Invariant Vα14⁺ NKT Cells Participate in the Early Response to Enteric *Listeria monocytogenes* Infection

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Invariant Vα14+ NKT Cells Participate in the Early Response to Enteric Listeria monocytogenes Infection

Thomas Ranson,2§ Søren Bregenholt,2,3* Agnes Lehuen,† Olivier Gaillot,‡ Maria C. Leite-de-Moraes,§ André Herbelin,§ Patrick Berche,§ and James P. Di Santo4*§

Invariant Vα14+ NKT cells are a specialized CD1-reactive T cell subset implicated in innate and adaptive immunity. We assessed whether Vα14+ NKT cells participated in the immune response against enteric Listeria monocytogenes in vivo. Using CD1d tetramers loaded with the synthetic lipid α-galactosylceramide (CD1d/αGC), we found that splenic and hepatic Vα14+ NKT cells in C57BL/6 mice were early producers of IFN-γ (but not IL-4) after L. monocytogenes infection. Adoptive transfer of Vα14+ NKT cells derived from TCRα° Vα14-Jα18 transgenic (TCRα°Vα14Tg) mice into lymphoid Rag°γ° mice demonstrated that Vα14+ NKT cells were capable of providing early protection against enteric L. monocytogenes infection with systemic production of IFN-γ and reduction of the bacterial burden in the liver and spleen. Rechallenge experiments demonstrated that previously immunized wild-type and Jα18 mice, but not TCRα° or TCRα°Vα14Tg mice, were able to mount adaptive responses to L. monocytogenes. These data demonstrate that Vα14+ NKT cells are able to participate in the early response against enteric L. monocytogenes through amplification of IFN-γ production, but are not essential for, nor capable of, mediating memory responses required to sterilize the host. The Journal of Immunology, 2005, 175: 1137–1144.

The primary control of infection by the intracellular pathogen Listeria monocytogenes relies on the ability of the host to mount an efficient Th1-like immune response (reviewed in Ref. 1). Production of IFN-γ in the early phases of infection is essential to enhance IL-12 production and activate bactericidal mechanisms in macrophages (2, 3). NK cells have been identified as a source of early IFN-γ production (4). Thus, SCID mice (T−, B−, NK+) are able to control primary L. monocytogenes infection in an IFN-γ-dependent manner. Eventually SCID mice succumb to chronic listeriosis, demonstrating that NK cells alone are unable to fully protect the host against L. monocytogenes (5, 6). Instead, sterilizing immunity relies on the generation of cytotoxic CD8+ T cells which clear infected macrophages and hepatocytes and thereby eliminate the bacteria (reviewed in Refs. 1 and 7). The participation of other cell types has been described in the protection against L. monocytogenes. Several studies have defined a role for CD4+ T cells in both primary and secondary L. monocytogenes infection (8, 9). In addition, γδ T cells play a role in the defense against L. monocytogenes, since they are able to control primary infections in the absence of αβ TCR cells. However, γδ TCR cells are not able to mediate sterilizing immunity after infection (10).

NKT cells constitute a heterogeneous subset of T cells expressing both NK and T cell surface markers. One well-characterized NKT subset includes a thymus-derived population expressing a canonical Vα14-Jα18 TCR α-chain associated with a limited set of TCRβ subfamilies (reviewed in Ref. 11). These invariant Vα14+ NKT cells, which are either CD4+ or CD4−CD8− double negative, are selected on the nonclassical MHC class I molecule CD1d. Vα14+ NKT cells recognize an endogenous lysosomal glycosphingolipid, isoglobotrihexosylceramide (12), and when activated through their TCR or by soluble factors (such as IL-12) can produce both IFN-γ and IL-4 (13, 14). Moreover, Vα14+ NKT cells have been shown to transactivate B, T, and NK cells in vivo (15, 16). Along these lines, Vα14+ NKT cells may act as sentinels to integrate initial signals following immune stimulation and thereby serve to orient subsequent immune responses. Vα14+ NKT cells have been implicated in a number of immune-mediated pathologies including graft-vs-host disease, autoimmune hepatitis, and in fetal loss (17–19). In addition, a disease-controlling role for NKT cells has been shown in Vα14-Jα18 transgenic (Tg)5 nonobese diabetic mice (20). Vα14+ NKT cells may participate in antitumor responses by counteracting invasion and metastasis (reviewed in Ref. 21). Finally, a role for Vα14+ NKT cells has been proposed for protection against parasites (Toxoplasma gondii, Plasmodium yoelli, and Plasmodium berghei) and intracellular pathogens (mycobacteria and L. monocytogenes) (reviewed in Ref. 22). Vα14+ NKT cells could provide a protective role via IFN-γ in sustaining Th1 responses (23). Alternatively, IL-4 production from Vα14+ NKT cells could either have a deleterious role by deviating Th2 responses toward Th2 or act as an amplifier of Th2 responses in the context of extracellular parasites (24, 25). The precise role of Vα14+ NKT cells in infection immunity is clearly not defined and could vary depending on the pathogen.

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Concerning *L. monocytogenes*, previous studies have demonstrated that NKT-deficient mice can resist infection by *L. monocytogenes* similar to wild-type mice (8, 26), excluding an essential role for these cells in antilisterial immunity. In contrast, Kaufmann and coworkers found that NKT cells are selectively depleted from the liver of *L. monocytogenes*-infected mice and that treatment of infected mice with CD1-specific Abs ameliorated the antilisterial response via increased IFN-γ, TNF-α, and IL-12 production (27, 28). This group proposed that NKT cells could play a negative role in the immunity against intracellular bacteria, possibly through production of TGF-β (28). Considering these contradictory findings, we decided to re-examine the role for Vα14⁺ NKT cells in the antilisterial response. Using several approaches in wild-type, Jα18⁺, and Vα14 transgenic mice, we demonstrate that invariant Vα14⁺ NKT cells clearly contribute to the pro-Th1 response following infection with *L. monocytogenes* but are not essential for or capable of mediating memory responses to this pathogen.

**Materials and Methods**

**Mice**

Rag²⁻ and Rag²⁻γ⁻ mice (29) were from the 10th backcross to the C57BL/6 background. TCRα⁺ mice and Vα14-Jo18Tg on the TCRα-deficient C57BL/6 background (TCRα⁺Vα14Tg) mice (20) as well as Jo18⁺ mice (30) have been previously described. C57BL/6 mice were purchased from the Institut Pasteur (Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 668) and at Necker Hospital (INSERM Unité 411). All animal studies were evaluated and approved by a local institutional review board.

**Abs and reagents**

Abs were obtained from BD Pharmingen and were used as FITC, PE, biotin, and allophycocyanin conjugates. Biotinylated Abs were revealed with FITC-, PE (Caltag Laboratories) or PerCP-conjugated streptavidin (BD Pharmingen). Anti-CD19 microbeads and LS⁺ magnetic separation columns were obtained from Miltenyi Biotec. RPMI 1640, FCS, and antibiotics were purchased from Invitrogen Life Technologies. Percoll was purchased from Pharmacia. Brain-heart infusion medium (BHI) was obtained from Acumedia.

**Preparation of bacterial strains**

*Listeria monocytogenes* (strain LO28) (31), was grown to exponential phase in BHI medium and harvested in the exponential growth phase, washed, and stored at −80°C in aliquots of 10⁶ bacteria/ml in PBS.

**Isolation of lymphoid cells**

For isolation of lymphoid cells from peripheral lymphoid organs, mice were sacrificed and the mesenteric lymph node (mLN), spleen, and liver were removed. Single-cell suspensions were generated from mLN and spleen by teasing the organs through a metal mesh followed by erythrocyte lysis. Single-cell suspensions were generated from liver by teasing the organs through a metal mesh followed by centrifugation on a Percoll gradient (40/80%) and erythrocyte lysis.

**Cell sorting and adoptive transfer into Rag⁻γ⁻ mice**

For electronic cell sorting, single-cell suspensions were generated from the mLN of TCRα⁺Vα14Tg mice. Following erythrocyte lysis, lymph nodes cells were depleted of B cells using MACS anti-CD19 microbeads and LS⁺ columns according to the manufacturer's instructions. Subsequently, the cells were incubated with biotinylated anti-CD5 mAb, PE anti-CD69 mAb and allophycocyanin anti-NK1.1 mAb as described below. Biotinylated Ab was revealed by incubation with FITC-streptavidin. NK1.1⁺ cells were sorted from NK1.1⁺ CD5⁻CD69⁻ cells using a MoFlo cell sorter (DakoCytomation). Post-sort analysis confirmed that these cells were >98% NK1.1⁺ and contained <0.4% contaminating NK cells. Nonirradiated Rag²⁻γ⁻ mice (3–6 wk of age) were transplanted i.v. with 5 × 10⁶ purified NK1.1⁺ T cells 4 days before infection.

**Infection and determination of CFU**

For intragastric (i.g.) infection with 5 × 10⁸ *L. monocytogenes* strain LO28, groups of mice were gavaged i.g. using an 18-gauge dumb-end feeding needle. For rechallenge experiments, mice were injected i.v. in the lateral tail vein with 2 × 10⁹ bacteria.

At the indicated time points after infection, mice were sacrificed and the livers and spleens were aseptically removed. Homogenates of liver and spleen were prepared by grinding organs in sterile PBS with a motorized Teflon pestle. Bacterial CFU were enumerated by plating organ homogenates in 10-fold, serial dilutions on BHI agar plates. After incubation at 35°C for 36–48 h, the bacterial colonies were counted.

**Flow cytometry**

For surface Ab staining, cells were washed twice in PBS supplemented with 1% BSA (PBS-BSA), incubated on ice for 30 min with Abs, and subsequently washed twice in PBS-BSA before analysis. When appropriate, cells were incubated with biotin-conjugated Abs, washed three times, and then incubated for 30 min with the relevant streptavidin conjugate and then washed three times before analysis. Samples were analyzed using a FACSCalibur flow cytometer (BD Biosciences) and the data were analyzed using CellQuest software (BD Biosciences).

For intracellular cytokine detection, total cell suspensions were incubated for 1 h in RPMI 1640/5% FCS containing brefeldin A (10 µg/ml) to block cytokine secretion. Surface-stained cells (TCRβ⁺, tetramer⁺) were fixed for 1 h in PBS containing 2% paraformaldehyde, and intracellular cytokines were detected using a PE-conjugated IFN-γ (XMG1.2) or control rat IgG1 (R3-34) mAbs in PBS containing 0.5% saponin.

**Tetramer staining**

Single-cell suspensions were stained for 20 min on ice with α-galactosylceramide (α-GC)-loaded allophyococyanin-conjugated CD1d tetramers (derived from mCD1dβ2m, microglobulin expression vector as described in Ref. 32). Cells were then washed twice with ice-cold PBS-BSA and subsequent Ab surface staining with FITC anti-TCRβ mAb and PE anti-NK1.1 mAb was performed as described above. Nonspecific binding was controlled by staining using CD1d tetramers without α-GC (data not shown).

**ELISA**

Serum was obtained (days 0 and 3 postinfection) and the concentrations of IFN-γ were determined using a specific sandwich ELISA kit (Genzyme) according to the manufacturer’s instructions.

**Statistics**

Statistical significance was evaluated using the Mann-Whitney U test. Values of p < 0.05 were considered to be significant.

**Results**

Vα14⁺ NKT cells participate directly in the antilisterial response in vivo

We used several independent and complementary approaches to assess the role of Vα14⁺ NKT cells in antilisterial immunity. We first used CD1d tetramers loaded with the synthetic lipid α-GC to follow Vα14⁺ NKT cell activation and cytokine production after i.g. *L. monocytogenes* infection of wild-type mice. Uninfected C57BL/6 mice harbored a population of CD1d/α-GC-reactive T cells which, on a percentage basis, were more abundant in the liver (6 ± 1.2%) than in the spleen (0.5 ± 0.1%; Fig. 1A and data not shown). These cells were mainly NK1.1⁻ and did not constitutively synthesize IFN-γ (Fig. 1, B and C). As early as 24 h after i.g. infection with *L. monocytogenes*, invariant Vα14⁺ NKT cells became activated and began to produce IFN-γ (Fig. 1B), but not IL-4 (data not shown). It should be emphasized that the protocol used for ex vivo analysis of cytokine production by Vα14⁺ NKT cells did not involve a TCR restimulation in vitro. By day 2 after *L. monocytogenes* infection, about one-half of the CD1d/α-GC-reactive T cells in the liver and spleen were active in IFN-γ production, and this fraction persisted at day 3 after infection (Fig. 1B and data not shown). Interestingly, the percentage of CD1d/α-GC-reactive T cells decreased by days 2 and 3 after infection, which was correlated with a decreased density of NK1.1 expression (Fig. 1, A and C), although CD1d tetramer staining was still clearly observed. This “loss” of Vα14⁺ NKT cells likely corresponds to a partial down-modulation of TCR and NK1.1 expression rather than an actual disappearance of the cells. These results
NKT cells participate in vivo in the early response to *L. monocytogenes*. Naïve C57BL/6 mice were infected i.g. with *5 × 10^8* *L. monocytogenes* strain LO28 and invariant Vα14+ NKT cells were analyzed using CD1d tetramers loaded with α-GC. A, Regions define the mean and SD of percentages of tetramer+ T cells in the liver of mice at indicated days after infection. B, Corresponding IFN-γ production by hepatic CD1d-reactive T cells in C57BL/6 mice after *L. monocytogenes* infection. Bars indicate percentages of IFN-γ-positive cells compared with staining with isotype control Abs. C, NK1.1 vs IFN-γ expression on tetramer+ T cells in the liver of control and infected mice.

Clearly demonstrate the participation of NKT cells in response to *L. monocytogenes* via IFN-γ production, a cytokine required for the control of this pathogen.

Vα14+ NKT cells transactivate NK cells for IFN-γ production after *Listeria* infection in vivo

Since Vα14+ NKT cells have been demonstrated to transactivate γδ T cells, NK cells, and CD8 αβ T cells after TCR stimulation (15, 16), we asked whether this transactivation also occurred after infection by *L. monocytogenes*. We therefore analyzed the kinetics of NK cell IFN-γ production in *L. monocytogenes*-infected C57BL/6 mice compared with Vα14+ NKT cell-deficient Jα18° mice (Fig. 2 and Table I). NK cells in the liver and spleen of uninfected wild-type mice did not constitutively produce IFN-γ, but became IFN-γ+ by day 1 after infection and continued to synthesize this cytokine throughout the time period analyzed (Table I). The peak of IFN-γ production by NK cells was day 2 after *L. monocytogenes* infection and paralleled the kinetics of the response of the invariant Vα14+ NKT cells (Fig. 1). In contrast, the kinetics of IFN-γ production by NK cells in Jα18° mice was clearly different. Production of IFN-γ by NK cells in Jα18° mice was significantly delayed in comparison to wild-type mice (no evidence for production at day 1 and peak production at day 3) and overall percentages of IFN-γ+ NK cells were reduced (Table I). These results suggest that Vα14+ NKT cells may be involved in amplifying the IFN-γ production capacity of NK cells after *L. monocytogenes* infection.

Antilisterial responses in Vα14 NKT cell-deficient mice

We next asked whether Vα14+ NKT cells were essential for immunity against *L. monocytogenes*. Previous studies have attempted to address this question using mice deficient in CD1 (26); however, since CD1+ mice are also unable to select non-Vα14 CD1d-reactive T cells (reviewed in Ref. 11), the unique roles for Vα14+ NKT cells were not unambiguously defined. We therefore infected Jα18° mice which have a selective deficiency in Vα14+ NKT cells (30). Both wild-type and Jα18° mice were able to control the initial infection (Fig. 3A), whereas alymphoid Rag° mice were highly susceptible as previously described (33). In recall experiments, previously immunized wild-type and Jα18° mice were protected against lethal challenge (10^6 bacteria i.v.), whereas naïve wild-type mice succumbed rapidly to infection (Fig. 3B). These results demonstrate that Vα14+ NKT cells are not essential for innate and adaptive responses to *L. monocytogenes*, despite their capacity to respond to this pathogen.

Antilisterial responses in Vα14 NKT cell Tg mice

We used mice harboring a productively rearranged TCR Vα14-Jα18 transgene on the TCRα-deficient background (TCRα Vα14Tg mice; Ref. 20) to assess whether increasing the

C57BL/6  Jα18°

Day 0

Day 3

< 1%

< 1%

13.5%

< 1%
frequency of \( \kappa 14^+ \) NKT cells would alter the antilisterial response in vivo. Lymphoid organs from these mice are enriched in invariant or “type I” \( \kappa 14^+ \) NKT cells, which can be detected using CD1d/α-GC-loaded tetramers (3, 4). TCR\(^{α} \kappa 14^+ \)Tg mice harbor increased percentages and absolute numbers of CD1d-reactive T cells in the liver, spleen, and lymph nodes (Fig. 4 and data not shown) compared with wild-type mice or TCR\(^{α} \) littermates as previously reported (20, 34). The CD1d-reactive T cells were comprised of a major population of NK1.1\(^+ \)CD5\(^+ \) T cells previously reported (20, 34). The CD1d-reactive T cells were composed of a major population of NK1.1\(^+ \)CD5\(^+ \) T cells previously reported (20, 34). The CD1d-reactive T cells were comprised of a major population of NK1.1\(^+ \)CD5\(^+ \) T cells previously reported (20, 34). The CD1d-reactive T cells were comprised of a major population of NK1.1\(^+ \)CD5\(^+ \) T cells previously reported (20, 34). The CD1d-reactive T cells were comprised of a major population of NK1.1\(^+ \)CD5\(^+ \) T cells previously reported (20, 34).

Nevertheless, in TCR\(^{α} \kappa 14^+ \)Tg animals, \( \kappa 8^+ \) Rag\(^{γc}\) mice harbor increased percentages and absolute numbers of CD1d-reactive T cells (Fig. 4 and data not shown), the latter of which could represent immature NKT cells. These V\(^{α} \kappa 14^+ \)NKT cells provide early protection against enteric L. monocytogenes infection. A highly purified population of CD1d-reactive V\(^{α} \kappa 14^+ \) NKT cells (> 98% CD5\(^+ \), NK1.1\(^+ \); Fig. 6A) was isolated from TCR\(^{α} \kappa 14^+ \)Tg mice and injected i.v. into nonirradiated Rag\(^{γc}\) mice. After 4 days (during which the transferred V\(^{α} \kappa 14^+ \) NKT cells underwent hemostatic expansion; Ref. 36), these V\(^{α} \kappa 14^+ \)NKT-reconstituted mice were infected orally with L. monocytogenes (Fig. 6A). Bacterial burdens were assessed 4 days later. Transfer of \( 5 \times 10^5 \) purified NKT cells was able to provide almost 2 logs of protection against L. monocytogenes in the liver and spleen of Rag\(^{γc}\) mice (Fig. 6B).

The reduced bacterial burden in V\(^{α} \kappa 14^+ \)NKT-reconstituted mice was correlated with enhanced survival after L. monocytogenes infection. Unmanipulated Rag\(^{γc}\) mice succumbed to L. monocytogenes dissemination by ~10 days, whereas adoptive transfer of \( 5 \times 10^5 \) purified V\(^{α} \kappa 14^+ \) NKT cells protected these mice for >20 days (Fig. 6C). This early protection against L. monocytogenes was associated with an increase in serum IFN-\( γ \) levels at day 3 postinfection (NKT-reconstituted Rag\(^{γc}\) mice: \( 190 \pm 132 \) pg/ml vs Rag\(^{γc}\) mice: < 25 pg/ml), and intracellular staining demonstrated that CD1d-reactive V\(^{α} \kappa 14^+ \) NKT cells were producing IFN-\( γ \) after L. monocytogenes infection (Fig. 6D).

Table I. IFN-\( γ \) production by NK cells following oral infection with L. monocytogenes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Organ</th>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>Liver</td>
<td>0 ± 0°</td>
<td>18 ± 9</td>
<td>14 ± 9</td>
<td>26 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0 ± 0°</td>
<td>31 ± 30</td>
<td>14 ± 5</td>
<td>14 ± 5</td>
<td></td>
</tr>
<tr>
<td>Jo18°</td>
<td>Liver</td>
<td>0 ± 0°</td>
<td>14 ± 3</td>
<td>14 ± 3</td>
<td>14 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0 ± 0°</td>
<td>34 ± 2.2</td>
<td>2.2 ± 0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Percentages of NK1.1\(^+ \)CD3\(^+ \) NK cells with intracellular IFN-\( γ \) staining above levels revealed using isotype control Abs. Data represent the mean of groups of four to six mice. SD values are indicated.

FIGURE 3. Role of invariant V\(^{α} \kappa 14^+ \) NKT cells in primary and recall responses to L. monocytogenes infection. A, Survival of control C57BL/6 (●), Jo18-deficient (Jo18°, ■), and alymphoid Rag\(^{γc}\) mice (○) to an i.g. infection of \( 5 \times 10^9 \) L. monocytogenes strain LO28. B, Mice were microbioid by i.g. infection as above and after 6 wk were rechallenged i.v. with a lethal dose (\( 2 \times 10^9 \)) of L. monocytogenes strain LO28. Survival of C57BL/6 (●), Rag\(^{γc}\) (■), and naive C57BL/6 mice (○) are shown. Experiments involved groups of four to six mice per genotype and were at least repeated twice.
TCRa° mice were challenged systemically with an elevated dose (2 × 10⁶ i.v.) of *L. monocytogenes* (Fig. 7). Resistance to this protocol of infection correlates with successful generation of adaptive immune responses (reviewed in Refs. 1 and 7). As expected, naive mice, irrespective of their genotype, rapidly succumbed to infection with bacterial dissemination in the liver, spleen, and brain (Fig. 6 and data not shown). In contrast, immunized wild-type mice were able to control the infection and survived the 15-day observation period (Figs. 3 and 7). Immunized TCRα° and TCRα°/Val14Tg mice, however, failed to control the infection (Fig. 7), demonstrating their inability to generate an adaptive immune response to *L. monocytogenes*.

**FIGURE 4.** Phenotype of CD1d-reactive T cells in C57BL/6, TCRα-deficient, and TCRα°/Val14Tg mice. Spleen, lymph node, and hepatic lymphocytes were isolated from control C57BL/6, TCRα-deficient (TCRα°), and TCRα°/Val14Tg mice and analyzed for TCRβ expression and reactivity with α-GC-loaded CD1d tetramers. Region indicates percentages of tetramer+ T cells. Liver tetramer+ T cells were further analyzed for NK1.1 and CD5 expression. Percentages of NK1.1° and NK1.1°/CD5° T cells are indicated. Representative results of six independent mice are presented.

**FIGURE 5.** TCRα°/Val14Tg and TCRα° mice are resistant to enteric *L. monocytogenes* infection. A, Wild-type, Rag°γ°, TCRα°/Val14Tg, and TCRα° mice were infected i.g. with 5 × 10⁸ *L. monocytogenes* strain LO28. CFU in the liver and spleen were determined 7 days postinfection. Data represent the mean from groups of six mice, and SD values are indicated. Similar results were obtained in a second experiment. Asterisk indicates significant difference from Rag°γ° mice, p < 0.005. B, Survival of wild-type, Rag°γ°, TCRα°/Val14Tg, and TCRα° mice after i.g. infection with 5 × 10⁸ *L. monocytogenes* strain LO28.

**Discussion**

Using a combination of approaches, including analysis with CD1d tetramers, Val14° NKT cell transgenic and knockout mice and selective reconstitution of lymphoid mice with highly purified Val14° NKT cells, we have reassessed the role of Val14° NKT cells in the immunity against enteric infection with the intracellular bacterium *L. monocytogenes*. Although previous reports suggested a negative impact of NKT cells on antilisterial immunity (27, 28), we found that Val14° NKT cells were stimulated to produce IFN-γ in vivo following enteric *L. monocytogenes* infection and were able to provide early protection of highly susceptible lymphoid mice against *L. monocytogenes*. In contrast, we demonstrated that Val14° NKT cells do not provide adaptive immunity to this pathogen under conditions of recall stimulation.

The capacity of α-GC-loaded CD1d tetramers to unambiguously identify invariant Val14° T cells provided an essential tool for our studies. Previous reports have demonstrated the specificity of this reagent in wild-type mice and in transgenic mice bearing a functionally rearranged Val14-Jα18 TCRα chain that develops increased numbers of Val14° NKT cells (32, 34). These TCRα°/Val14Tg mice provided us with the means to directly assess the functional capacity of Val14° NKT cells to provide early protection against *L. monocytogenes* infection. One caveat of our experiments is whether the NKT cells derived from TCRα°/Val14Tg mice faithfully represent their counterparts from wild-type mice. Previous studies have shown that CD1d-reactive NK1.1° T cells from TCRα°/Val14Tg mice have a TCRβ repertoire and cell surface phenotype that closely matches NK1.1° T cells from C57BL/6 mice (34). Moreover, NKT cells from TCRα°/Val14Tg mice, like their normal counterparts, have the capacity to rapidly produce cytokines (IL-4, IFN-γ) following in vitro stimulation (20, 34). Thus, by several distinct criteria, the Val14° NKT cells from TCRα°/Val14Tg mice appear to faithfully represent their normal C57BL/6 counterparts.

<table>
<thead>
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<th>Genotype</th>
<th>Day 0 (pg/ml)</th>
<th>Day 3 (pg/ml)</th>
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<td>&lt;25</td>
<td>&lt;25</td>
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<tr>
<td>TCRα°</td>
<td>&lt;25</td>
<td>126 ± 69</td>
</tr>
<tr>
<td>Val14Tg</td>
<td>&lt;25</td>
<td>450 ± 228</td>
</tr>
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</table>

a Data represent the mean of groups of four to six mice. SD values are indicated.
FIGURE 6. Invariant Vα14⁺ NKT cells mediate innate protection against enteric L. monocytogenes infection. A, Lymph node cells from control C57BL/6, TCRα-deficient (TCRα°), and TCRα°/Vα14Tg mice were analyzed for NK1.1 and CD5 expression. CD5⁺ NK1.1⁺ T cells were further analyzed for TCRαβ expression and reactivity with α-GC-loaded CD1d tetramers. Sorted Vα14⁺ NKT cells (98% pure) from C57BL/6, TCRα°/Vα14Tg, and TCRα° mice were adoptively transferred to Rag°/° mice. 3 days later, mice were infected i.p. with 5 × 10⁶ L. monocytogenes strain LO28 and rested 6 wk. Naive (○) or immunized mice (●) were then challenged with a lethal dose of 2 × 10⁶ L. monocytogenes strain LO28 i.v. and their survival was monitored for a period of 15 days.

CD1d-reactive Vα14⁺ T cells from both C57BL/6 and TCRα°/Vα14Tg mice harbor a subset of NK1.1⁺ cells. Previous studies from Benlagha et al. (14) have demonstrated that these cells in C57BL/6 mice likely represent precursors of the NK1.1⁺ cells. Using CD1d tetramers, these authors found that the NK1.1⁺ subset of Vα14⁺ T cells bore an immature phenotype and selectively produced IL-4, but not IFN-γ, after stimulation. The presence of NK1.1⁺ Vα14⁺ T cells in the spleen suggested that these precursors could exit the thymus and further differentiate into NK1.1⁺ IFN-γ secreting mature Vα14⁺ NKT cells in the periphery. Additional experiments showed that purified NK1.1⁺ CD1d-reactive T cells could give rise after adoptive transfer to NK1.1⁺ progeny. The presence of two phenotypically and functionally distinct Vα14⁺ T cell subsets in the periphery of mice could allow for flexibility in the ways that immune responses could be oriented.

The ability of TCRα° mice to control primary L. monocytogenes infection is consistent with the previously recognized capacity of TCRγδ and NK cells to participate in innate immunity against this pathogen (3, 6, 10). No difference in the bacterial burden or early survival was observed among wild-type, TCRα°, and TCRα°/Vα14Tg mice following enteric L. monocytogenes infection. This observation argues against any predominant regulatory role for Vα14⁺ NKT cells in the immunity against enteric L. monocytogenes, in contrast with previous studies (27, 28) that reported an amelioration of listeriosis in mice treated with anti-CD1 mAbs. These authors deduced that the blockade of CD1 interfered with the activation of NKT cells, resulting in decreased TGF-β levels and increased IFN-γ, TNF-α, and IL-12 production. Since TCRα°/Vα14Tg mice were as resistant as TCRα° mice to primary infection, our results are incompatible with a dominant negative activity of Vα14⁺ NKT cells during L. monocytogenes infection. Still, NKT cells could impact on L. monocytogenes infections under conditions when NK and/or γδ T cells are limiting.

i.g. infection with 5 × 10⁶ L. monocytogenes strain LO28. D, Synthesis of IFN-γ by CD1d-reactive T cells in NKT-reconstituted Rag°/° mice 3 days after L. monocytogenes infection. Bars in upper histograms indicate percentages of IFN-γ-positive cells as compared with staining with isotype control Abs.

FIGURE 7. Vα14⁺ NKT cells do not mediate sterilizing memory responses to L. monocytogenes. C57BL/6, TCRα°/Vα14Tg, and TCRα° mice were immunized by i.g. infection with 5 × 10⁶ L. monocytogenes strain LO28 and rested 6 wk. Naive (○) or immunized mice (●) were then challenged with a lethal dose of 2 × 10⁶ L. monocytogenes strain LO28 i.v. and their survival was monitored for a period of 15 days.
We used adoptive transfer of Vα14+ NKT cells from TCRα-Vα14Tg mice to assess the capacity of these cells to confer protection against L. monocytogenes when transplanted into allogenic Ragαγ− mice. We observed a beneficial effect of Vα14+ NKT cells in this setting, which correlated with IFN-γ (but not IL-4) production. It is interesting to consider our results in light of the observations that Vα14+ NKT cells can produce both IFN-γ and IL-4 following TCR stimulation in vitro. In contrast, Vα14+ NKT cells can preferentially produce either IL-4 or IFN-γ following stimulation with cytokines (37). The restricted biological activity of NKT cells after L. monocytogenes infection could indicate that these cells do not receive TCR stimulation via CD1d molecules reconstituted with NKT cells are able to resist early L. monocytogenes infection (T. Ranson and J. P. Di Santo, unpublished observations). These results would suggest that Vα14+ NKT cells are recruited to respond to certain types of intracellular infections dependent on the cytokine milieu; a pro-Th1 (IL-12)-rich environment would then favor Vα14+ NKT production of IFN-γ. Following L. monocytogenes infection, TCRα-Vα14Tg mice displayed systemic IFN-γ levels comparable to those of wild-type mice and 3- to 4-fold higher levels than found in TCRα−/− mice. Early IFN-γ production by Vα14+ NKT cells therefore represents a likely antilisterial mechanism in our experiments, although direct NKT cell-mediated killing of L. monocytogenes-infected macrophages cannot be ruled out (39).

In our transfer experiments, we found that NKT cells were able to substantially reduce the bacterial burden in the liver and spleen of the Ragαγ− hosts (by almost 2 logs) after enteric L. monocytogenes infection. The level of protection afforded by the injected NKT cells is even more impressive considering the limited number of NKT cells transferred and the fact that host-mediated expansion of these cells only results in the generation of ~105 NKT cells in the liver and spleen of the recipient hosts (36). In addition, the transplanted Vα14+ NKT cells might have undergone apoptosis following stimulation in vivo (40). Thus, despite being unable to completely eradicate the bacterial inoculum, NKT cells demonstrated potent antilisterial activity which resulted in protection of the reconstituted mice for at least 3 wk. Vα14+ NKT cells have been shown to “cross-talk” with other lymphocytes, including NK, B, and T cells (15, 16). In particular, it has been shown that NKT-NK cell interactions may play an important role in tumor surveillance in vivo (reviewed in Ref. 21). Our results using adoptive transfer showed that NKT cells alone provide early protection after L. monocytogenes infection. Still, functional synergy between NKT and NK cells may allow for an even better protection after infectious challenge. The use of CD1d/α-GC tetramers allowed us to directly demonstrate that Vα14+ NKT cells in C57BL/6 mice respond after L. monocytogenes infection by production of IFN-γ. Comparisons of C57BL/6 and Vα18+ mice revealed a major difference in NK cell IFN-γ production after L. monocytogenes infection, consistent with Vα14+ NKT cell transactivation of NK cells in vivo. The fact that TCRα− and TCRα-Vα14Tg mice did not mount functional memory responses to L. monocytogenes is consistent with previous reports demonstrating a pivotal role for cytotoxic CD8+ αβ T cells in the generation of antilisterial memory responses (reviewed in Refs. 1 and 7). Our observations indicate that NKT cells do not play an essential role in recall responses to L. monocytogenes. Nevertheless, NKT cells could amplify memory responses via transactivation of previously established CD8 memory T cells. The capacity for NKT cells to rapidly produce IFN-γ and to potentiate its production by other lymphocytes (NK cells, γδ T cells, CD8 memory T cells) after L. monocytogenes infection provides an important physiological example of the important role of NKT cells as a bridge between innate and adaptive immunity.

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Disclosures

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References


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