Methods for Supplemental Figures

Plate bound α–GalCer-loaded CD1d1-Ig dimer stimulation assay

Recombinant soluble murine dimeric CD1d-Ig (Dimer XI; BD Biosciences) was loaded with 40 molar excess lipid antigen according to the manufacturer's instructions. The antigen, α-Galactosylceramide (α-GalCer) was purchased from Alexis Biochemicals. The lipid-loaded CD1d dimers were diluted in PBS (or anti-CD3 as a control) and used to coat 96-well flat bottom plates (1μg/well). The plates were incubated at 37^{o}C for 2 hr and then washed extensively. NKT cell hybridomas, DN32.D3, N37-1A12, & N38-3C3 were incubated in the coated plate for 20-24hr. Culture supernatants were harvested and standard sandwich ELISA was used to measure IL-2 production.

CD1d–Ig dimer staining of *i*NKT cells

To detect *i*NKT cells, recombinant soluble dimeric human CD1d–Ig fusion proteins (DimerX; BD Biosciences) were loaded with 40 molar excess α-GalCer according to the manufacturer's instructions. Then the lipid-loaded CD1d dimers were incubated with PE-labeled anti-mouse IgG (CalTag). Human NKT (Vα24^{+}) cell lines (5×10^{5}) (graciously provided by Dr. Mark Exley, Harvard Medical School) were stained with either empty or lipid-loaded CD1d-Ig dimers for 2h at 4°C. Liver mononuclear cells and splenocytes were harvested from C57BL/6 mice and stained with PE-aGalCer loaded CD1d tetramer (generously provided by Dr. Zhiping Li), anti-CD4-PerCp, anti-CD3-allophycocyanin, anti-CD44-FITC, and anti-CD69-FITC, all purchased from BD Pharmingen, and analyzed by flow cytometry using the BD FacsCalibur device and the FCS-Express software from DeNovo.

Supplemental Figure Legends

Supplemental Fig. 1. (A) NKT cells stained and activated with α-GalCer-loaded CD1d1-Ig dimers. Human NKT (Vα24+) cell lines were stained with either empty (black) or lipid-loaded CD1d-Ig dimers (red) and analyzed by FACS. (B) α-GalCer-loaded CD1d dimers coated plates were used to stimulate NKT cell hybridomas, DN32.D3, N37-1A12, & N38-3C3 for 20-24hr. Culture supernatants were harvested and standard sandwich ELISA was used to measure IL-2 production.

Supplemental Fig. 2. Expression of costimulatory molecules on freshly isolated NKT cells. (A) Liver and (B) splenic MNC were harvested from C57BL/6 mice and were stained with α-GalCer loaded tetramer and mAbs specific for CD3, CD4, CD44, and CD69. The CD1d-tetramer^{+}CD3^{+} population was gated and expression of CD4, CD44, and CD69 on NKT cells was assessed by flow cytometry.