Invariant Vα14+ NKT Cells Participate in the Early Response to Enteric Listeria monocytogenes Infection1

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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 infection 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-Ja18 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 Ja18 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.

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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 allowing mediation of memory responses to this pathogen.

Materials and Methods

**Mice**

Rag− and Rag−γ− 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- NKT mice (30) have been previously described. C57BL/6 mice were purchased from Iffa-Credo. Mice were housed at 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, 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-CD8 mAb and allophycocyanin anti-NK1.1 mAb as described below. Biotinylated Ab was revealed by incubation with FITC-streptavidin. NK1.1+ cells were sorted as NK1.1+CDα−CD5− cells using a MoFlo cell sorter (DakoCytomation). Post-sort analysis confirmed that these cells were >98% NKT cells and contained <0.4% contaminating NK cells. Nonirradiated Rag−γ− 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 × 106 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 aliphophycocyanin-conjugated CD1d tetramers (derived from mCD1dβ2, 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 tetramer-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 tetramer-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
and SD of percentages of tetramer Vγ14 expression is decreased in the absence of isotype control Abs. NK cells after L. monocytogenes infection (15, 16), we asked whether this transactivation also occurred after days after infection.

Indicated percentages of IFN-γ-reactive T cells in C57BL/6 mice after L. monocytogenes infection. Percentages of IFN-γ-lyzed at the indicated time after infection. Bars indicate percentages of IFN-γ-positive cells compared with staining with isotype control Abs are indicated.

FIGURE 1. NKT cells participate in vivo in the early response to L. monocytogenes. Naive C57BL/6 mice were infected i.g. with 5 × 10^6 L. monocytogenes strain LO28 and invariant Vγ14+ 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.


demonstrate 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°γ°α° mice were highly susceptible as previously described (33). In recall experiments, previously immunized wild-type and Jα18° mice were protected against lethal challenge (10^9 bacteria i.v.), whereas naive 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
frequency of $\alpha 14^+\gamma$ NK cells would alter the antilisterial response in vivo. Lymphoid organs from these mice are enriched in invariant or "type I" $\alpha 14^+\gamma$ NK cells, which can be detected using CD1d/αGalC-loaded tetramers (3, 4). TCR$\alpha\alpha$/$\gamma$ NK cells 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$\alpha\alpha$ littermates as previously reported (20, 34). The CD1d-reactive T cells were comprised of a major population of NK1.1$^+$, CD4$^+$, CD8$^+$, and CD5$^+$ T cells and a smaller fraction of NK1.1$^+$CD5$^+$ T cells (Fig. 4 and data not shown), the latter of which could represent immature NKT cells. These V$\alpha 14$ T cells were comprised of a major population of NK1.1$^+$ previously reported (20, 34). The CD1d-reactive T cells were common in the course of infection. Wild-type mice mounted a strong systemic response in vivo. Lymphoid organs from these mice are enriched in invariant or "type I" $\alpha 14^+\gamma$ NK cells, which can be detected using CD1d/αGalC-loaded tetramers (3, 4). TCR$\alpha\alpha$/$\gamma$ NK cells 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$\alpha\alpha$ littermates as previously reported (20, 34). The CD1d-reactive T cells were comprised of a major population of NK1.1$^+$, CD4$^+$, CD8$^+$, and CD5$^+$ T cells and a smaller fraction of NK1.1$^+$CD5$^+$ T cells (Fig. 4 and data not shown), the latter of which could represent immature NKT cells that have recently exited the thymus (14, 35). CD1d-reactive T cells from TCR$\alpha\alpha$V$\alpha 14$Tg mice expressed CD122, 2B4, and DX5 markers at levels similar to their wild-type counterparts (data not shown).

We orally infected wild-type, TCR$\alpha\alpha$V$\alpha 14$Tg, TCR$\alpha\alpha$, and alymphoid Rag$\gamma$N$\gamma$, mice and evaluated the bacterial burden in the liver and spleen 7 days later. Rag$\gamma$N$\gamma$ mice accumulated high bacterial levels in the liver and spleen (Fig. 5A) and succumbed to disseminated infection by day 10 (Fig. 5B). In contrast, wild-type mice efficiently controlled the enteric infection with bacterial clearance from the target organs (Fig. 4A) and survived at least 8 wk (Fig. 5B). Concerning TCR$\alpha\alpha$V$\alpha 14$Tg and TCR$\alpha\alpha$, both types of mice could control the early infection by L. monocytogenes (Fig. 5A) and survived this infection protocol (Fig. 5B). No obvious differences were noted in terms of efficiency of the response or in the kinetics of bacterial clearance (data not shown).

We analyzed the serum levels of IFN-γ before and during the course of infection. Wild-type mice mounted a strong systemic IFN-γ response upon infection with L. monocytogenes, whereas IFN-γ could not be detected in the serum from Rag$\gamma$N$\gamma$ mice (Table II). TCR$\alpha\alpha$ mice showed reduced IFN-γ levels as compared with wild-type mice, whereas TCR$\alpha\alpha$V$\alpha 14$Tg mice had systemic IFN-γ levels comparable to those observed in control mice (Table II). These data are consistent with the potential of V$\alpha 14$ NK cells to provide an early source of IFN-γ in response to enteric L. monocytogenes infection and their capacity to transactivate other cell types for enhanced IFN-γ production (Ref. 16 and Table I). Nevertheless, in TCR$\alpha\alpha$ mice, γδ T cells and/or NK cells appear sufficient to control early L. monocytogenes infection (5, 10) in the absence of V$\alpha 14$ NK cells.

Purified V$\alpha 14$ NK cells provide early protection against L. monocytogenes in vivo

To directly evaluate a role for V$\alpha 14$ NK cells in early protection against enteric L. monocytogenes infection, Rag$\gamma$N$\gamma$ mice were adoptively transferred with highly purified invariant V$\alpha 14$ NK cells. These V$\alpha 14$ NK-reconstituted mice offer the possibility to directly test effector functions of NKT cells, since Rag$\gamma$N$\gamma$ mice are devoid of all lymphocytes (29). A highly purified population of CD1d-reactive V$\alpha 14$ NK cells (> 98% CD5$^+$, NK1.1$^+$; Fig. 6A) was isolated from TCR$\alpha\alpha$V$\alpha 14$Tg mice and injected i.v. into nonirradiated Rag$\gamma$N$\gamma$ mice. After 4 days (during which the transferred V$\alpha 14$ NK cells underwent homeostatic expansion; Ref. 36), these V$\alpha 14$-NTK-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 alymphoid Rag$\gamma$N$\gamma$ mice (Fig. 6B).

The reduced bacterial burden in V$\alpha 14$-NTK-reconstituted mice was correlated with enhanced survival after L. monocytogenes infection. Unmanipulated Rag$\gamma$N$\gamma$ mice succumbed to L. monocytogenes dissemination by ~10 days, whereas adoptive transfer of $5 \times 10^5$ purified V$\alpha 14$ NK 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 (NTK-reconstituted Rag$\gamma$N$\gamma$ mice: 190 ± 132 pg/ml vs Rag$\gamma$N$\gamma$ mice: < 25 pg/ml), and intracellular staining demonstrated that CD1d-reactive V$\alpha 14$ NK cells were producing IFN-γ after L. monocytogenes infection (Fig. 6D). Under these conditions, we were unable to detect any IL-4 production from the transferred V$\alpha 14$ NK cells after exposure to L. monocytogenes (data not shown).

V$\alpha 14$ NK cells do not mediate adaptive immunity to L. monocytogenes

Having shown that V$\alpha 14$ NK cells can participate in innate immune responses, we next asked whether these cells could mediate adaptive immunity to L. monocytogenes. "Naïve" (uninfected) and "immunized" (orally infected 4 wk previously with $5 \times 10^5$ L. monocytogenes) wild-type, TCR$\alpha\alpha$V$\alpha 14$Tg, and

### Table 1. IFN-γ production by NK cells following oral infection with L. monocytogenes

| Genotype | Organ | Day | 0 | 1 | 2 | 3 |
|----------|-------|-----|---|---|---|---|
| C57BL/6  | Liver | 0 ± 0 | 10 ± 9 | 40 ± 19 | 26 ± 9 |
|          | Spleen| 0 ± 0 | 31 ± 30 | 30 ± 5 | 14 ± 5 |
| Jα18+    | Liver | 0 ± 0 | 0.6 ± 0.1 | 1 ± 0.3 | 1.4 ± 0.2 |
|          | Spleen| 0 ± 0 | 0.3 ± 0.1 | 3.4 ± 2.2 | 2.2 ± 0.5 |

*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$\alpha 14$ NK cells in primary and recall responses to L. monocytogenes infection. A. Survival of control C57BL/6 (●), Jα18-deficient (Jα18+, □), and alymphoid Rag$\gamma$N$\gamma$ mice (○) to an i.g. infection of $5 \times 10^8$ L. monocytogenes strain LO28. B. Mice were infected i.g. as above and after 6 wk were rechallenged i.v. with a lethal dose ($2 \times 10^7$) of L. monocytogenes strain LO28. Survival of immunized C57BL/6 (●), immunized Jα18-deficient (Jα18+, □), and naive C57BL/6 mice (○) is 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 × 10^6 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.

Discussion

Using a combination of approaches, including analysis with CD1d tetramers, Va14° NKT cell transgenic and knockout mice and selective reconstitution of alymphoid mice with highly purified Va14° NKT cells, we have reassessed the role of Va14° 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 Va14° 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 al lymphoid mice against L. monocytogenes. In contrast, we demonstrated that Va14° 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 Va14° 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 Va14-Jα18 TCRα chain that develops increased numbers of Va14° NKT cells (32, 34). These TCRα°Val14Tg mice provided us with the means to directly assess the functional capacity of Va14° NKT cells to provide early protection after 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 Va14° NKT cells from TCRα°Val14Tg mice appear to faithfully represent their normal C57BL/6 counterparts.

Table II. Serum IFN-γ levels following oral infection with L. monocytogenes

| Genotype   | Day 0 (pg/ml) | Day 3 (pg/ml) |
|------------|---------------|---------------|
| C57BL/6   | <25           | 550 ± 200     |
| Rag°γ°    | <25           | <25           |
| TCRα°     | <25           | 126 ± 69      |
| Va14Tg    | <25           | 450 ± 228     |

Data represent the mean of groups of four to six mice. SD values are indicated.

![FIGURE 4](image-url)  
**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 TCRα 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](image-url)  
**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 × 10^8 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 × 10^8 L. monocytogenes strain LO28.
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 × 10^6 L. monocytogenes strain LO28. D. Synthesis of IFN-γ by CD1d-reactive T cells in NKT-reconstituted Ragα- 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.
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 allogeneic Ragα-/- 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 Ragα-/- hosts (by almost 2 logs) after enteric L. monocytogenes infection. The level of protection afforded by the injected NKT cells from Vα14-/- mice was not different from the Ragα-/- mice (which lack expression of CD1 molecules) reconstituted with NKT cells. Nevertheless, NKT cells might have undergone apoptosis following stimulation in vivo (40). Thus, despite being unable to provide protection in the Ragα-/- mice, the adoptively transferred NKT cells were able to substantially reduce the bacterial burden in the liver and spleen of the recipient hosts (36). In addition, the transferred 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 are not completely T cells reciprocally regulate T-cell-dependent pathway of macrophage activation, defined in the scid mouse. Immunol. Rev. 124: 5–24.

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