Antibodies induced by Glycosphingolipids

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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.

Keywords: 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.

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