



REVIEW ARTICLE

Physiology of gangliosides and the role of antiganglioside antibodies in human diseases

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Gangliosides are structurally and functionally polymorphic sialic acid containing glycosphingolipids that are widely distributed in the human body. They play important roles in protecting us against immune attacks, yet they can become targets for autoimmunity and act as receptors for microbes, like the influenza viruses, and toxins, such as the cholera toxin. The expression patterns of gangliosides vary in different tissues, during different life periods, as well as in different animals. Antibodies against gangliosides (AGA) can target immune attack e.g., against neuronal cells and neutralize their complement inhibitory activity. AGAs are important especially in acquired demyelinating immune-mediated neuropathies, like Guillain-Barré syndrome (GBS) and its variant, the Miller-Fisher syndrome (MFS). They can emerge in response to different microbial agents and immunological insults. Thereby, they can be involved in a variety of diseases. In addition, antibodies against GM3 were found in the sera of patients vaccinated with *Pandemrix*[®], who developed secondary narcolepsy, strongly supporting the autoimmune etiology of the disease.

Keywords: ganglioside; Guillain-Barre syndrome; complement; narcolepsy; sialic acid

Cellular & Molecular Immunology (2020) 17:313–322; <https://doi.org/10.1038/s41423-020-0388-9>

INTRODUCTION

The neuronal and immune systems represent the two most complicated systems in the vertebrate bodies. The brain has traditionally been considered as an immune privileged organ. However, both humoral and cell-mediated immunity can attack different cells and structures to cause autoimmune diseases. These include multiple sclerosis, narcolepsy, optic neuritis, limbic encephalitis, and Guillain-Barré syndrome. Immunoinflammatory mechanisms are believed to be involved in various forms of dementia and neurodegenerative diseases, as well.

Our understanding of many neurological and neurodegenerative pathological processes has increased a lot, despite the fact that the brain is not an easily accessible organ for studies, and that the actual disease processes have usually taken place before symptoms appear. The recent interest in the interactions between the nervous system and the immune system has been boosted by better opportunities to image intracerebral phenomena and by the identification of the network of dural lymphatic vessels.^{1,2}

Molecular targets for neuronal autoimmunity are being better recognized, but in some diseases, like multiple sclerosis (MS) and narcolepsy, uncertainty about the real targets still exists. The targets are not always exclusively proteins, but could include lipids or glycolipids, as well. Our aim in the present review is to summarize and highlight the possibility that antiganglioside antibodies (AGAs) could have a pathophysiological role and help in the diagnostics or prognostic evaluation of the diseases. AGAs are autoantibodies produced against gangliosides, which are sialic acid containing glycosphingolipids (Box 1). Since their discovery, AGAs have been linked to a wide variety of pathologies, notably inflammatory neuropathies following an immunological insult.^{3,4}

In recent years, gangliosides received renewed interest as targets for cancer immunotherapy. It is known that the GD3 ganglioside is strongly expressed by melanoma cells.^{5,6} The clearest pathophysiological role for AGAs has been demonstrated in Guillain-Barré Syndrome (GBS). GBS and AGA appearance have been associated with different infectious agents such as *Campylobacter jejuni*,⁷ cytomegalovirus,⁸ Epstein-Barr virus and *Haemophilus influenzae*.⁹

The present review will discuss the multiple roles of gangliosides and their key components, sialic acids, in shielding human and microbial cells from immune attack, determining species-specificity of certain infections and as targets for autoimmunity.

AN OVERVIEW OF GANGLIOSIDES

Structure, expression and function of gangliosides

Glycosphingolipids contain a hydrophobic ceramide or sphingoid lipid tail, which is usually anchored to the outer leaflet of the plasma membrane (Fig. 1a). They also contain an oligosaccharide moiety and are classified according to this carbohydrate structure (ganglio, isoganglio, lacto etc.).^{5,19} Gangliosides are a peculiar and particularly important subclass of glycosphingolipids, because they contain negatively charged sialic acids (N-acetylneuraminic acid or N-glycolylneuraminic acid) linked to the lipooligosaccharide moiety. Gangliosides are named and classified according to the number of sialic acid residues attached (M for one, D for two, T for 3 and Q for 4) to the inner sugar moiety and according to their chromatographic mobility (Fig. 1b).²⁰ The somewhat illogical numbering of the gangliosides (5-x) is based on the number (x) of the inner sugar moieties (glucose, galactose or GalNAc) according to the original experimental classification of Svennerholm.²¹ Thus,

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Received: 20 January 2020 Revised: 23 February 2020 Accepted: 23 February 2020
Published online: 9 March 2020

Box 1. Sphingolipids and sialic acids

Sphingolipids are constituents of plasma membranes that fulfill various functions, like membrane stability, adhesion, signaling and cell protection. They also act as receptors for hormones or toxins.¹⁰ A particular derivative of sphingolipids, sphingosine-1-phosphate, has signaling functions to regulate the growth and survival of various cell types.^{11–13}

Sialic acids are nine carbon acidic sugars that are enriched in the membranes of all vertebrate cells, and also in a few invertebrates. They can be attached to lipids or proteins.¹⁴ They owe their name to the term "saliva" (Greek σάλιο) because they were first identified in 1955 in the bovine salivary mucins.¹⁵ Given their negative charge, sialic acids can prevent unwanted interactions of erythrocytes in the blood circulation. They are necessary for maintaining the filtering function of the glomerular basement membranes of the kidney. They are also believed to affect neuronal plasticity.¹⁶ The functions of gangliosides remain related to the cells and the molecules which carry them.¹⁷ Sialic acids are also exploited by viruses like the influenza viruses, to be internalized into the host cells through interactions between the viral hemagglutinin and neuraminidase with sialic acids on the surfaces of e.g., the host respiratory epithelial cells.¹⁸

if x is 4, the gangliosides are: GM1, GD1, GT1, $x = 3$ for GM2, GD2, GT2, and $x = 2$ for GM3, GD3, and GT3 (Fig. 1b).

Gangliosides are ubiquitously expressed throughout the body tissues and fluids, but they are particularly abundant in the brain and in the nervous system. They participate in the maintenance and repair of neuronal cells²², memory formation²³ and synaptic transmission²⁴. They also take part in the development and regeneration of neurons.^{25,26} During brain development, ganglioside expression undergoes massive qualitative and quantitative changes.²⁷ The ganglioside expression profile changes so that in embryonic rodent and human brain the predominant gangliosides are simple gangliosides like GM3 and GD3, while in adults more complex species such as GM1, GD1a, GD1b and GT1b predominate²⁸ and comprise over the 90% of the adult brain gangliosides.^{20,29,30} This difference in ganglioside expression reflects changes in the expression of various glycosyltransferases, i.e., the enzymes needed for the synthesis of gangliosides.^{20,31} Vajn et al.²⁹ have used immunochemical techniques to localize the presence of the above-mentioned gangliosides in the brains of C57bl/6 mice (Table 1).

In cells, gangliosides are concentrated in the outermost leaflets of the plasma membranes (PMs) in transient structures referred to as lipid rafts.^{32,33} Gangliosides are attached to the membrane so that their hydrophobic ceramide tail is embedded in the lipid membrane, and the sugar moiety is protruding out from the membrane. Lipid rafts are specific and transient subdomains rich in glycosphingolipids (e.g., gangliosides), cholesterol and distinct proteins, e.g., caveolins, flotillins and glycosphosphoinositol (GPI)-linked proteins. They also contain molecules involved in signaling, like low molecular weight and hetero-trimeric G proteins, EGF receptors, PDGF receptors, endothelin receptors, MAP kinase and protein kinase C.³⁴ Gangliosides interact with phospholipids, cholesterol and transmembrane proteins of the lipid rafts both in cis and trans. They are involved in many cellular processes such as regulation of many cellular functions, neurotransmission, interaction with regulatory proteins of the nervous system and in cell-cell recognition, proliferation, as well as in the modulation of signal transduction pathways.¹⁰

IMMUNOLOGICAL ROLES OF GANGLIOSIDES

Gangliosides have multiple important structural and functional roles that are related with various immunological functions. Because of their location on the outer leaflet of the plasma membrane, gangliosides are critical components in the protection of host structures against the autologous immune system. By virtue of their robust negative charges, sialic acids shield self-surfaces and repulse the attachment of other cells or macromolecules to the cellular surfaces. On the other hand, certain viruses, bacteria and parasites, like blood merozoites of the

deadliest malaria parasite *Plasmodium falciparum*, have learnt to exploit sialic acids/gangliosides as their receptors. Thus, gangliosides can function as attachment sites for pathogens. Some bacterial toxins (cholera toxin, *Salmonella typhi* toxin) also use sialic acids as their receptors. Since the binding of these toxins is specific for sialic acids in humans, the diseases they cause also occur primarily in humans.

Another special example is the influenza virus. Influenza viruses bind to sialic acids via their hemagglutinin (H) proteins, and after entry e.g., to respiratory epithelial cells, the neuraminidase (N) enzymes of the virus, will cut the sialic acids from the cell surface to prevent entry of further viruses to the cell. Of great importance, different types of influenza viruses carry H proteins with different sialic acid specificities. Thus, only certain influenza viruses (H1, H2, H3) are infecting humans, while H1 and H3 have more preference for pigs and all types from H1 to H16 can infect birds. The H5 and H7 types are particularly dangerous influenza virus types for birds and a potential risk for humans, as well.³⁵ Variation in the H and N protein structures in pandemic influenza strains forces the adoption of new vaccines against the H and N proteins for seasonally occurring new types of influenza virus during epidemics.

Another major immunological function of gangliosides and sialic acids is to protect our cells from our own complement attack and from autoimmunity. In general, sialic acids are not very immunogenic, although in some cases and with specific sialic acids immunization may occur and cause immune damage (see below). Gangliosides function in the recognition and protection of host organs and tissues from complement attack by binding the complement regulatory protein factor H.³⁶ The complement system has a potentially strong cytotoxic and inflammation-inducing activity. Sialic acids provide a robust protection against complement killing of autologous cells. This is mediated by the binding of the soluble complement inhibitor factor H to the surface sialic acids, when the surfaces are threatened by C3b deposition.³⁷

Structural studies have shown that the specific targets for factor H include the α 2–3 linked sialic acid glycans of the GD3 ganglioside.³⁸ Thus, on surfaces, where the complement component C3b has become accidentally bound, the neighboring sialic acids provide the additional affinity to bind the complement inhibitory factor H instead of the activation promoting factor B, which does not bind to sialic acids.³⁶

Gangliosides play an important role in maintaining the integrity of lipid rafts, particular cell membrane microdomains.^{22,33} Changes in ganglioside composition of lipid rafts may cause alterations in the modes of interactions with individual counterpart proteins, or phospholipid and cholesterol molecules.²² These may be involved in signaling, generation of focal synapse-like points of interactions and removal of membrane components by internalization or exocytosis.³⁹ Such events may take place in T lymphocytes during their activation by various signals.⁴⁰ Thus, defects in membrane lipid rafts could contribute to dysfunction of CD4+ T helper cells and consequent autoimmunity.

A set of mammalian proteins, more than 150, are anchored to the outer leaflets of cell membranes via a GPI-lipid anchor, which is a standard means of membrane protein anchoring in protozoan parasites. GPI-anchored proteins are enriched in lipid rafts and they can signal via cell membrane tyrosine kinases.⁴¹ In humans, GPI-anchored proteins include two complement-regulatory proteins: CD55 (decay accelerating factor; DAF) inhibiting the C3 convertases⁴² and CD59 (protectin) that inhibits the membrane attack complex formation by binding to the terminal C5b-8 and C5b-9 complexes.⁴³ Alterations in ganglioside profiles may cause changes in the properties of these protective proteins. A deficiency in the anchoring mechanism causes a hematological disorder, paroxysmal nocturnal hemoglobinuria (PNH), where affected blood cells become the target for complement attack.

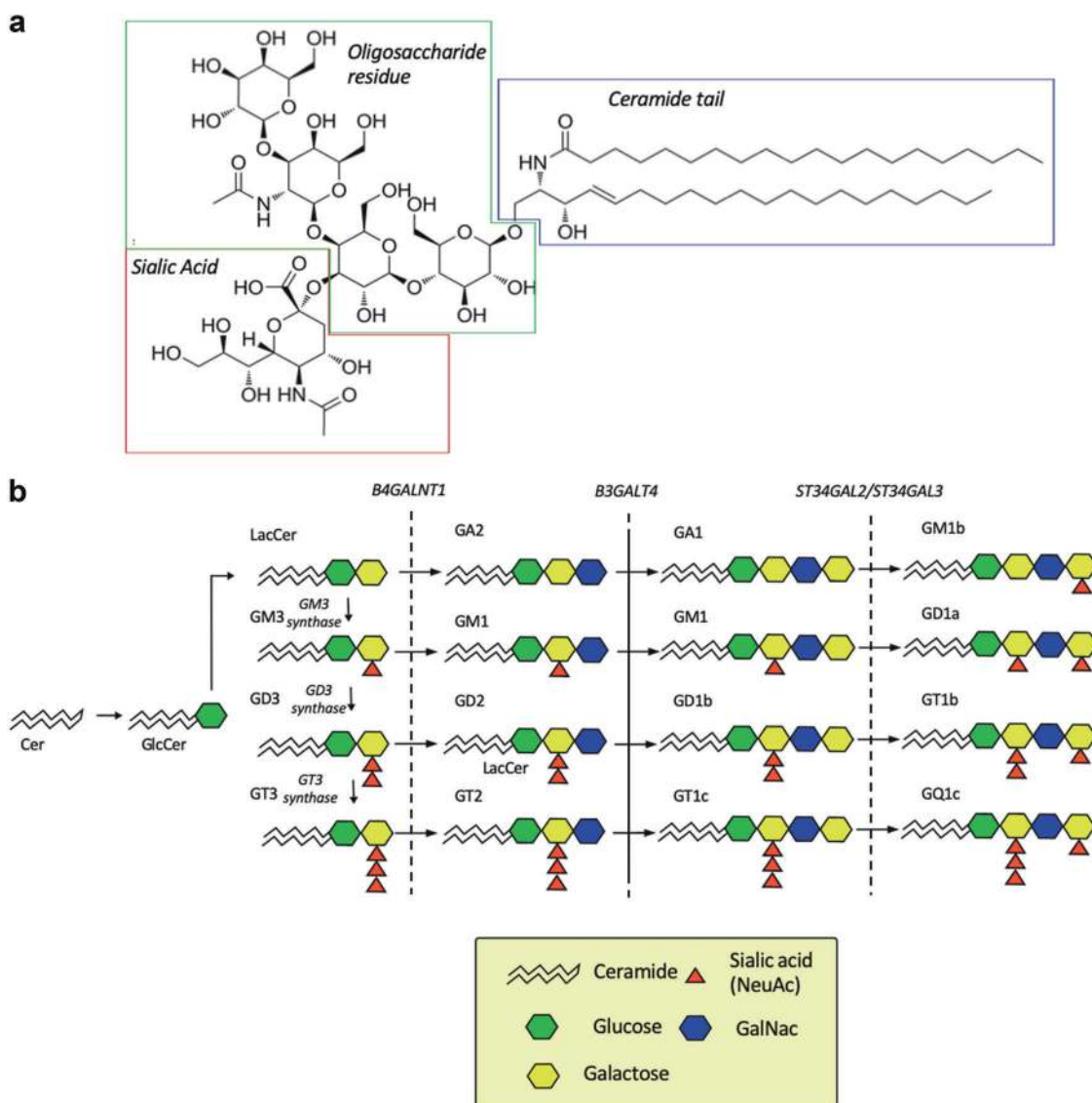


Fig. 1 Structures and biosynthesis of gangliosides. **a** Structure of the GM1 ganglioside. The sialic acid (red) is linked to the oligosaccharide residue (green) ultimately connected to the ceramide tail (blue) that is embedded in the outer leaflet of the cell membrane. **b** A schematic representation of the biosynthetic pathway and structures of gangliosides. Gangliosides are sialylated glycosphingolipids. Their synthesis consists of the sequential addition of sugars and/or sialic acids (neuraminic acid; NeuAc) by two main groups of enzymes, i.e., sialyltransferases (GM3/GD3/GT3 synthases and ST3GAL2/ ST3GAL3) and glycosyltransferases (B4GALNT1 and B3GALT4).^{19,148} The letters M (1), D (2), T (3) and Q (4) indicate the number of sialic acid residues, respectively. The numbers, on the other hand, indicate indirectly the number of sugar residues subtracted from 5: e.g., GM1 contains 5–1 = 4 sugar residues, and GD3 contains 5–3 = 2 sugar residues. Abbreviations: beta-1,4-N-acetyl-galactosaminyl transferase 1 (B4GALNT1), beta-1,3-galactosyltransferase 4 (B3GALT4), ST3 beta-galactoside alpha-2,3-sialyltransferase 2 (ST3GAL2), ST3 beta-galactoside alpha-2,3-sialyltransferase 3 (ST3GAL3)

A critical feature of the disease is also an increased tendency for thromboses. Most commonly, the disease is caused by an acquired mutation in the X-chromosomal *PIG-A* gene, whose product is involved in the GPI-anchor synthesis.⁴⁴ When cells are attacked by the complement membrane attack complex, they release vesicles enriched in GPI-anchored proteins. Overall, the GPI-lipid anchored proteins together with the attached gangliosides are only relatively loosely bound to cell membranes and can easily become detached. For example, high density lipoproteins in human plasma can transfer GPI-anchored CD59 between cells.⁴⁵ It is likely that the same applies for gangliosides, as well. Also, *Helicobacter pylori*, a long living companion in the human gastric ventricular mucosa, may acquire the human CD59 molecules to its surface to help the bacteria to survive attacks by the human complement system.⁴⁶ Analogously, lipopolysaccharides (LPS) of

Gram-negative bacteria may become transferred to the surfaces of human cells. Transfer of cell membrane glycolipids, including gangliosides and LPS, between human and microbial cells could—via an adjuvant type effect—be involved in the generation of antibody responses against these structures.

PATHOLOGIES INVOLVING ANTIGANGLIOSIDE ANTIBODIES (AGAS)

Neurological diseases

As stated above, gangliosides are particularly enriched on the outer leaflets of neuronal membranes. Lipid rafts and gangliosides therein have been suggested to serve important neuronal functions, such as modulation of ion channels and transporters, neuronal interactions and recognition, Ranvier node stability and

Table 1. Areas of localization of the main gangliosides

Ganglioside	Area of localization	Reference
GM1	Mainly in the white matter following the expression of MAG (a myelin marker). Present also in some nuclei, especially abundant in the hypothalamic nuclei but absent in amygdala and basal nuclei Abundant also in gut epithelial cells	Vajn ²⁹ Aleadini ¹⁴⁹
GD1a	Predominantly expressed in the gray matter Strong presence in the olfactory bulb and substantia nigra Weak expression in thalamus (in the reticular nucleus)	Vajn ²⁹
GD1b	Predominantly expressed in the gray matter (like GD1a) Strong expression in the epithalamus Widely expressed in both white matter and gray matter of the spinal cord	Vajn ²⁹
GT1b	Strong expression in the pallidus and raphe magnus	Vajn ²⁹
GM3	A major endothelial cell ganglioside (angiogenesis suppressor) Especially present in "glycosignaling domains" Hypothalamus	Yu ²⁰ , Kanda ³⁰ , Hakomori ¹⁵⁰
GQ1b	Paranodal region Schwann cells	Rodella ¹⁵¹ Fehmi ⁴

synaptic transmission. In general, gangliosides seem to have important roles in the maintenance of the nervous system.

Various diseases, especially those involving the neurological system and many neurodegenerative diseases may arise from disturbed ganglioside metabolism or alternatively from the action of antibodies directed against single or multiple targets. In 2004 mutations to GM3 synthase were identified as the cause of an autosomal recessive infantile-onset epilepsy syndrome.⁴⁷ In addition, mutations in ST3GAL5, which codes for an enzyme necessary for ganglioside biosynthesis, result in an early-onset seizure disorder with motor and cognitive disturbances. Furthermore, mutations in B4GALNT1, a gene encoding for another enzyme of the ganglioside biosynthetic pathways, result in hereditary spastic paraplegia and intellectual deficits.¹⁰

In addition to gangliosidoses, ganglioside accumulation diseases, also diseases like infantile epilepsy, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and multiple sclerosis (MS) may involve disturbed ganglioside metabolism.⁴⁸ On the other hand, the peripheral neuropathy Guillain-Barré syndrome (GBS) is an example of a disease associated with production of autoreactive antiganglioside antibodies (AGAs).⁴⁹ As studied by Ceccarelli et al. already in the 70's, gangliosides can promote the regeneration of injured neurons.²⁶ Exogenously administered gangliosides mimic endogenous gangliosides through their insertion into the plasma membranes after first adhering as micelles.²⁵ During recent years, exogenous purified gangliosides have been used experimentally to treat certain neurological diseases.⁴⁸

AGAs have been found in a variety of disorders classically considered to be of exclusive neurological interest. Anti-GM1 AGAs have been found in Alzheimer's disease (AD) patients.⁵⁰ AD has been also related to IgM anti-GD1b^{51,52} and anti-GQ1b antibodies, although the latter association has received discordant views in the literature.^{53,54} It appears that GM1-amyloid-beta protein complexes have the capacity to increase Aβ assembly.⁵⁵ To further complicate the picture, Svennerholm et al in 2002, reported a slowing of the degenerative process in Alzheimer's disease patients treated with exogenous GM1.²¹ Similarly to AD, association to anti-GM1 antibodies has been found also in Parkinson disease⁵⁶ and GM1 supplementation has been studied as a possible treatment strategy.⁵⁷ The presence of AGA in amyotrophic lateral sclerosis (ALS) has been investigated, but recent studies found no differences between ALS patients and healthy controls.⁵⁸ AGA were also sporadically identified in cases of myasthenia gravis and Lambert-Eaton syndrome^{59,60} and in a

single case of small fiber neuropathy⁶¹. Because of their ubiquitous nature in the neuronal system, it is not surprising that gangliosides have potentially multiple pathological roles in neurological diseases. Some of them play key roles in disease development, whereas in other cases ganglioside abnormalities are secondary to the actual disease process.

Immune-mediated diseases

The presence of AGAs has been investigated in many immune-mediated diseases, especially in those with neurological manifestations. In MS patients, AGAs have been detected in several studies, but their role in disease progression remains unclear.⁶² In fact, the presence of anti-GM1 antibodies appears to be unrelated to the level of brain atrophy.^{63,64} However, patients with primary MS have been shown to have higher plasma levels of anti-GM3 and anti-GQ1b antibodies compared to patients with relapsing-remitting MS, healthy controls and patients with other neurological diseases. This has led to the hypothesis that unconventional T cell reactions against GM3 and GQ1b could contribute to axonal damage.^{62,65}

AGAs have been found in systemic lupus erythematosus (SLE) patients with neuropsychiatric manifestations of the disease. In particular, anti-GM1 antibodies were associated with neuropsychiatric manifestations, highlighting their predictive and, potentially, prognostic value.^{66,67} Also celiac disease (CD), which can have a variety of neurological manifestations, e.g cerebellar ataxia, peripheral neuropathy, epilepsy and depression^{68,69}, has been associated with anti-GM1 antibodies.⁷⁰ The trigger that induces the generation of AGAs is not known. However, it is possible that the formation of complexes between gliadin and GM1 leads to the generation of anti-GM1 antibodies as a "side product".^{71,72} AGAs have also been found in patients with type 1 diabetes but their correlation with the development of diabetic neuropathy has not been confirmed.^{73,74}

AGAs in healthy individuals

The presence of AGAs in the general population has not been studied thoroughly. However, estimates can be drawn from the data of healthy control groups from many AGA studies. The incidence of AGAs in healthy individuals in control groups appear to range from 1 to 9%.^{75,76} Sometimes, IgM class anti-GM1 antibodies can be found in the sera of healthy individuals after bacterial infections, while the presence of IgG antibodies, especially of IgG1 and IgG3 subclasses, are generally linked to pathological processes.⁷⁷

Guillain-Barré syndrome

Gangliosides have many roles in the development of infectious diseases and neurological conditions triggered by infections. In the beginning of infection, gangliosides and other glycosphingolipids can serve as receptors for the entry of many viruses, and for binding of bacteria and fungi, or for toxins produced by bacteria. Pathogens may also carry structural elements on their surfaces, e.g., lipo-oligosaccharides (LOS) which resemble gangliosides. This structural similarity may protect pathogenic bacteria from both innate and adaptive immune defense reactions of the host. On the other hand, in the absence or upon loss of tolerance to these structures, the microbial infections may lead to production of autoantibodies against gangliosides. These would be due to immunological cross-reactions that lead to production of AGAs, which recognize also host gangliosides.

Since the first reports of the disease by Guillain, Barré and Strohl in 1916⁷⁸ our knowledge of Guillain-Barré syndrome (GBS) has exponentially increased over the last century.⁷⁹ In particular, IgG AGAs are found in more than half of GBS patients and are strongly indicative of the disease, while the role of IgM is still not clear.⁸⁰ GBS is the most common cause of a flaccid paralysis worldwide. It is considered to be a postinfectious, inflammatory, peripheral neuropathy.⁸¹ Clinically, it is characterized by weakness and areflexia or hyporeflexia in all four limbs.^{82,83} GBS has a plethora of variants but the most relevant ones are an acute inflammatory demyelinating polyneuropathy (AIDP), the axonal forms (AMAN and AMSAN), Miller-Fisher syndrome (MFS) and Bickerstaff brainstem encephalitis (BEE).

AIDP is the most common form of GBS. It is characterized by demyelination, probably mediated by antibodies against antigens in the paranodal region or directly on the Schwann cells. AIDP has been associated with antibodies against paranodal proteins like neurofascin 155 and contactin 1.^{4,84} Associations of AIDP with AGAs, such as GT1b⁸⁵ are still controversial in the literature because a substantial number of AIDP cases could be reclassified as AMAN or AMSAN.^{86,87} As previously mentioned, axonal forms of the disease have been described, in which direct axonal damage is mediated by the attack of AGAs against gangliosides located in the nodes of Ranvier.⁴ Two main axonal forms of GBS are recognized: acute motor axonal neuropathy (AMAN) and acute motor and sensory axonal neuropathy (AMSAN). According to Hughes and Cornblath, antibodies to GM1, GM1b, GD1a, and GalNac-GD1a are in particular implicated in acute motor axonal neuropathy and also, with the exception of GalNac-GD1a, in acute motor and sensory axonal neuropathy.^{81,88,89}

Miller-Fisher syndrome (MFS) was described for the first time in 1956 by Charles Fisher Miller.⁹⁰ It is characterized by the clinical triad of ophthalmoplegia, ataxia and areflexia. Bickerstaff brainstem encephalitis (BEE), clinically very similar to MFS, is characterized by hypersomnolence, ophthalmoplegia and ataxia.⁸² Both syndromes are associated with anti-GQ1b antibodies, suggesting that they are two distinct manifestations of a single disease spectrum.^{91,92} Subsequent testing of the anti-GQ1b antibodies revealed that they react also with GD3 and GT1b gangliosides, making it difficult to identify a clear target for the immunological attack.⁹³

As is apparent from the above, there is no doubt that anti-ganglioside antibodies may cause a variety of serious neurological diseases. However, antibodies alone rarely cause target cell damage. They recruit the complement system, leukocytes and other effector mechanisms into the area of antibody deposition. They mediate inflammation and cell injury, whose extent depends on the types of antibodies (class, subclass, target specificity and affinity), individual patterns or reactivity (including the MHC-type determining the extent of T cell help) and target cell sensitivity to damage. Importantly, sialic acids on gangliosides usually protect cells from complement attack, but this protection may fail, if AGAs neutralize the protective layer on cell surfaces. As pointed out

above, the complement inhibitor factor H is a key player in protecting host cells from complement attack, because it binds directly to gangliosides in the context of prior antibody-mediated C3b deposition. Inhibition of factor H binding to neuronal cells predisposes them to complement-mediated cell damage and attack by inflammatory leukocytes.

INFECTIONS ASSOCIATED WITH GBS DEVELOPMENT

Campylobacter jejuni

Numerous infectious agents have been associated with the onset of GBS and consequently with the production of AGAs. The most important pathogen associated with GBS is *Campylobacter jejuni*.^{9,94} Almost three-quarters of the GBS patients developed the disease after a gastrointestinal or respiratory infection.⁹⁴

The onset of GBS can be explained by the molecular mimicry that occurs between gangliosides and the campylobacter's lipo-oligosaccharides (LOS).⁷ Although GM1 in particular is indicated as the target for AGAs in GBS, there are many other homologs between the campylobacter LOS and human gangliosides.⁹⁵ As stated by Shahrizaila and Yuki the association between GBS and *C. jejuni* is the first human autoimmune disorder that fulfills the criteria to conclude that GBS is caused by molecular mimicry.⁹⁶ The criteria include (1) the establishment of an epidemiological association between infectious agent and the immune-mediated disease. In general, epidemiological studies have shown 30–35% positivity for *C. jejuni* in GBS patients as compared to 4.4% in controls.⁹⁷ The second (2) criterium is to identify antibodies or T cells directed against the target antigen. As previously stated, AGAs were identified in patients with GBS and recognized as the key pathological element in the pathogenesis. Depending on the ganglioside targeted a different manifestation of GBS may occur. Interestingly, also activated T cells and macrophages have been identified around the nerves of GBS patients.⁸⁸ Apparently, patients develop T cell responses against the exogenous LOS and endogenous glycolipids (gangliosides). Further studies should address the nature of the responding T cells. Possible candidates include innate lymphoid cells, gamma-delta T cells or NK cells. As the third criterium (3) microbial mimics to the target antigens have been identified. They include the low molecular weight LOS of *C. jejuni*. It has been observed that only certain specific *C. jejuni* serotypes are associated with GBS, in particular O:19 and O:41.^{94,98} These serotypes correspond to the development of AGAs especially against GM1 but also to other gangliosides, like GD1a and GD3. As the final criterium (4) it has been shown that GBS can be induced in a rabbit model by an injection with either the GM1 ganglioside or the homologous LOS of *C. jejuni*.⁹⁹ The antibodies were found to disrupt lipid rafts, nodal structures and ion channel clusters in the peripheral motor nerves.⁹⁹ Interestingly, in the animal model the neuronal damage could be inhibited with the synthetic serine protease inhibitor nafamostat mesilate that also acts as a complement inhibitor.¹⁰⁰

Pathogens other than *C. jejuni*

GBS and AGA development can be a post-infectious condition associated with many pathogens other than *C. jejuni*. These include *M. pneumoniae*, *H. influenzae*, cytomegalovirus and Epstein-Barr virus.^{9,101–103} *M. pneumoniae* has been linked to the development of GBS. These patients developed antibodies not only against gangliosides but also against anti-galactocerebrosides.^{104–106} *H. influenzae* has GM1-like structures on its surface that can elicit a mimicry-type antibody response similar to the one seen in *C. jejuni* infections.^{107–109} Analyses of the antecedent infections in GBS and MFS have indicated that *C. jejuni* is the most common causative agent for infections preceding the occurrence of the neurological disease, being usually observed in approximately 21–32% of the cases.^{101,110}

Interestingly, it has become clear that also viral infections can predispose to GBS, although the mechanisms may be different than in the case of bacterial infections. Cytomegalovirus has been mainly associated with the development of IgM class anti-GM2 antibodies.^{111,112} The Varicella-Zoster virus, also known as HHV-3, has been associated with GBS in rare cases.^{113,114} Recently, GBS has been associated with arboviruses infections, like Zika virus and chikungunya, but the mechanisms are still debated.⁴⁹

Zika virus (ZIKV) has caused recent outbreaks in Latin America, Africa and French Polynesia. It has been associated with neurological sequelae such as microcephaly in newborns and GBS in adults.^{115,116} Microcephaly seems to be related to a direct neurotropic effect of the virus on the neural cells of the newborn, given its ability to cross the placenta, while the pathogenesis of GBS is far from being understood.^{115,117} An autoimmune etiology, through a mechanism of molecular mimicry similar to the one observed in *C. jejuni*, has been hypothesized, but the mechanism may differ.¹¹⁸ In a few studies, AGAs have been found in GBS patients having antibodies against ZIKV. Nico et al. reported anti-GD3 antibodies in acute ZIKV infections.¹¹⁹ Baskar and colleagues showed anti-GM1 antibodies in 50% of ZIKV-positive patients.¹²⁰ However, Cao-Lormeau and his team showed that only a fraction of patients with GBS after ZIKV had AGAs, and their sera (anti-GA1 and GD1a) displayed no antibody competition between ZIKV proteins and GA1, a.k.a. asialo GM1.¹¹⁶ In addition to ZIKV, also other arbovirus infections have been associated with GBS, like dengue, West Nile and chikungunya with sporadic reports in the literature. Case reports have been published on GBS in patients with hepatitis E¹²¹ or herpes simplex virus infection.¹²² Parvovirus B19 infections are associated with a broad spectrum of neurological complications. Anti-GD1b AGAs were described in a case report by Sequeira et al. in a 23-year-old immunocompetent woman with a PB19 infection associated with hemolytic anemia and cranial polyneuropathy.¹²³

While in some cases the associations between viral infections and GBS may be coincidental, it is possible that viral infections could lead to autoimmunity e.g., by exposing endogenous structures. One possibility is that a viral infection of cells may force them to release cell membrane particles containing gangliosides, e.g., from the membrane rafts, which then, in association with viral structures, could lead to a bystander-type autoimmune response.

Possible functional roles of AGAs

It has been suggested that in Guillain-Barré syndrome AGAs play a role in the destruction of the neuromuscular junction (NMJ) of nerve cells. NMJ is rich in gangliosides and resides outside the blood-nerve barrier thus making it more vulnerable to attack by antibodies. In the Miller-Fisher variant of GBS, characterized by ataxia, areflexia and ophtalmoplegia, high titer AGAs against GQ1b are present in the majority of patients in the acute phase. These AGAs cross-react often with GT1a, and may react with GD3 and GD1b gangliosides, as well. They activate the human complement system and mediate complement-dependent injury in the motor nerve terminal.^{124,125} Complement activation will also attract leukocytes to neurons and contribute to activation of neutrophils and macrophages. Often, also T lymphocytes have been detected in the area of damage indicating a cell-mediated immune response. The activated immune cells can release cytokines and oxygen radicals to further damage the target cells. As a result, the protective myelin sheath and even the neuronal axons may become destroyed.

Experimental data by Kanda has revealed that AGAs in vitro can disrupt the integrity of the blood nerve barrier (BNB), possibly foreshadowing a similar effect on the blood brain barrier (BBB).¹²⁶ Breakdown of BBB or changes in its permeability can be critical events in the pathogenesis of many neurological diseases and AGAs may be key players of the neurological damage.^{127,128}

Secondary narcolepsy following Pandemrix® vaccination

Type I narcolepsy (NT1) is a chronic sleep disorder characterized by excessive daytime sleepiness, cataplexy and, commonly, parasomnias.^{129,130} The prevalence of narcolepsy is circa 30 per 100,000. It occurs in a bimodal distribution with two peaks at the ages of 15 and 35 years.^{130,131} Cataplexy is defined as a sudden loss of bilateral muscular tone, usually brought on by a strong emotional response. It is essentially unique to narcolepsy type I.¹³²

A clue to the etiology of narcolepsy was obtained, when narcoleptic dogs were found to be genetically deficient in the orexin receptor type 2.¹³³ Human NT1 is caused by a reduced orexinergic signaling, probably due to a loss of orexinergic neurons in the hypothalamus.^{131,132} The neuropeptide product of the neurons, orexin, is also known as hypocretin, encoded by the *HCRT* gene. It is involved in maintaining the sleep-wake cycle and regulating muscle tone and appetite.

There are different genetic traits related to the human narcoleptic phenotype, i.e., mutations in the T-cell-receptor α chain and purinergic receptor subtype 2Y11.¹³¹ It associates strongly with the HLA-DQB1*06:02 allele, present in practically all narcoleptic patients.¹³⁴ However, the etiology of NT1 is still under investigation, but in the majority of cases it is an autoimmune disease. No direct evidence is available for the pathogenetic mechanisms in humans but evidence from studies in mice indicate that CD8+T cells can mediate killing of orexinergic neurons to give a narcolepsy-like phenotype.¹³⁵

Surprisingly, narcolepsy has been associated with the pandemic H1N1 flu vaccine. In Finland and Sweden, between October 2009 and August 2010, and later in other countries, after the vaccination campaign against the H1N1-type influenza A with the *Pandemrix* vaccine (GSK), a clear increase in narcolepsy cases was reported. The cases were observed in particular in children and adolescents, ranging from 4 to 19 years of age. The vaccination increased the risk of developing narcolepsy 6.5–14.4-fold in vaccinated subjects compared to unvaccinated ones.^{136–138}

An increase in narcolepsy was not restricted to the flu vaccine. Interestingly, in China, in the absence of vaccination, a 6-fold increase in the incidence of narcolepsy was observed after the 2009 influenza pandemics.¹³⁹

The mechanisms and potential autoantigen underlying the development of H1N1 vaccine- or virus infection-induced narcolepsy have remained elusive. The receptor for the H1N1-virus hemagglutinin H1 is sialic acid, α 2–3 or α 2–6 linked to galactose, structures commonly present on gangliosides, like GD3. Furthermore, the other main protein on the virus particle surface is neuraminidase N1, capable of cleaving sialic acids. A molecular mimicry between orexin and the viral proteins leading to T cell cross-reactivity has been suggested.¹⁴⁰ *Pandemrix* is an AS03-adjuncted vaccine that contains α -tocopherol, which in vitro can increase the expression of orexin, upregulating the Nrf2 pathway.¹⁴¹ In this way it can potentially favor cross-reactive antigen presentation by MHC class II.¹⁴² Another influenza A (H1N1) vaccine, *Arepanrix* (also by GSK) with a similar composition, same adjuvant and antigenicity as *Pandemrix*,¹⁴³ did not show any increase in narcolepsy incidence.¹⁴⁴ This could have been due to a different protein antigen composition in the vaccine.¹⁴⁵

In our own study¹³⁷ we found AGAs, in particular anti-GM3 and anti-GM4, in the sera of 18.1% of patients with *Pandemrix*-induced narcolepsy (compared to 7.3% in unvaccinated controls). The origin of these antibodies could be an immune response against the vaccine adjuvant (squalene) preparation that is from shark liver, a source rich in the GM3 and GM4 gangliosides. The role of anti-ganglioside antibodies in the pathological process is not clear. While the disease most likely is a specific autoimmune response to distinct self antigen(s) expressed by the orexin producing neurons the anti-ganglioside antibodies could have an additional role. A model for the potential disease development is depicted in Fig. 2. Anti-GM3 or anti-GM4 antibodies could (1) directly damage the

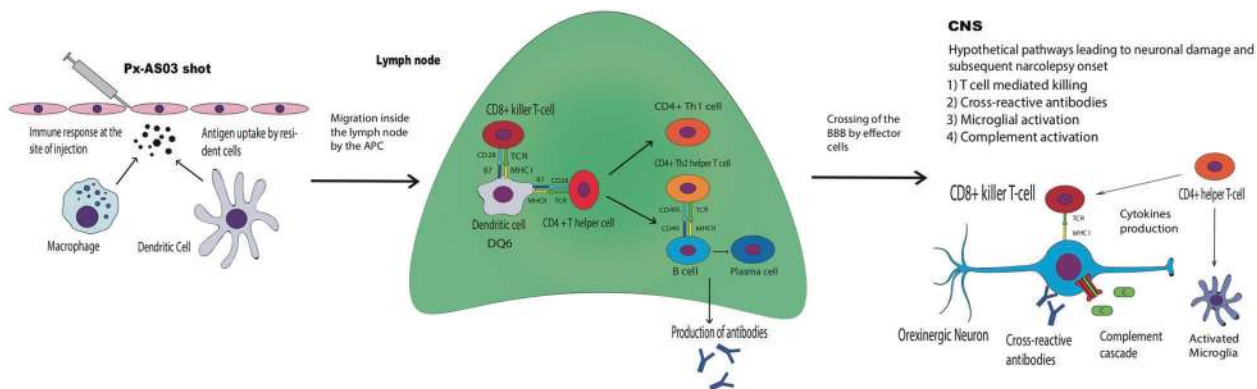


Fig. 2 A model for the pathogenesis of secondary narcolepsy following Pandemrix® vaccination. In susceptible recipients, i.e., in those who harbor the HLA-DQB1*06:02 allele, antigen presenting cells (APCs, dendritic cells and macrophages) gather at the injection site after the vaccine shot. Following the uptake of and response to vaccine components (e.g., H1 and N1 proteins, squalene, α -tocopherol, possible nucleoprotein or ganglioside impurities) the APCs migrate to the lymph node presenting the antigens to the T-cells through the MHC class II, inducing their activation. Eventually, through a process of molecular mimicry this mechanism could lead to a targeted attack against hypothalamic orexinergic neurons and to the subsequent narcolepsy onset. Antiganglioside antibodies could emerge upon complex formation between H1N1 proteins and the gangliosides, which would be picked up by the ganglioside responding B cells. T cells specific for the H1N1 proteins would provide the necessary help for antibody formation by B cells, and subsequently by plasma cells. Antiganglioside antibodies have an ability to both activate complement and to neutralize the complement inhibitory gangliosides on neuronal cell surfaces

hypothalamic neurons by binding to their gangliosides and activating the complement system or inflammatory cells. This is unlikely to be a major mechanism given the presence of these autoantibodies only in a proportion of the patients. On the other hand, they are frequently observed in the context of neurological disorders and could have an effect on the aforesaid nervous damage. (2) Since GM3 is a major endothelial ganglioside, anti-GM3 AGAs could damage the cerebral endothelium and BBB increasing its permeability to immune cells and mediators. (3) Furthermore, anti-GM3 autoantibodies could bind to lipid raft gangliosides and activate a signaling pathway that leads to the recruitment of molecules like integrins that can potentially facilitate the chemotaxis of the leukocytes.¹⁴⁶ A similar effect has been observed in melanoma cell lines, in which GD3 activation leads to the recruitment of β 1 integrins.¹⁴⁷ These potential mechanisms could play a role in any disease, where antiganglioside antibodies are formed (Table 2).

CONCLUSIONS

Gangliosides and especially their key variable components, sialic acids, are at the border of immune tolerance. In general, sialic acids protect and shield human cell and tissue structures against immune attacks. In consequence, certain microbes have learnt to mimic or exploit these structures to escape immune attack or to use them as receptors for entry into human tissues. On some occasions, however, the tolerance is broken, and the immune system succeeds in responding to the microbial mimics of sialic acids with consequent autoimmunity as a byproduct. Examples include neuronal autoimmune diseases like Guillain-Barré and Miller-Fisher syndromes.

The role of AGAs in the pathogenesis of many different diseases is not fully understood. AGAs could (i) participate in the direct damage to the structure that they bind to, (ii) be a sequela of different types of infectious diseases, (iii) facilitate many immune-mediated pathological mechanisms, including complement activation and the enhancement of its functional effects because of neutralization of a basic complement control mechanism of the cells.

Relative to proteins, immune responses to carbohydrates and glycolipids have often received less attention in studies of autoimmunity despite the fact that they could have a direct or an important adjunct role. Future studies should address the

Disease(s) associated	AGAs	References
Alzheimer's disease	GM1, GD1b (IgM strongly correlates with AD), GQ1b α (association still debated)	Chapman ⁵⁰ , Hatziflippu ⁵² , Koutsouraki ⁵¹
Multiple sclerosis	GM3, GQ1b	Pender ⁶⁵
Systemic lupus erythematosus	GM1	Costellat ¹⁵² , Galeazzi ⁶⁷
Celiac disease	GM1 (or GM1-gliadin complexes)	Przybylska ⁷⁰
HIV (asymptomatic patients)	GM2, GD1a, GQ1b	Nicolae ¹⁵³
Parvovirus B19	GD1b	Sequeira ¹²³
Type I diabetes	GM1, GM2, GM3, GD1b, GD1a	Lucchetta ⁷³
Secondary narcolepsy	GM3, GM4	Saariaho ¹³⁷
Guillain-Barré syndrome	AIDP: GT1b AMAN: GM1, GM1b, GD1a, GalNac-GD1a AMSAN: GM1, GM1b, GD1a MF: GQ1b BE: GQ1b	Hughes and Cornblath ⁸⁸ , Naik ⁸⁵
Breast cancer	GM3, GD3, 9-O-Ac GD3, 9-O-Ac GT3	Groux-Degroote ¹⁵⁴

abilities of antibodies to neutralize the functions of gangliosides, including their roles as receptors or protective molecules against immune attack. Therapeutic inhibition of complement activation could be considered in cases, where the protective ability of gangliosides has become compromised, and complement causes serious injury. Because of varying expression during different life periods, gangliosides could explain relative sensitivities to diseases in different age groups, especially in the elderly people. Their roles as receptors for infectious agents and initiators of immunological cross-reactions still needs further exploration. Also, studies on the roles of gangliosides

in T cell-mediated immune responses and in regulating activities of nonconventional T cells, NK cells and innate lymphoid cells could prove useful and interesting in the future.

ACKNOWLEDGEMENTS

This work was supported by the Sigrid Jusélius Foundation, the Academy of Finland (292393) and Helsinki University Central Hospital (TYH2018313, TYH2019311) grants.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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