

Novel bacteria-derived glycolipids and sulfatide analogues as potential HIV vaccine adjuvants act through stimulation of CD1d-restricted NKT cells

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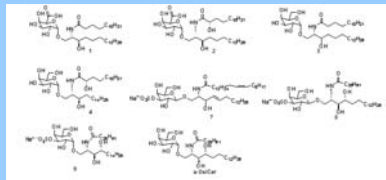
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Introduction

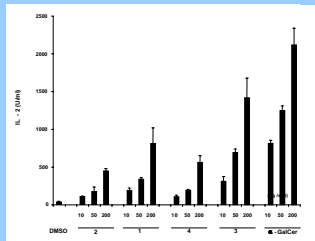
CD1 molecules are a family of highly conserved antigen presenting proteins that are similar in function to classic MHC molecules. Whereas classic MHC molecules present peptides, CD1 proteins present a variety of lipids and glycolipids to T lymphocytes. When loaded with antigenic glycolipids, CD1d binds the lipid portion in a hydrophobic groove leaving the hydrophilic sugar moiety available to make contact with the T cell receptor. α -Galactosyl ceramide (α GalCer), a lipid found in the marine sponge *Agelas mauritanicus*, has been the most extensively studied ligand for CD1d. α GalCer, when bound to CD1d, stimulates rapid IFN- γ (Th1) and IL-4 (Th2) cytokine production by V α 14i natural killer T (V α 14i NKT) cells in mice and the human homolog V α 24i NKT cells. However, the physiological significance of response to α GalCer in mammals remains unclear because it is enigmatic why an antigen of marine origin is such a potent agonist. Some α GalCer analogs with different lipid chain lengths and linkages have also been reported to have significant activities toward NKT cells.

Objectives

Given the rapid and vigorous production of IFN- γ induced by glycolipid-stimulated NKT cells, we are studying the potential for glycolipid antigens to serve as adjuvants to be used in vaccination strategies against HIV. We have synthesized a number of glycolipids and tested them for NKT cell activation. These included glycolipids of bacterial origin, α -GalCer analogs modified on the galactose moiety and acyl group, and variations of sulfatide, the only known promiscuous ligand for CD1. The bacterial glycolipids included those isolated from the outer membrane of *Sphingomonas wittichii* and glycolipids from *Borrelia burgdorferi*.

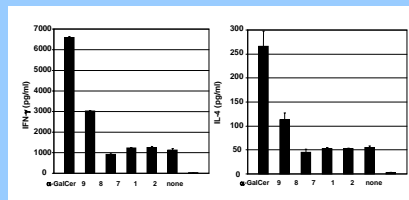


Glycolipid antigens studied: **1** and **2** *Sphingomonas* glycolipids GSL-1 and GSL-2; **3** Carboxyl group on sugar; **4** alpha hydroxyl group on lipid; **7** and **8** β -linked sulfatides; **9** 3''-O-sulfoGalCer, **α GalCer**

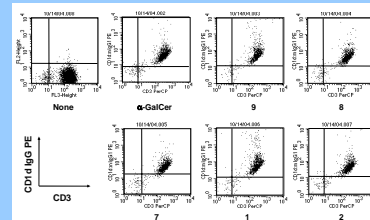


Dose dependent secretion of IL-2 by Glycolipid antigens. CD1d molecules (10 μ g/ml in PBS) were coated in a 96-well plate by incubation for 1 h at 37C. IL-2 release was measured after 16 h of culture in a sandwich ELISA.

Results/graphs



Human V α 24i NKT cells respond to glycolipid antigens. IFN- γ and IL-4 release after 16 hours of culture by 1×10^5 V α 24i human NKT cell line in response to culture with 4×10^5 autologous immature CD14+ dendritic cells pulsed with the glycolipid antigens at 10 μ g/ml. The negative control wells contained similar numbers of NKT cells and dendritic cells, cultured without added glycolipid. Data are mean \pm S.D. of duplicate wells.



Human V α 24i NKT cells bind to glycolipids in the context of CD1d. Flow cytometric analysis of a human V α 24i human NKT cell line with human CD1d dimers that were unloaded or loaded with 10M of the indicated glycolipid antigen. The cells were also stained with anti-human CD3-PerCP.

Methods

1.2 Hybridoma Assay: CD1d reactive T cell hybridomas expressing an invariant V α 14i TCR α chain were stimulated with glycolipids that were added to CD1d transfected A20 B lymphoma cells. As a measure of T cell activation, IL-2 release into the tissue culture medium was measured after 16 h culture by ELISA.

Generation of V α 24i human NKT cell line: Anti-CD161 and anti-CD14 monoclonal antibodies, each coupled to magnetic beads, were used to isolate CD161+ cells and CD14+ cells from leukopaks. Immature dendritic cells (DCs) were generated from the CD14+ cells. Following irradiation with 2000rads, the immature DCs were co-cultured with syngeneic CD161+ cells in the presence of 100 ng/ml of α GalCer and 10 IU/ml of IL-2 for 10-14 days. After stimulating the CD161+ cells a second time with α GalCer-pulsed, irradiated immature DCs, NKT cell lines were shown to express both CD161+ and V24i TCR (99% purity).

In vitro cytokine secretion assay using human NKT cell lines: 1×10^5 V24i human NKT cells were co-cultured with 4×10^5 irradiated, immature DCs, in the presence of the glycolipid compounds at 10 μ g/ml in a 96-well flat-bottom plate. IFN- γ and IL-4 secretion was quantified by ELISA

In vitro CD1d-dimer assay using a human NKT cell line: 1 μ g of soluble divalent human CD1d-IgG₁ fusion protein were incubated overnight with 10M of each glycolipid. The glycolipid-loaded CD1d-IgG₁ dimers were incubated with human NKT cells for 60 min, followed by incubation with PE-coupled anti-mouse IgG₁ mAb. The cells were also surface stained with a PerCP-coupled anti-CD3 mAb.

Conclusions

Given the rapid secretion of IFN- γ by NKT cells stimulated with bacteria-derived glycolipids and sulfatide analogues, administration of these antigens may represent novel and efficacious adjuvants in HIV vaccination strategies.

