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Original article

Reversible NK1.1 surface expression on invariant liver natural killer T cells during *Listeria monocytogenes* infection

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Abstract

The invariant (i) natural killer (NK)T cells consistently express the Vα14 chain of the T cell receptor (TCR) and recognize α-galactosylceramide (α-GalCer) presented by the nonpolymorphic presentation molecule CD1d. Despite their name, the iNKT cells represent a heterogeneous population, which can be divided on the basis of NK1.1 surface expression. Here we show that NK1.1 surface expression on liver iNKT cells in mice fluctuates during *Listeria monocytogenes* infection. At early stages of listeriosis, iNKT cells expressing NK1.1 were numerically reduced and those lacking NK1.1 were increased. At later time points, the NK1.1$^-$ iNKT cell population contracted, whereas NK1.1$^+$ iNKT cells re-emerged. Alterations in NK1.1 surface expression on iNKT cells were paralleled by numerical changes of interleukin (IL)-12 producers in the liver and were completely prevented by endogenous IL-12 neutralization, whereas NK1.1 surface alterations on iNKT cells following α-GalCer stimulation were not prevented. Adoptive cell transfer experiments revealed that the liver NK1.1$^-$ iNKT cells from NK1.1$^+$ cell-depleted *L. monocytogenes*-infected mice accumulated in the liver of recipient recombination-activating gene-1-deficient mice where they acquired NK1.1 surface expression. Thus, we present first evidence that NK1.1 surface expression on liver iNKT cells is reversible during *L. monocytogenes* infection, and that different mechanisms underlie stimulation by TCR and IL-12.

Keywords: *Listeria monocytogenes*; NK1.1; NKT cell; IL-12

1. Introduction

Listeria monocytogenes is a facultative intracellular bacterium that resides in Kupffer cells as well as in hepatocytes in the liver [1]. Effective protection depends on the ability of the host to induce type 1 immune responses [1]. Interleukin (IL)-12 produced by macrophages, dendritic cells as well as granulocytes, and interferon (IFN)-γ secreted by natural killer (NK)1.1$^+$ cells and T helper 1 lymphocytes of CD4 and CD8 phenotype are key cytokines that stimulate type 1 immune effector functions and hence, are crucial for defense against *L. monocytogenes* infection [1–5]. Because the vast majority (>90%) of *L. monocytogenes* microorganisms are trapped in the liver immediately after systemic infection [1], immunocompetent cells, which reside in the liver, play a crucial role in local defense [1]. Although conventional T cells are mandatory for sterile eradication of this pathogen [1], granulocytes infiltrating the liver play a pivotal role as a first line of defense before conventional T cells become activated [1]. In addition to these inflammatory cells, NK1.1$^+$ cells seem to participate in early protection against *L. monocytogenes* infection through IFN-γ secretion [1,2].

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NKT cells are a unique T cell population expressing NKR-P1B and C (NK1.1) of the type II C-type lectin super family [6]. In the mouse, the majority of NKT cells express an invariant (i) T cell receptor (TCR) α chain encoded by Vα14/Jα18 gene segments and a highly skewed TCRVβ towards Vβ8.2, 7, and 2 [6]. Development of these iNKT cells depends on the nonpolymorphic antigen-presenting molecule CD1d, which is surface expressed in conjunction with β2-microglobulin (β2m) [6]. The iNKT cells recognize α-galactosylceramide (α-GalCer), a synthetic glycolipid, originally isolated from a marine sponge in a CD1d-dependent manner [7]. The iNKT cells are abundant in the liver [8] and they promptly secrete both IFN-γ and IL-4 after stimulation [3,8–10].

The iNKT cells become undetectable immediately after activation [3,9–23]. Although it was proposed that the disappearance of iNKT cells was caused by Fas-mediated activation-induced cell death or apoptosis [15,19,22,23], more recent studies suggest that iNKT cells proliferate in situ robustly rather than undergoing apoptosis in some, but not all, cases [3,13,18,21]. The failure of iNKT cell detection is due to the loss of NK1.1 and/or TCR, which was previously considered as a reliable marker for detection of iNKT cells [3,13,18,21]. Although the loss of surface expression of NK1.1 and/or TCR and subsequent reexpression of the marker(s) have been observed in iNKT cells after in vivo treatment with their agonist, α-GalCer [13,18,21], it remains elusive whether this holds true for natural situations, e.g. microbial infections.

In the present study, we analyzed the NK1.1 surface expression on iNKT cells in the liver of mice during the course of listeriosis using α-GalCer/CD1d tetramers. We found that numbers of α-GalCer/CD1d tetramer-reactive (α-GalCer/CD1d$^+$) T cells lacking the NK1.1 marker increased immediately after *L. monocytogenes* infection with the capacity to acquire NK1.1 surface expression. Our data demonstrate that disappearance of iNKT cells during bacterial infection is, at least in part, caused by the loss of surface expression of NK1.1. Moreover, our data suggest that the NK1.1 marker can be reexpressed on iNKT cells after its loss in response to bacterial infection and that different mechanisms underlie stimulation via TCR and IL-12 signaling.

2. Materials and methods

2.1. Mice

Breeding pairs of recombination-activating gene (RAG)-1$^{-/-}$ mice were purchased from the Jackson Laboratories (Bar Harbor, ME). Mutant mice backcrossed onto C57BL/6 (>15th generation) and C57BL/6 mice were maintained under specific pathogen-free conditions, and female mice were used at 8–10 weeks of age.

2.2. Antibodies

MAbs against TCRα/β (H57-597), Fcγ receptor (R) (2.4G2), IL-12 (p40/p70) (C17.8), IL-12 (p40)(C15.6.7), and NK1.1 (PK136) were purified from hybridoma culture supernatants by ammonium sulfate precipitation and affinity chromatography on Protein A- or G-Sepharose (Amersham Biosciences, Freiburg, Germany). MAb against IL-12 (p40) (C15.6.7) was biotinylated, and mAb against TCRα/β was conjugated with fluorescein isothiocyanate (FITC) by standard methods. FITC-conjugated mAbs against CD54 (H9.2B8), CD25 (7D4), CD69 (H1.2F3), NK1.1 (PK136) and active caspase-3 (C92-605), phycoerythrin (PE)-conjugated mAb against TCRα/β, and biotinylated mAb against NK1.1 (PK136) were purchased from BD PharMingen (Hamburg, Germany).

2.3. Bacteria and infection

L. monocytogenes (strain EGD) organisms recovered from infected liver were grown in tryptic soy broth (Difco Laboratories, Detroit, MI) at 37 °C for 18 h and aliquots were frozen at −80 °C until used. The final concentration of viable bacteria was enumerated by plate counts on tryptic soy agar (Difco). Mice were infected intravenously (i.v.) with 2×10^3 *L. monocytogenes* organisms. Heat-killed *L. monocytogenes* (HKL) were prepared by heating bacteria in a water bath at 80 ° C for 3 h. HKL were washed three times with phosphate-buffered saline (PBS) and frozen at −20 ° C until used.

2.4. Mouse α-GalCer/CD1d tetramers

Mouse α-GalCer/CD1d tetramers were prepared using the baculovirus expression system as described previously [3,20].

2.5. Cell preparation and cell surface phenotype analysis

Mice were killed by cervical dislocation and organs were collected. Hepatic leukocytes (HLs) were prepared as described previously [8]. For staining with mAb, cells were incubated with anti-FcγR mAb and then stained with conjugated mAbs at 4 ° C for 15 min. Biotinylated mAbs were visualized by streptavidin (SA)-conjugated Cy5 (BD PharMingen). Stained cells were washed with PBS containing 0.1% bovine serum albumin (Serva, Heidelberg, Germany) and 0.1% sodium azide (Merck, Darmstadt, Germany), fixed with 1% paraformaldehyde (Merck), acquired by FACScan® or FACSCalibur® (BD Biosciences, Mountain View, CA), and the lymphoid cells were analyzed with CellQuest software (BD Biosciences). For staining with α-GalCer/CD1d tetramers, cells were stained with PE-labeled α-GalCer/CD1d tetramers for 15 min at room temperature after blocking.

2.6. Enzyme-linked immunospot assay

Frequencies of IL-12-producing cells were measured by the enzyme-linked immunospot (ELISPOT) method as described previously [3,17]. Briefly, cells were cultured for 18 h in the presence or absence of HKL (1×10^6) in ELISPOT plates (Millipore, Eschborn, Germany) coated with anti-IL-12 (p40/70) mAb (C17.8). After washing, plates were incubated with biotinylated anti-IL-12 (p40) mAb (C15.6.7). For developing spots, SA-conjugated alkaline phosphatase (Dianova,