eBioscience

The Journal of Immunology

Cutting Edge: The Mechanism of Invariant NKT Cell Responses to Viral Danger Signals

Aaron J. Tyznik, Emmanuel Tupin, Niranjana A. Nagarajan, Min J. Her, Chris A. Benedict and Mitchell Kronenberg

This information is current as of August 24, 2011

J Immunol 2008;181;4452-4456

References This article **cites 22 articles**, 8 of which can be accessed free at:
http://www.jimmunol.org/content/181/7/4452.full.html#ref-list-1

Article cited in:
http://www.jimmunol.org/content/181/7/4452.full.html#related-urls

Subscriptions Information about subscribing to *The Journal of Immunology* is online at
http://www.jimmunol.org/subscriptions

Permissions Submit copyright permission requests at
http://www.aai.org/ji/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at
http://www.jimmunol.org/etoc/subscriptions.shtml/

Downloaded from www.jimmunol.org on August 24, 2011

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.
Copyright ©2008 by The American Association of
Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Cutting Edge

THE JOURNAL OF IMMUNOLOGY

Cutting Edge: The Mechanism of Invariant NKT Cell Responses to Viral Danger Signals[1]

Aaron J. Tyznik,* Emmanuel Tupin,[2,3]* Niranjana A. Nagarajan,[2,4]* Min J. Her,† Chris A. Benedict,[2,5]† and Mitchell Kronenberg[2,5]*

Invariant NK T (*i*NKT) cells influence the response to viral infections, although the mechanisms are poorly defined. In this study we show that these innate-like lymphocytes secrete IFN-γ upon culture with CpG oligodeoxynucleotide-stimulated dendritic cells (DCs) from mouse bone marrow. This requires TLR9 signaling and IL-12 secretion by the activated DCs, but it does not require CD1d expression. *i*NKT cells also produce IFN-γ in response to mouse CMV infection. Their mechanism of mouse CMV detection is quite similar to that of CpG, requiring both TLR9 signaling and IL-12 secretion, while the need for CD1d expression is relatively minor. Consequently, *i*NKT cells have the ability to respond to a variety of microbes, including viruses, in an Ag-independent manner, suggesting they may play a broad role in antipathogen defenses despite their limited TCR repertoire. ***The Journal of Immunology,* 2008, 181: 4452–4456.**

Invariant NK T (*i*NKT)[6] cells are a subpopulation of innate-like T lymphocytes that express an invariant T cell Ag receptor α-chain. *i*NKT cells rapidly mount a response to synthetic glycolipid Ags presented by CD1d that is characterized by the production of large amounts of IFN-γ and IL-4 (1, 2). Additionally, *i*NKT cells respond to several bacteria that have glycolipids that engage their invariant TCRs. *i*NKT cells probably also can be activated by microbes that do not encode Ags for their TCRs (3). For example, *i*NKT cells have been reported to influence the course of some viral infections. In fact, several viruses down-regulate human CD1d expression, suggesting a key role for *i*NKT cells in antiviral defenses (4, 5).

It is well established that stimulation of APCs with bacterial products or the TLR ligand LPS activate *i*NKT cells in the absence of foreign Ag for their TCR (6, 7). Until recently, however, it was not known whether other TLR ligands, such as those that detect viral danger signals, also activate *i*NKT cells. Therefore, in this study we investigated the response of *i*NKT cells to a TLR9 ligand, CpG oligodeoxynucleotide (ODN), and the response to virus infection with mouse CMV (MCMV) (8). MCMV is a β-herpesvirus that is a widely studied model with viral control dependent on innate immune responses by stromal cells, dendritic cells (DCs), and NK cells (9, 10). Despite studies assessing the potential contribution of *i*NKT cells in innate control of MCMV (11), direct analysis of *i*NKT activation, and the cytokine requirements for this activation, has not been performed. In the present study we show that the mechanism of *i*NKT cell activation by MCMV is similar to that of in vitro activation by CpG ODN-stimulated APCs.

Materials and Methods

Mice

C57BL/6 (B6), B6.129S1-*Il12b^{tm1Jm}*/J (*IL-12p40$^{-/-}$*), and B6.129P2-*Il18^{tm1Aki}*/J (*IL-18$^{-/-}$*) mice were purchased from The Jackson Laboratory. *CD1d$^{-/-}$* mice were a gift from Dr. L. Van Kaer (Vanderbilt University, Nashville TN). *Tlr9^{cpg1}* mice were a gift from Dr. B. Beutler (The Scripps Research Institute, La Jolla CA) (8). All mice were housed in specific pathogen-free conditions.

Cell preparation

Liver lymphocytes were isolated as described previously (12). For preparation of DCs, bone marrow was cultured in medium supplemented with 100 ng/ml recombinant human fms-like tyrosine kinase-3 ligand (hFlt3L; Amgen) for 8 days. DCs were treated with 10 μg/ml type B CpG ODN 1826 or control 1982 ODN (Alexis Biochemicals), and infected with MCMV Smith strain at a multiplicity of infection of 3, or mock infected, for 2 h. DCs were cultured with *i*NKT cells for 48 h before cytokine detection (7). *i*NKT cells were obtained from spleen by selecting for NK1.1$^+$ TCRβ^+ cells by flow cytometry as previously described (7). The purity of the cells collected, typically >98%, was assessed using CD1d tetramers loaded with α-galactosylceramide (αGalCer; provided by Kirin Pharma).

ELISA

A standard sandwich ELISA was performed to measure mouse IFN-γ, TNF, and IL-4 following the manufacturer's instructions (R&D Systems).

*Division of Developmental Immunology and †Division of Molecular Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037

Received for publication June 18, 2008. Accepted for publication July 31, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grants AI 69296, AI 71922 (to M.K.), and F32 AI 80087 (to A.J.T.).

[2] E.T. and N.A.N. contributed equally to this manuscript; C. A. B. and M. K. share senior authorship.

[3] Current address: Vironova AB, Smedjegatan 6, 131 34 Nacka, Sweden.

[4] Current address: University of California Berkeley, Department of Molecular and Cell Biology, 425 Life Sciences, Addition no. 3200, Berkeley, CA 94720-3200.

[5] Address correspondence and reprint requests to Dr. Chris Benedict or Dr. Mitchell Kronenberg, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037. E-mail addresses: benedict@liai.org and mitch@liai.org

[6] Abbreviations used in this paper: *i*NKT, invariant NK T (cell); DC, dendritic cell; αGalCer, α-galactosylceramide; hFlt3L, human fms-like tyrosine kinase-3 ligand; MCMV, mouse CMV; ODN, oligodeoxynucleotide; WT, wild type.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

Downloaded from www.jimmunol.org on August 24, 2011

Viral infections

Salivary gland extract stocks of Smith strain MCMV were prepared as previously described (13). Mice were infected with 5×10^4 PFU of virus in 500 μl of PBS via i.p. injection.

Monoclonal Ab treatment

To block CD1d in vitro, 30 μg of anti-mouse CD1d clone 1B1 mAb was added to cultures. For blocking in vivo, mice received 300 μg of mAb on day −1, 200 μg on day 0, and 100 μg on day 1 of 1B1 mAb or rat IgG controls (eBiosciences) in PBS via i.p. injection (14).

Flow cytometry and intracellular cytokine staining

Lymphocytes were stained with αGalCer/CD1d tetramers labeled with streptavidin-allophycocyanin, anti-NK1.1-PerCp PE-cyanin (PECy)5, anti-CD8-PECy7, anti-CD11b-PECy7, anti-TCRβ-allophycocyanin-AF750, and CD25-FITC. All Abs and isotype controls, except anti-TCRβ-allophycocyanin-AF750 from eBioscience, were purchased from BD Biosciences. As a positive control for *i*NKT cell responses, mice were injected with 2 μg of αGalCer. Cells were fixed and permeabilized using Cytofix/Cytoperm buffer and stained for intracellular IFN-γ with PE-labeled clone XMG1.2. The data were collected on a LSR II flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star).

Results and Discussion

TLR9-stimulated DCs activate i*NKT cells*

Previous studies have demonstrated that mouse *i*NKT cells were activated following stimulation of DCs with the TLR4 ligand LPS (7). To further investigate the potential role of *i*NKT cells in regulating the immune response to viral danger signals, we evaluated the ability of TLR9-stimulated or MCMV-infected DCs to activate *i*NKT cells in vitro.

Because triggering the TLR9 pathway with CpG-ODN serves as a model for innate recognition of viral DNA, we tested the ability of DCs stimulated with CpG ODN to activate *i*NKT cells in culture. DCs derived from bone marrow by culture with hFlt3L were exposed to CpG ODNs and cocultured with highly purified splenic *i*NKT cells. High levels of IFN-γ, but no IL-4 or TNF, were detected in the stimulated cocultures (Fig. 1*A* and data not shown). Similar results were obtained when the DCs were stimulated with TLR7 agonists (data not shown). The production of IFN-γ in the absence of IL-4 was similar to previous results obtained from analyzing *i*NKT cell responses to LPS (7), but it contrasts with the results obtained with *i*NKT cell activation by TCR agonists, which elicits all three cytokines. IFN-γ secretion by *i*NKT cells was dependent on TLR9 activation of the DCs, as nonstimulated DCs or DCs stimulated with an inactive CpG ODN did not induce it. Additionally, hFlt3L-derived DCs from $TLR9^{CpG1}$ mutant mice were unable to induce IFN-γ secretion by *i*NKT cells (Fig. 1*B*). IFN-γ production in these cultures was dependent on *i*NKT cells, although TLR9-stimulated DCs in the absence of *i*NKT cells produced IL-12p70 (data not shown).

To define the mechanism of *i*NKT cell activation by CpG ODN-treated DCs, DCs were generated from mice deficient for IL-12p40, IL-18, or CD1d. Similar to our previous results with LPS-stimulated DCs, *i*NKT cells did not produce IFN-γ when the TLR9-stimulated DCs were made from $IL\text{-}12p40^{-/-}$ mice. Somewhat surprisingly, $IL\text{-}18^{-/-}$ DCs treated with CpG ODN did induce IFN-γ production by *i*NKT cells, contrasting with the results from LPS stimulation (7). DCs derived from CD1d-deficient mice were equally effective at causing IFN-γ secretion, and similar results were obtained when a CD1d-blocking mAb was added to cocultures containing wild-type (WT) DCs or $CD1d^{-/-}$ DCs to block the contribution of

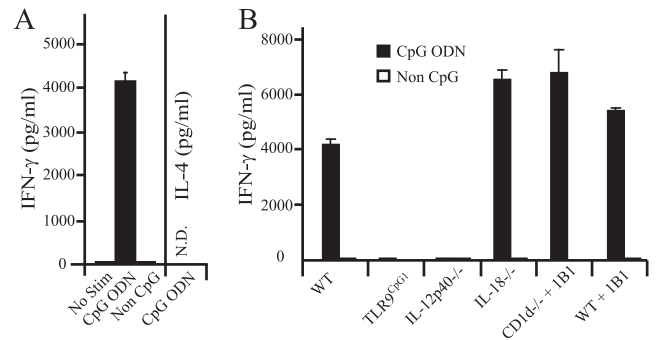

FIGURE 1. IFN-γ production by *i*NKT cells in response to CpG-stimulated DCs. *A*, WT DCs were stimulated with CpG ODN or nonactivating CpG ODN (Non CpG), cocultured with purified splenic *i*NKT cells, and cytokine production was measured by ELISA. No Stim, Not stimulated. *B*, Purified *i*NKT cells were cocultured with CpG or non-CpG ODN-stimulated DCs derived from the indicated mouse strains or with DCs from $CD1d^{-/-}$ or WT mice in the presence of anti-CD1d blocking mAb (1B1). Cytokine production was measured in the supernatant by ELISA. Shown is a representative experiment of five performed. Results represent the mean of one experiment with three replicate cultures measured in triplicate for ELISA. Error bars represent SEM ($n = 9$ for each set of DCs).

CD1d expression by the *i*NKT cells themselves. These results indicate that *i*NKT cells can be activated in response to IL-12 induced by endosomal TLR ligands independently of either foreign or self-lipid Ag presentation by CD1d.

Our results are consistent with those from several recent reports showing *i*NKT cell activation following exposure to TLR9 agonists. In one of these, however, activation in vivo did not occur unless the CpG ODN was encapsulated in liposomes (15). In studies using human *i*NKT cells, activation marker expression was increased but cytokine release was not observed (16), whereas in another study TLR3, 7, and 9 agonists were shown to cause the in vitro expansion of human *i*NKT cells in cultures of PBMCs (17). Furthermore, a recent report demonstrated that liver-derived mouse *i*NKT cells were activated by DCs exposed to TLR7 or TLR9 agonists. However, in that study *i*NKT cell activation was dependent upon type I IFN secretion, not IL-12, despite the production of both of these cytokines by the activated DCs (18). Furthermore, *i*NKT cell IFN-γ secretion was found to be at least partially dependent upon CD1d expression and the presentation by CD1d of a charged glycosphingolipid autologous Ag of undefined structure (18). The reasons for this discrepancy are not known but could be due either to differences in DCs generation or to the use of liver mononuclear cells as opposed to purified splenic *i*NKT cells. In our studies, hFlt3L cultured DCs were used whereas the previous study used 14-day cultured GM-CSF DCs. As described below, hFlt3L-cultured DCs may more appropriately model in vivo infection with virus.

MCMV-infected DCs activate i*NKT cells in vitro*

The engagement of endosomally expressed TLR9 by CpG ODN in DCs is a model for some of the early events leading to innate immune activation following infection with a DNA virus. To test this, we evaluated the in vitro *i*NKT cell response to DCs infected with MCMV. Infected hFlt3L DCs were cocultured with purified *i*NKT cells, and the supernatants were measured for cytokine production (Fig. 2, *A* and *B*). MCMV infection induced rapid production of IFN-γ but not IL-4 (Fig. 2*A*).

Downloaded from www.jimmunol.org on August 24, 2011

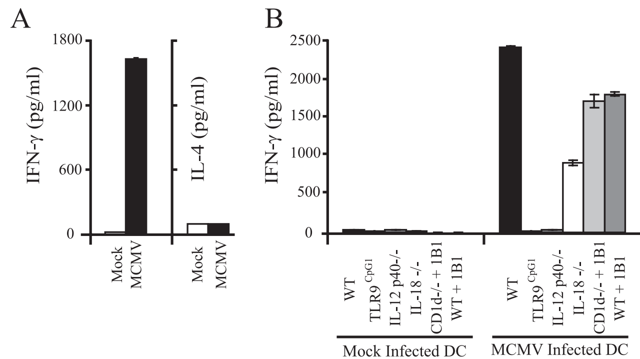

FIGURE 2. Cytokine production by *i*NKT cells in response to MCMV-infected DCs. *A*, DCs were infected with MCMV and cultured with sorted *i*NKT cells before cytokine measurement by ELISA. *B*, Sorted *i*NKT cells were cocultured with MCMV or mock-infected DCs from the indicated mouse strains or DCs from $CD1d^{-/-}$ or WT mice in the presence of an anti-CD1d-blocking mAb. Cytokine production was measured by ELISA. Shown is a single experiment of eight performed. Results show the mean of one experiment with three replicate cultures of DCs measured in triplicate for ELISA. Error bars repesent SEM ($n = 9$ for each set of DCs).

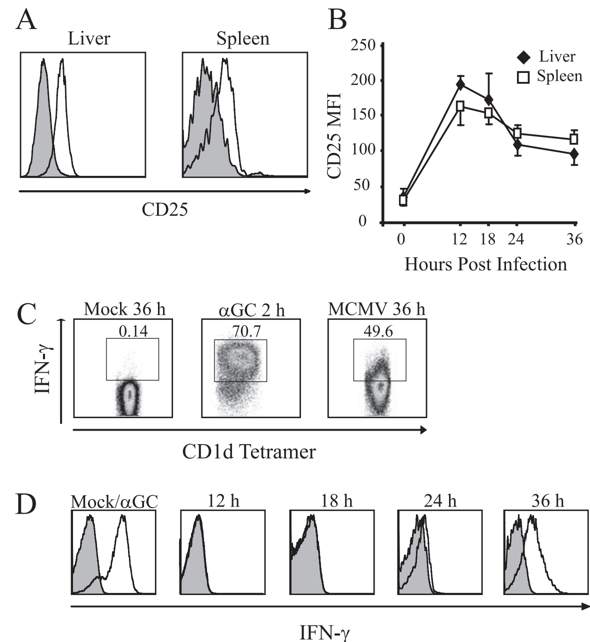

FIGURE 3. MCMV infection activates liver *i*NKT cells in vivo. *A*, WT mice were infected with MCMV i.p. and αGalCer/CD1d tetramer^{+} *i*NKT cells were analyzed for CD25 expression (open histograms) compared with PBS injected controls (shaded histograms) at12 h. Data are representative of four independent experiments on two or three mice per group. *B*, Time course of the mean fluorescence intensity (MFI) of CD25 expression after infection. Time 0 is the average MFI of PBS-injected controls. Average MFI of *i*NKT cells in one of four independent experiments is shown. Error bars represent SEM ($n = 3$ for each time point). *C*, At the indicated times, liver mononuclear cells were stained for intracellular IFN-γ and the data were gated for αGalCer (αGC)/CD1d tetramer^{+} *i*NKT cells. Numbers represent the percentage of IFN-γ^{+} cells in the *i*NKT cell population. *D*, Time course of the MCMV-activated *i*NKT cell IFN-γ response. MFI values of intracellular IFN-γ (open histograms) were compared with those of isotype controls (shaded histograms). *Far left panel* represents the *i*NKT cell response 2 h after i.p. injection of αGalCer. Results are representative of three independent experiments of 3–5 mice per group.

IFN-γ secretion required MCMV infection, as uninfected DCs (Fig. 2*A*) or infection with a mouse-adapted strain of Dengue virus (19) (data not shown) did not lead to IFN-γ production. MCMV-infected DCs from $TLR9^{CpG1}$ mutant mice or from $IL\text{-}12p40^{-/-}$ mice also did not induce IFN-γ production (Fig. 2*B*). By contrast, MCMV-infected, GM-CSF-cultured DCs did not activate *i*NKT cells (data not shown).

MCMV has been shown to provoke innate immune responses that lead to IL-12 secretion and NK cell activation (9, 20). Our data suggest that the recognition of MCMV by TLR9 induces IL-12 production by DCs, leading to IFN-γ production by *i*NKT cells. Unlike in vitro stimulation with CpG-ODN, however, optimal production of IFN-γ by cocultures with *i*NKT cells was dependent on the ability of the DCs to produce IL-18 (Fig. 2*B*). In no case did the absence of CD1d expression reduce the amount of IFN-γ produced by *i*NKT cells to the levels observed with DCs deficient for TLR9 or IL-12. In the experiment shown, IFN-γ release was reduced by ~30% when WT DCs were treated with an anti-CD1d mAb or when mAb blockade was combined with the use of $CD1d^{-/-}$ DCs (Fig. 2*B*). Comparing multiple experiments, the reduction in IFN-γ when CD1d was blocked or deleted, although variable, was generally minor and in several experiments there was little or no effect of inhibiting the interaction with CD1d (data not shown).

MCMV infection rapidly activates i*NKT cells in vivo*

Upon Ag recognition or exposure to the combination of IL-12 and IL-18, *i*NKT cells rapidly become activated in vivo (21). As early as 12 h postinfection with MCMV, tetramer^{+} *i*NKT cells showed increased surface expression of CD25 (Fig. 3*A*). The increased expression peaked by 12–18 h and rapidly declined by 36 h in both the liver and spleen (Fig. 3*B*). A similar temporal expression profile was observed when CD69 expression was analyzed (data not shown). This early activation of *i*NKT cells parallels very closely the induction kinetics of initial innate cytokine production by stromal cells upon MCMV infection (13). Liver and spleen *i*NKT cells produced IFN-γ 36 h postinfection when analyzed directly ex vivo without restimulation (Fig. 3*C* and data not shown). No production of IL-4 or TNF was observed. A previous study demonstrated that a $TCR^{+}NK1.1^{+}$ population produced IFN-γ at this time point (8), but in the absence of tetramer staining these data could not unequivocally show the activation of *i*NKT cells. A time course analysis revealed that *i*NKT cells were capable of producing IFN-γ as early as 24 h postinfection (Fig. 3*D*), a time preceding the viral spread within infected organs. Therefore, similar to results in vitro, the activation of *i*NKT cells post-MCMV infection in vivo leads to early CD25 and CD69 up-regulation, as well as IFN-γ production somewhat later.

i*NKT cell responses to MCMV infection require TLR9 and IL-12*

Consistent with in vitro results, MCMV infection of $TLR9^{CpG1}$ mice did not result in a marked activation of *i*NKT cells to increase CD25 expression at 18 h or to secrete IFN-γ at 36 h after infection (Fig. 4). Also similar to the in vitro results, $IL\text{-}12p40^{-/-}$ (Fig. 4) or IL-12Rβ2$^{-/-}$ mice (data not shown) did not induce up-regulation of CD25 in *i*NKT cells shortly after infection (18 h) or IFN-γ production at 36 h. $IL\text{-}18^{-/-}$ mice also had reduced CD25 expression, but IFN-γ production was not reduced, suggesting that in vivo IL-18 may be dispensable for the induction of *i*NKT cell effector function in response to MCMV.

Downloaded from www.jimmunol.org on August 24, 2011

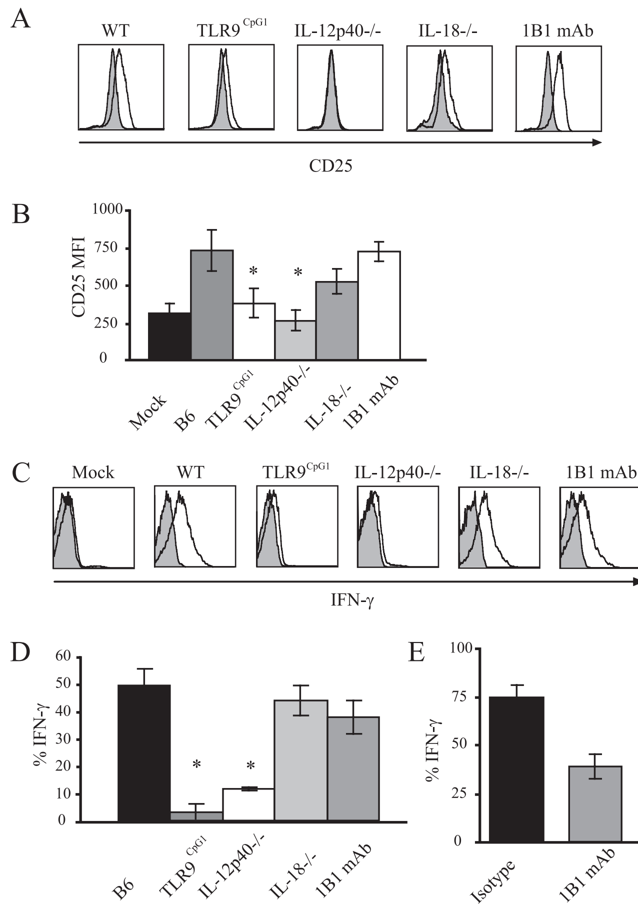

FIGURE 4. *i*NKT cell activation in vivo following MCMV infection. *A*, CD25 expression on *i*NKT cells from the indicated mouse strains 18 h postinfection with MCMV (open histograms) compared with PBS controls (shaded histograms). Histograms are representative plots of three independent experiments of 3–8 mice per group. WT mice treated with a CD1d mAb (*far right panels* in *A* and *C*) are also indicated. *B*, Mean CD25 mean fluorescence intensity (MFI) of 3–8 mice per group 18 h postinfection with MCMV from a minimum of three independent experiments. Statistically significant differences using the equal variance Student *t* test are indicated with an asterisk (*, $p \leq 0.01$) comparing WT to either mutant strains or anti-CD1d mAb-treated mice. *C*, IFN-γ production by *i*NKT cells measured directly ex vivo 36 h postinfection with MCMV (open histograms) compared with isotype controls (shaded histograms). Histograms are representative plots of a minimum of three independent experiments of 3–8 mice per group. *D*, Average percentage of IFN-γ^+ *i*NKT cells 36 h post-MCMV infection. Values plotted are the mean of 3–8 mice per group from a minimum of three independent experiments. Error bars represent SEM (n = 3–8 for each group). Statistically significant differences using Student's *t* test are indicated with an asterisk (*, $p \leq 0.01$). *E*, Average percentage of IFN-γ^+ *i*NKT cells 2 h after αGalCer injection in isotype control or CD1d mAb treated mice. Values plotted are the mean of three mice per group from two independent experiments.

To determine whether CD1d was required in vivo for viral activation of *i*NKT cells, WT mice were treated with a CD1d-blocking mAb and infected with MCMV. As a control for the efficacy of blocking, the mAb was administered to WT mice that received the potent agonist αGalCer, and IFN-γ synthesis by liver and splenic *i*NKT cells was measured directly ex vivo 2 h later. Anti-CD1d-treated mice showed a 50% reduction in *i*NKT cell IFN-γ production (Fig. 4*E*). Treatment with the CD1d-blocking mAb did not alter the induction of *i*NKT cell CD25 expression following MCMV infection, however, and although the treated mice trended lower for IFN-γ production, this decrease was not statistically significant ($p = 0.063$). Self-Ags presented by CD1d during viral infection are likely to be much less potent than αGalCer, and thus the response to these Ags should be more effectively inhibited. Therefore, we conclude that CD1d-mediated Ag presentation is not likely to be a key player in MCMV-induced activation of *i*NKT cells in vivo.

Although *i*NKT cells are early responders to LPS, they are not the first responders because they depend on IL-12 and IL-18 produced by other cell types. Likewise, the kinetics of the *i*NKT cell response indicates that these cells also are not the first responders to MCMV infection. Previous work has shown that the initial IFN-αβ response to i.p. infection with MCMV peaks at ~8 h postinfection in the spleen and is dependent upon B cells expressing lymphotoxin that cross-talk with type I IFN-producing stromal cells (13). A second wave of TLR-dependent activation occurs at ~36 h postinfection in the spleen, shortly after MCMV completes its first round of replication (13, 22). Given the time course of increased activation marker expression and IFN-γ production by *i*NKT cells, we propose a two-stage activation process, with the initial response to viral danger signals being sufficient to induce increased surface marker expression by *i*NKT cells. However, a second TLR-dependent wave of activation and cytokines may be necessary to induce the relatively high levels of IFN-γ secretion needed to detect cytokine production by *i*NKT cells directly ex vivo. This hypothesis is consistent with data demonstrating that *i*NKT cells express slightly increased CD69 and CD25 expression in $TLR9^{CpG1}$ mice 12 h postinfection, but this was reduced by 18 h, and we were unable to detect *i*NKT cell IFN-γ production at later times (Fig. 4 and data not shown).

There are multiple pathways for the foreign Ag-independent activation of *i*NKT cells, which requires, to varying extents, IL-12, IL-18, and type I IFN, as well as CD1d presentation of self-Ags. We speculate that the pathway used will depend on the nature of the inciting stimulus and the type(s) of APCs. Our findings indicate, however, that the mechanism of *i*NKT cell activation following MCMV infection in vivo is similar to that we observed with TLR9 ligands in vitro or with virus infection in vitro using Flt3L DCs. The data suggest that the *i*NKT cell response to viral danger signals in vivo from MCMV infection is mediated by TLR9 engagement leading to IL-12 production and that this response is not highly dependent upon CD1d Ag presentation. Clearly, this pathway, along with the others described that do not depend on foreign Ag, endow innate-like *i*NKT cells with the ability to respond to diverse pathogens despite a highly constricted TCR repertoire.

Acknowledgments

We acknowledge A. Khurana for assistance with the generation of CD1d tetramers. We acknowledge Dr. Sujan Shresta for help with experiments and advice. This is publication no. 1033 from La Jolla Institute for Allergy and Immunology.

Disclosures

The authors have no financial conflict of interest.

References

1. Bendelac, A., P. B. Savage, and L. Teyton. 2007. The biology of NKT cells. *Annu. Rev. Immunol.* 25: 297–336.
2. Van Kaer, L. 2007. NKT cells: T lymphocytes with innate effector functions. *Curr. Opin. Immunol.* 19: 354–364.
3. Kinjo, Y., and M. Kronenberg. 2005. Vα14i NKT cells are innate lymphocytes that participate in the immune response to diverse microbes. *J Clin. Immunol.* 25: 522–533.

Downloaded from www.jimmunol.org on August 24, 2011

4. Van Kaer, L., and S. Joyce. 2006. Viral evasion of antigen presentation: not just for peptides anymore. *Nat. Immunol.* 7: 795–797.
5. Raftery, M. J., M. Hitzler, F. Winau, T. Giese, B. Plachter, S. H. Kaufmann, and G. Schonrich. 2008. Inhibition of CD1 antigen presentation by human cytomegalovirus. *J. Virol.* 82: 4308–4319.
6. Tupin, E., Y. Kinjo, and M. Kronenberg. 2007. The unique role of natural killer T cells in the response to microorganisms. *Nat. Rev. Microbiol.* 5: 405–417.
7. Nagarajan, N. A., and M. Kronenberg. 2007. Invariant NKT cells amplify the innate immune response to lipopolysaccharide. *J. Immunol.* 178: 2706–2713.
8. Tabeta, K., P. Georgel, E. Janssen, X. Du, K. Hoebe, K. Crozat, S. Mudd, L. Shamel, S. Sovath, J. Goode, et al. 2004. Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. *Proc. Natl. Acad. Sci. USA* 101: 3516–3521.
9. Biron, C. A., and L. Brossay. 2001. NK cells and NKT cells in innate defense against viral infections. *Curr. Opin. Immunol.* 13: 458–464.
10. Vidal, S. M., and L. L. Lanier. 2006. NK cell recognition of mouse cytomegalovirus-infected cells. *Curr. Top. Microbiol. Immunol.* 298: 183–206.
11. van Dommelen, S. L., H. A. Tabarias, M. J. Smyth, and M. A. Degli-Esposti. 2003. Activation of natural killer (NK) T cells during murine cytomegalovirus infection enhances the antiviral response mediated by NK cells. *J. Virol.* 77: 1877–1884.
12. Tupin, E., and M. Kronenberg. 2006. Activation of natural killer T cells by glycolipids. *Methods. Enzymol* 417: 185–201.
13. Schneider, K., A. Loewendorf, C. De Trez, J. Fulton, A. Rhode, H. Shumway, S. Ha, G. Patterson, K. Pfeffer, S. A. Nedospasov, et al. 2008. Lymphotoxin-mediated crosstalk between B cells and splenic stroma promotes the initial type I interferon response to cytomegalovirus. *Cell Host Microbe* 3: 67–76.
14. Brigl, M., L. Bry, S. C. Kent, J. E. Gumperz, and M. B. Brenner. 2003. Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. *Nat. Immunol.* 4: 1230–1237.
15. Suzuki, Y., D. Wakita, K. Chamoto, Y. Narita, T. Tsuji, T. Takeshima, H. Gyobu, Y. Kawarada, S. Kondo, S. Akira, et al. 2004. Liposome-encapsulated CpG oligodeoxynucleotides as a potent adjuvant for inducing type 1 innate immunity. *Cancer Res.* 64: 8754–8760.
16. Montoya, C. J., H. B. Jie, L. Al-Harthi, C. Mulder, P. J. Patino, M. T. Rugeles, A. M. Krieg, A. L. Landay, and S. B. Wilson. 2006. Activation of plasmacytoid dendritic cells with TLR9 agonists initiates invariant NKT cell-mediated cross-talk with myeloid dendritic cells. *J. Immunol.* 177: 1028–1039.
17. Raftery, M. J., F. Winau, T. Giese, S. H. Kaufmann, U. E. Schaible, and G. Schonrich. 2008. Viral danger signals control CD1d de novo synthesis and NKT cell activation. *Eur. J. Immunol.* 38: 668–679.
18. Paget, C., T. Mallevaey, A. O. Speak, D. Torres, J. Fontaine, K. C. Sheehan, M. Capron, B. Ryffel, C. Faveeuw, M. Leite de Moraes, et al. 2007. Activation of invariant NKT cells by toll-like receptor 9-stimulated dendritic cells requires type I interferon and charged glycosphingolipids. *Immunity* 27: 597–609.
19. Shresta, S., K. L. Sharar, D. M. Prigozhin, P. R. Beatty, and E. Harris. 2006. Murine model for dengue virus-induced lethal disease with increased vascular permeability. *J. Virol.* 80: 10208–10217.
20. Andrews, D. M., A. A. Scalzo, W. M. Yokoyama, M. J. Smyth, and M. A. Degli-Esposti. 2003. Functional interactions between dendritic cells and NK cells during viral infection. *Nat. Immunol.* 4: 175–181.
21. Velazquez, P., T. O. Cameron, Y. Kinjo, N. Nagarajan, M. Kronenberg, and M. L. Dustin. 2008. Cutting edge: activation by innate cytokines or microbial antigens can cause arrest of natural killer T cell patrolling of liver sinusoids. *J. Immunol.* 180: 2024–2028.
22. Krug, A., A. R. French, W. Barchet, J. A. Fischer, A. Dzionek, J. T. Pingel, M. M. Orihuela, S. Akira, W. M. Yokoyama, and M. Colonna. 2004. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity* 21: 107–119.

Downloaded from www.jimmunol.org on August 24, 2011