Abstract  NKT cells are responsible for hepatitis induced either by concanavalin A (Con-A) or α-galactosylceramide (α-GalCer), and they are also profoundly involved in the generalised Shwartzman reaction (GSR) induced by consecutive injections of interleukin (IL)-12 and lipopolysaccharide (LPS). In the present study, using NC/Nga (NC) mice and SJL mice lacking the Vβ8 gene, we examined the role of Vβ8+NKT cells in hepatitis models and in the GSR. The absence of Vβ8+NKT cells in the liver mononuclear cells (MNC) was confirmed by the α-GalCer/CD1d/Ig dimer. Unexpectedly, other dimer+NKT cells including Vβ7+NKT cells in these mice were found to decrease in comparison to that of C57BL/6 mice. No significant hepatocyte injury was observed after α-GalCer or Con-A administration in either mice. The serum interferon (IFN)-γ, IL-4 and tumour necrosis factor (TNF) levels did not increase in these mice after α-GalCer injection, however these cytokines substantially increased after Con-A administration, thus suggesting that the roles of NKT cells differ between the two hepatitis models. However, in GSR, although neither mice showed lower IFN-γ levels after a priming IL-12 injection, they showed TNF levels comparable to those in normal mice after LPS injection, and thus resulted in a decreased but substantial mortality. Although liver MNC from IL-12-injected SJL mice showed an impaired antitumour cytotoxicity, liver MNC of NC mice exhibited a greater antitumour cytotoxicity than that of C57BL/6 mice because liver NK cells proportionally increased in NC mice. These results confirm the critical role that Vβ8+NKT cells play in both liver and multi-organ injury.

Key words  Natural killer T cells • α-Galactosylceramide • Vβ8+NKT cells • Concanavalin A • Shwartzman reaction

Introduction

Mouse NKT cells have both an NK cell marker (NK1.1) and T-cell receptor (TCR) and also functionally stand between NK cells and T cells. In contrast to conventional T cells, the TCR of NKT cells comprises an invariant Vα14Jα18 gene product combined with Vβ8, Vβ7 or Vβ2 chains [1, 2], which recognises the glycolipid Ags presented with CD1d [3]. Interleukin (IL)-12, which is a cytokine produced by macrophages and Kupffer cells, is important for the host defence against tumours as well as bacteria [4]. IL-12-activated liver NKT cells produce IFN-γ, acquire MHC-unrestricted NK cell-like cytotoxic activity and inhibit haematogenous tumour metastases in the liver, kidney and lung [3, 5, 6]. However, interferon (IFN)-γ produced by IL-12-stimulated NKT cells primes the mice and triggers multi-organ failure associated with disseminated intravascular coagulation after a challenge with lipopolysaccharide (LPS) of gram-negative bacteria [7], which is a lethal septic shock model and is called the generalised Shwartzman reaction (GSR) [8–10]. NKT cells
stimulated with their synthetic ligand, α-galactosylceramide (α-GalCer), produce IFN-γ, which activates NK cells as well as CD8+T cells and inhibits both liver and lung metastases in mice [11, 12]. However, α-GalCer-activated NKT cells themselves in turn cause hepatocyte injury in a tumour necrosis factor (TNF)- and Fas-ligand dependent manner [13, 14]. NKT cells are also involved in the severe hepatic injury induced by concanavalin-A (Con-A), which resembles human autoimmune hepatitis [15–18]. Whereas the activation of NKT cells by α-GalCer may inhibit or retard the onset of diseases in some autoimmune models of mice [19–21], activated NKT cells in turn either induce or aggravate diseases in other autoimmune mouse models [22, 23]. NKT cells therefore have autoreactivity, which can be a double-edged sword.

NC mice were established as an inbred strain in 1965 by Kondo and are considered an animal model of human atopic dermatitis [24]. They spontaneously develop dermatitis similar to human atopic dermatitis if they are fed under conventional conditions [24]. We previously reported that NC mice lack the Vβ8 gene and thereby lack Vβ8+T cells and Vβ8+NKT cells, which may be involved in the onset of dermatitis and their T helper 2 (Th2)-dominant state [25]. Vγ14 gene or CD1d gene KO mice lacking all invariant NKT cells [26–29] have shown decreased antitumour immunity [3, 30], a deficient response to α-GalCer [26, 31], complete resistance to Con-A hepatitis [32, 33] and GSR [34]. In contrast to these gene KO mice, NC mice lack the Vβ8 gene but have Vβ7 and Vβ2 genes and therefore lack Vβ8+NKT cells, but they have been suggested to have other NKT cells [25]. Therefore, these mice are useful for estimating the function of Vβ8+NKT cells and observing the effect of the absence of them on immune functions. In the present study, we used NC mice and another Vβ8 gene-deficient inbred strain, Th1-dominant SJL mice [35, 36], to examine the role of Vβ8+NKT cells in hepatic injury and GSR, and discuss the unique immunologic characteristics of these cells.

Materials and methods

Mice

C57BL/6 (B6), BALB/c and NC mice at 8 weeks of age were obtained from Japan SLC Inc. (Hamamatsu). SJL mice at 8 weeks of age were obtained from Charles River Laboratories Japan (Yokohama).

Reagents

α-GalCer was kindly provided by the Pharmaceutical Research Laboratory of Kirin Brewery Company [37, 38]. The original solution of α-GalCer (220 µg/ml) was prepared with 0.5% polysorbate 20 (Nikko Chemical, Tokyo, Japan) in saline and then was subsequently diluted either with this solution (vehicle) or with saline. Con-A was purchased from Vector Laboratories, Inc. (Burlingame, CA). LPS (Escherichia coli 0111: B4) was purchased from Sigma (St. Louis, MO). Mouse recombinant IL-12 was purchased from R&D (Minneapolis, MN).

Induction of hepatic injury and GSR

The mice were injected i.v. with 100 µg/kg of body mass of α-GalCer and blood samples were obtained from retro-orbital plexus at the indicated time points to measure cytokines and ALT. For Con-A hepatitis, Con-A (0.3 mg/mouse) was i.v. injected into mice and blood samples were obtained at 1, 3, 12 and 24 h after Con-A injection. To induce the GSR, the mice were i.p. injected with IL-12 (0.5 µg/mouse) and 50 µg of LPS was i.v. injected into mice 16 h after IL-12 priming.

Isolation of liver mononuclear cells (MNC)

Hepatic MNC were prepared essentially as described previously [7].

Flow cytometric analysis

The surface phenotypes of liver MNC were characterised by a two- or three-colour flow cytometric analysis. An FITC-conjugated mAb to mouse TCR αβ (H57-597), mAb to Vβ8 (F23.1) and mAb to NK1.1 (PK136) were obtained from BD PharMingen. IL-18 levels were measured by an ELISA kit (BD PharMingen). Before staining with Abs, the MNC were incubated for 10 min at 4°C with Fc-blocker (2.4 G2; BD PharMingen) to prevent any nonspecific binding. Flow cytometry was performed using the EPICS XL (Coulter, Miami, FL).

Measurement of IFN-γ, IL-4, TNF and serum alanine aminotransferase (ALT)

The peripheral blood of the individual mice was collected at the indicated time points from the retro-orbital sinus. The IFN-γ, IL-4 and TNF levels of the sera were measured by ELISA kits (BD PharMingen). IL-18 levels were measured by an ELISA kit (MBL, Nagoya, Japan). The activity of ALT in the sera was determined using a DRICHEM 3000V device (Fuj Medical Systems, Tokyo, Japan).

Cytotoxicity assay

NK cell-sensitive YAC-1 lymphoma cells and EL-4 lymphoma cells were used as target cells. Cytotoxic assays were performed as described previously [5, 40].