue of anergy induction in designing treatment regimens that use specific

activators of *i*NKT cells [102,103]. Similarly, it has been demonstrated that *i*NKT cells activated in response to multiple bacterial microorganisms acquire a hyporesponsive phenotype, which can significantly impact subsequent *i*NKT cell–mediated immune responses and the efficacy of *i*NKT cell–based immunotherapy [104]. Therefore, therapeutic approaches that specifically stimulate *i*NKT cells might need to be combined with systems that target inhibitory receptors such as programmed cell death 1 or the neutralization of IL-10 [105].

Finally, non-invariant CD1d restricted T cells contribute significantly to the innate immune response to several pathogens, illustrated by the different phenotypes observed when the immune response from Jα18$^{-/-}$ and CD1d$^{-/-}$ mice is compared. For instance, CD1d deficient mice are more sensitive to MCMV and ECMV infections than Jα18$^{-/-}$ mice [57,97]. Therefore the characterization of non-classical NKT cells as well as identification of their specific ligands warrant further investigations.

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