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Supplemental Data

Liver Autoimmunity Triggered by Microbial

Activation of Natural Killer T Cells

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Supplemental Experimental Procedures

Identification of alpha-glucuronosylceramide (GSL-1) in *Novosphingobium aromaticivorans*

N. aromaticivorans was grown up on TS broth and pelleted via centrifugation. Lipids were extracted and base-labile lipids were removed using the procedure reported by Naka et al. (2000). The crude lipid extract was dissolved in methanol for LC/MS analysis. LC parameters: column-Hypersil SAxNormal phase (50 x 2.1 mm); eluent-ethyl acetate, methanol, water gradient (100% ethyl acetate to ethyl acetate:methanol:water 7:2.7:0.3); flow rate 1 ml/min. MS detection at 352.709, 703.523, 704.531, 721.557, and 726.513 mass units. Agilent LC/MSD (TOF).

NKT cell hybridoma stimulation assays

Bone marrow-derived DCs were prepared in RPMI/10 % FCS supplemented with rmGM-CSF and rmIL- 4 (R&D Systems, Minneapolis, MN) as described (Mattner et al., 2005). 100,000 DCs/well were pulsed for 8 hours with titrated doses of heat killed or live *N. aromaticivorans* or synthetic GSL-1 and exposed to 100,000 DN32.D3 NKT hybridoma cells. Cells were co-cultured in 96-well round-bottom plates for 24 hours and cell culture supernatants were assayed for IL- 2 release by ELISA (R&D Systems, lower detection limit of 15 pg/ml).

Supplemental References

Chiu, Y.H., Park, S.H., Benlagha K., Forestier C., Jayawardena-Wolf J., Savage P.B., Teyton L., and Bendelac A. (2002). Multiple defects in antigen presentation and T cell development by mice expressing cytoplasmic tail – truncated CD1d. Nat. Immunol., *3*, 55-60

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Mattner, J., Debord, K. L., Ismail, N., Goff, R. D., Cantu, C., 3rd, Zhou, D., Saint-Mezard, P., Wang, V., Gao, Y., Yin, N.*, et al.* (2005). Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature *434*, 525-529.

Figure S1. *N. aromaticivorans*-derived alpha-glucuronosylceramide (GSL-1) stimulates NKT cells.

(A and B) Characterization of alpha-glucuronosylceramide (GSL-1) by liquid chromatography and mass spectrometry. A, Liquid chromatograms of synthetic GSL-1 (left panel) and GSL-1 isolated from *N. aromaticivorans* (right panel). B, Mass spectra of synthetic GSL-1 (left panel) and GSL-1 isolated from *N. aromaticivorans* (right panel). Ions at 704 and 726 mass units correspond to $[M+H]^+$ and $[M+Na]^+$, respectively.

(C and D) NKT cell stimulation by N. aromaticivorans alpha-glucuronosylceramide. Dendritic cells from wild type B6, CD1d-deficient B6 (*CD1*$^{-/-}$), or tail-deleted CD1d (TD) B6 (Chiu et al., 2002) were pulsed for 8 hours with heat-killed (C, left panel) or live (C, right panel) *N. aromaticivorans* at the indicated bacteria:DC ratios or with synthetic GSL-1 (D) at the indicated concentrations and exposed to the NKT cell hybridoma DN32.D3. The release of IL-2 was measured 24 hours later using ELISA and the results of two independent experiments were combined. Levels of IL-2 release in unstimulated samples were below the detection limit of the ELISA (not shown). Error bars represent the standard error of the mean for each group.