Antibodies induced by Glycosphingolipids

Subjects: Biochemistry & Molecular Biology | Biotechnology & Applied Microbiology | Immunology
Contributor: Tetsuya Okuda

Glycosphingolipids containing very-long-chain fatty acids (VLCFAs) regulate several immune responses, such as cytokine production, immune signaling, and antibody induction. Here, we report that immunization with glycosphingolipids containing-VLCFAs can efficiently induce the production of anti-glycan antibodies by B cells.

antibody glycosphingolipid globoside/Gb4Cer Gb3Cer IgG3 IgM

1. Glycosphingolipids (GSLs)

Glycosphingolipids (GSLs) are cell membrane components composed of oligosaccharides and ceramides. Oligosaccharides and ceramides in GSLs are structurally diverse, and recent studies have revealed that GSLs containing very-long-chain fatty acids (VLCFAs) in the ceramide portion are involved in immune responses in mammalian tissues [1],[2],[3],[4]. For example, α-linked monosaccharyl ceramides such as α-galactosylceramide, which is isolated from the marine sponge *Agelas mauritianus*, contain VLCFAs in the ceramide portion [1] that activate mammalian natural killer T (NKT) cells [1][5] and promote cytokine production in a VLCFA-dependent manner [1][2]. GSLs containing VLCFAs also play an important role in the association of Src family kinases with membrane microdomains in neutrophils, which mediates signal transduction associated with neutrophil migration and phagocytosis [3]. The stimulation of vascular endothelial cells (ECs) with lipopolysaccharide (LPS) activates Toll-like receptor signaling, resulting in inflammatory responses in the ECs. LPS stimulation also promotes the production of GSLs containing VLCFAs by ECs, and these molecules are thought to reduce excess EC inflammatory responses via the inhibition of inflammatory signaling [4].

2. Previous Study

We previously reported that the stimulation of human umbilical vein ECs with an inflammatory mediator, TNF-α, promotes GSL production in ECs via the transcriptional regulation of genes related to GSL synthesis [6]. Further structural analyses revealed that the primary component of these GSLs is globotetraosylceramide/globoside (Gb4Cer) containing VLCFAs (Gb4Cer-VLCFAs) [6][7]. To characterize the function of these GSLs in ECs, we generated anti-Gb4Cer antibodies and found that Gb4Cer-VLCFAs exhibits efficient antibody-inducing activity in mice. The immunization of mice with Gb4Cer-VLCFAs immediately induced the production of serum antibodies that specifically reacted with Gb4Cer. Analyses of hybridoma cells generated from splenocytes isolated from a mouse immunized with Gb4Cer-VLCFAs revealed that hybridoma clones producing the anti-Gb4Cer antibodies could be easily isolated, and that these clones produced both IgM- and IgG-class

antibodies. Furthermore, some of these antibodies reacted with both Gb4Cer and its precursor, globotriaosylceramide (Gb3Cer), indicating that these antibodies recognize a shared epitope in these GSLs. These results indicate that Gb4Cer-VLCFAs function as immunity inducers for the production of anti-Gb4Cer and -Gb3Cer antibodies in mice.

3. Findings

Antibodies that recognize Gb4Cer and its precursor Gb3Cer can be easily produced by immunizing mice with Gb4Cer-VLCFAs. The antibodies produced include IgM and IgG3, indicating that Gb4Cer-VLCFAs induce class switching in B cells. The ability to induce class switching is associated with T-cell-independent type-type 2 polysaccharide antigens that induce potent B-cell responses [8]. Although previous studies described several mAbs that specifically react with Gb4Cer [9][10][11], no IgG-class mAbs were isolated. Furthermore, the PA7 mAb isolated in the present study reacted with an epitope shared by Gb4Cer and Gb3Cer. Such unique specificity has not been observed in previously described antibodies that react with Gb4Cer or similar GSLs.

Although this study revealed that Gb4Cer-VLCFAs induces the production of antibodies that react with Gb4Cer and Gb3Cer, the immunologic role of these antibodies remains unknown. Gb4Cer and Gb3Cer are expressed on the surface of mammalian cells and function as receptors for toxins produced by enterohemorrhagic *Escherichia coli* [12][13]. However, other infectious bacteria, such as *Neisseria gonorrhoeae* and *Haemophilus influenzae*, express Gb4- and Gb3-type oligosaccharides on the cell surface [14][15][16]. As these Gram-negative bacteria also produce the inflammatory mediator LPS, infection with these organisms elicits tissue inflammation. As inflammation promotes Gb4Cer-VLCFA synthesis in the tissues, antibodies induced by Gb4Cer-VLCFAs may play a role in host defense against these microbial pathogens. Previous studies also reported that cell surface Gb4Cer is recognized by parvovirus B19, which utilizes the molecule as a receptor for entry into the cell [17]. As viral entry can be blocked by treating cells with anti-Gb4Cer antibodies [17], anti-Gb4Cer antibodies induced by Gb4Cer-VLCFAs may inhibit the interaction of cell surface GSLs with viruses and infectious bacteria.

References

1. Tetsu Kawano; Junqing Cui; Yasuhiko Koezuka; Isao Toura; Yoshikatsu Kaneko; Kazuhiro Motoki; Hitomi Ueno; Ryusuke Nakagawa; Hiroshi Sato; Eisuke Kondo; et al.Haruhiko KosekiMasaru TaniguchiSarah H. TolbertAli FirouziGalen D. StuckyBradley F. Chmelka CD1d-Restricted and TCR-Mediated Activation of Vα14 NKT Cells by Glycosylceramides. *Science* **1997**, *278*, 1626-1629, 10.1126/science.278.5343.1626.
2. Randal D. Goff; Ying Gao; Jochen Mattner; Dapeng Zhou; Ning Yin; Carlos Cantu; Luc Teyton; Albert Bendelac; Paul B. Savage; Effects of Lipid Chain Lengths in α-Galactosylceramides on

Cytokine Release by Natural Killer T Cells. *Journal of the American Chemical Society* **2004**, *126*, 13602-13603, 10.1021/ja045385q.

3. Kazuhisa Iwabuchi; Hitoshi Nakayama; Chihiro Iwahara; Kenji Takamori; Significance of glycosphingolipid fatty acid chain length on membrane microdomain-mediated signal transduction. *FEBS Letters* **2009**, *584*, 1642-1652, 10.1016/j.febslet.2009.10.043.
4. Yuji Kondo; Kazutaka Ikeda; Noriyo Tokuda; Chiaki Nishitani; Umeharu Ohto; Sachiko Akashi-Takamura; Yasutomo Ito; Makoto Uchikawa; Yoshio Kuroki; Ryo Taguchi; et al.Kensuke MiyakeQing ZhangKeiko FurukawaKoichi Furukawa TLR4–MD-2 complex is negatively regulated by an endogenous ligand, globotetraosylceramide. *Proceedings of the National Academy of Sciences* **2013**, *110*, 4714-4719, 10.1073/pnas.1218508110.
5. Jamie Rossjohn; Daniel G. Pellicci; Onisha Patel; Laurent Gapin; Dale Godfrey; Recognition of CD1d-restricted antigens by natural killer T cells.. *Nature Reviews Immunology* **2012**, *12*, 845-57, 10.1038/nri3328.
6. Tetsuya Okuda; Data set for characterization of TNF-α–inducible glycosphingolipids in vascular endothelial cells. *Data in Brief* **2018**, *21*, 29-35, 10.1016/j.dib.2018.09.059.
7. Tetsuya Okuda; Sin-Ichi Nakakita; Ken-Ichi Nakayama; Structural characterization and dynamics of globotetraosylceramide in vascular endothelial cells under TNF-α stimulation. *Glycoconjugate Journal* **2010**, *27*, 287-296, 10.1007/s10719-009-9277-2.
8. C M Snapper; T M McIntyre; R Mandler; L M Pecanha; F D Finkelman; A Lees; J J Mond; Induction of IgG3 secretion by interferon gamma: a model for T cell-independent class switching in response to T cell-independent type 2 antigens.. *Journal of Experimental Medicine* **1992**, *175*, 1367-1371, 10.1084/jem.175.5.1367.
9. A. E. G. Kr. Von Dem Borne; M. J. E. Bos; N. Joustra-Maas; J. F. Tromp; R. Van Wijngaarden-Du Bois; P. A. T. Tetteroo; A murine monoclonal IgM antibody specific for blood group P antigen (globoside). *British Journal of Haematology* **1986**, *63*, 35-46, 10.1111/j.1365-2141.1986.tb07492.x.
10. M. Kotani; I. Kawashima; H. Ozawa; K. Ogura; T. Ariga; T. Tai; Generation of One Set of Murine Monoclonal Antibodies Specific for Globo-Series Glycolipids: Evidence for Differential Distribution of the Glycolipids in Rat Small Intestine. *Archives of Biochemistry and Biophysics* **1994**, *310*, 89-96, 10.1006/abbi.1994.1144.
11. Tetsuya Okuda; PUGNAc treatment provokes globotetraosylceramide accumulation in human umbilical vein endothelial cells. *Biochemical and Biophysical Research Communications* **2017**, *487*, 76-82, 10.1016/j.bbrc.2017.04.019.
12. Tetsuya Okuda; Shin-Ichiro Numata; Masafumi Ito; Michio Ohta; Kumiko Kawamura; Joëlle Wiels; Takeshi Urano; Orie Tajima; Keiko Furukawa; Koichi Furukawa; et al.Noriyo Tokuda Targeted

Disruption of Gb3/CD77 Synthase Gene Resulted in the Complete Deletion of Globo-series Glycosphingolipids and Loss of Sensitivity to Verotoxins. *Journal of Biological Chemistry* **2006**, *281*, 10230-10235, 10.1074/jbc.m600057200.

13. Nadine Legros; Gottfried Pohlentz; Daniel Steil; Johannes Müthing; Shiga toxin-glycosphingolipid interaction: Status quo of research with focus on primary human brain and kidney endothelial cells. *International Journal of Medical Microbiology* **2018**, *308*, 1073-1084, 10.1016/j.ijmm.2018.09.003.
14. C M John; J M Griffiss; M A Apicella; R E Mandrell; B W Gibson; The structural basis for pyocin resistance in Neisseria gonorrhoeae lipooligosaccharides.. *Journal of Biological Chemistry* **1991**, *266*, 19303-19311.
15. Hussein Masoud; E. Richard Moxon; Adèle Martin; Don Krajcarski; James C. Richards; Structure of the Variable and Conserved Lipopolysaccharide Oligosaccharide Epitopes Expressed byHaemophilus influenzaeSerotype b Strain Eagan†,‡. *Biochemistry* **1997**, *36*, 2091-2103, 10.1021/bi961989y.
16. Anna Risberg; Gunvor Alvelius; E K Schweda; Structural analysis of the lipopolysaccharide oligosaccharide epitopes expressed by Haemophilus influenzae strain RM.118-26. *JBIC Journal of Biological Inorganic Chemistry* **2001**, *265*, 1067-1074, 10.1046/j.1432-1327.1999.00832.x.
17. K. Brown; S. Anderson; N. Young; Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science* **1993**, *262*, 114-117, 10.1126/science.8211117.