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Guillain-Barré syndrome: expanding the concept of molecular mimicry

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Abstract

Guillain-Barré syndrome (GBS) is a rapidly progressive, monophasic, and potentially devastating immune-mediated neuropathy in humans. Preceding infections trigger the production of cross-reactive antibodies against gangliosides concentrated in human peripheral nerves. GBS is elicited by at least five distinct common bacterial and viral pathogens, speaking to the notion of polymicrobial disease causation. This Opinion emphasizes that GBS is the best-supported example of true molecular mimicry at the B-cell level. Moreover, we argue that mechanistically, single and multiplexed microbial carbohydrate epitopes induce IgM, IgA, and IgG subclasses in ways that challenge the classic concept of thymus-dependent (TD) versus thymus-independent (TI) antibody responses in GBS. Finally, we discuss how GBS can be exemplary for driving innovation in diagnostics and immunotherapy for other antibody-driven neurological diseases.

GBS, true molecular mimicry at the B-cell level

In molecular mimicry, the antigenic structures of pathogens and humans are sufficiently similar to induce an autoreactive response of T or B lymphocytes after infection, contributing to disease pathogenesis [1]. A well-known example of a disease likely induced via molecular mimicry is rheumatic fever caused by *Streptococcus pyogenes* [2]. Although the concept of molecular mimicry is intuitive and mechanistically appealing, it is

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exceedingly hard to prove [3, 4]. Historically, much work on molecular mimicry focuses on T-cell cross-reactivity, and much less on B cells or antibodies.

Guillain-Barré syndrome (GBS) is an immune-mediated neuropathy causing a rapidly progressive weakness that may affect respiratory muscles for which patients need ventilation at an ICU (Box 1 Clinician's corner, and websites for patients, neurologists and other resources[i], [ii], [iii], [iv]). GBS can develop within 1–3 weeks after infection with several commonly found pathogens, including viruses and bacteria. These include *Campylobacter jejuni,* involved in approximately 30% of GBS cases and a common cause of bacterial gastroenteritis, *Mycoplasma pneumoniae*, hepatitis E virus (HEV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and Zika virus (ZIKV)[5]. The associations between these pathogens and GBS have all been proven in comparative case-control studies [6, 7]. Other infections may trigger GBS as well, including influenza virus [8], but case-control studies are lacking. Recent studies have indicated that there is a small risk that SARS-CoV-2 infections may precede GBS [9, 10], which may be higher than after vaccination against SARS-CoV2 [9]. Of note, GBS is a rare disease with an incidence rate of approximately 1–2 cases per 100,000 per year, worldwide. However, pandemic or outbreaks of infections might increase the incidence of GBS temporarily or locally, as was observed in French Polynesia during the ZIKV outbreak and recently, in Peru with *C. jejuni* infection[6, 11]. Nevertheless, the risk for developing GBS following *C. jejuni* infection is estimated to be only 1 in 1,000–5,000 people, indicating that pathogen and host factors crucially determine susceptibility for developing GBS [12, 13].

There are several clinical variants of GBS that relate to the type of peripheral nerves involved. For instance, the classic **sensorimotor form** of GBS causes limb muscle weakness as well as sensory deficits, and is the most frequent manifestation in the Western world [14]. The **pure motor form** of GBS causes only muscle weakness of the limbs, whereas in the **Miller Fisher syndrome (MFS)**, weakness is restricted to muscles involved in eye movements which cause double vision [14]. Ataxia is also a prominent feature in MFS. In approximately half of GBS patients, serum antibodies against various gangliosides and other glycolipids are found [15–17]. Gangliosides are a family of sialylated glycolipids abundantly expressed in human cell membranes. Crucial to GBS is their high concentration in pre-synaptic membranes of the neuromuscular junction and their presence in the **axolemma** at the **nodes of Ranvier** which allow **saltatory nerve conduction** [18]. Peripheral nerves vary in their ganglioside composition, which contributes to explain the association between a patient's clinical variant and the specificity of the anti-ganglioside antibodies detected (Figure 2). For example the motor variant of GBS is highly associated with serum antibodies to the GM1a and GD1a gangliosides present in motor nerves [19]; by contrast, MFS is highly associated with the presence of antibodies directed against the GQ1b ganglioside present in oculomotor nerves [20]. In addition, prior *C. jejuni* infections are associated with the presence of antibodies to GM1, GD1a, and GQ1b [17]. The rare occurrence of GBS

[i]European and transcontinental websites for patients: https://www.gbs-cidp.org/
[ii]International websites for clinical neurologists on diagnosis, prognosis and treatment: https://rede.tghn.org/gbs-flowchart-sample/gbs-main-sub/
[iii]International GBS Outcome Study IGOS-consortium: https://gbsstudies.erasmusmc.nl/
[iv]Explanation of GBS for the general public by Dr. Ruth Huizinga: https://www.youtube.com/watch?v=XwosOoagyeg

following a relatively common infection such as *C. jejuni* suggests that the B-cell response to gangliosides is strictly controlled, and that a series of factors need to coalesce to develop a pathogenic cross-reactive antibody response. GBS has a typical monophasic clinical course which manifests as a rapid disease progression (within days to weeks) followed by a slow recovery (within weeks to months) which parallels the reduction in antibody titers [21]. Although in GBS, active disease lasts less than a week in most patients, nerve recovery is frequently incomplete and often results in residual disability and complaints.

While GBS is clearly immune-mediated, it lacks some typical general features of autoimmune diseases such as chronic disease course and association with certain HLA class I or II alleles (Box 2) [22]. Instead, GBS behaves more like a post-infectious disease because of the strong relationship with a preceding infection and the monophasic disease course [5–8]. Indeed, we have previously proposed that GBS is one of the best-documented examples of a disease caused by molecular mimicry at the B-cell level because all revisited **Witebsky's postulates** [23] – from epidemiological disease association to reproduction of disease in animal models – have been fulfilled [24]. Thus, in the case of GBS, similar epitopes in pathogens and human hosts have resulted in actual pathology stemming from cross-reactive antibodies; mimicry epitopes have been firmly established for *C. jejuni* within the **lipo-oligosaccharide** (LOS) inducing pathogenic antibodies to host gangliosides [25]. As shown in Figure 3, it is the carbohydrate part of LOS (and not the lipid part), that mimics the ganglioside. Of note, LOS from *C. jejuni* isolated from patients with pure motor GBS mimic GM1 and GD1a, while LOS from *C. jejuni* isolated from patients with MFS mimics GQ1b [25]. Similar cross-reactive antibodies to those found in GBS patients have been induced in mice and rabbits after immunization with LOS from *C. jejuni* isolates derived from GBS patients; moreover, such antibodies against several gangliosides have induced complement-mediated neural damage [25,26]. For other pathogens related to GBS, specific criteria remain to be fulfilled. However, in the case of *M. pneumoniae*, the epidemiological association between preceding infection and the development of GBS is firmly established and cross-reactive antibodies against galactocerebrosides that are present in human nerves have been identified [26, 27]; nevertheless, the exact microbial mimics remain to be identified.

Recently, several studies have deepened our understanding of the pathogenesis of GBS in terms of the role of carbohydrate mimicry, the cross-reactive antibody response, and its effect on nerves. In this Opinion, we describe how novel chemoenzymatic approaches can be used to create host epitopes and mimetics as well as multiplex assays; we argue that this approach can lead to a deeper understanding of the mechanism by which molecular mimicry can induce autoimmunity. Furthermore, we discuss how antibody responses against gangliosides shed new light on the classic **TI-TD paradigm.** Finally we outline recent progress in other neuroinflammatory disorders in which molecular mimicry may be involved.

Antibodies to complexes of heteromeric glycolipids

A groundbreaking discovery in 2004 documented (via ELISA) that patients with GBS could harbor serum antibodies against a heterodimer complex of GD1a and GD1b, instead

of harboring antibodies against each individual ganglioside alone [28]. Furthermore, the strength of antibody binding to a particular ganglioside could either be enhanced or reduced by close proximity to other gangliosides [26]. The relevance of this finding was demonstrated in a murine ex vivo muscle-nerve preparation showing that certain antibodies targeting GM1 did not bind to live nerve terminals due to the presence of other gangliosides in close proximity to GM1 [29]. Similar modifying effects have been described for other (glyco-)lipids, including cholesterol, galactocerebroside, and sulfatide [30], suggesting that this phenomenon is not restricted to gangliosides alone. Moreover, the optimal spacing of glycolipids might also be important for the formation of IgG hexamers against gangliosides, allowing proper binding of **C1q complement** [31]. This is relevant as complement activation contributes to nerve damage in GBS via the formation of the membrane attack complex (MAC) [32].

Protective effect of anti-glycolipid antibody endocytosis in cells

In GBS, antibodies to gangliosides bind both the peripheral nerve terminal and the nodes of Ranvier where binding is not prevented by myelin or **Schwann cells** [33]. Notably, neural tissues appear to be particularly sensitive to antibodies binding targets embedded in myelin and axonal membranes (evidence summarized in Box 3). Some nerve regions however are more vulnerable than others to damage, and in a landmark study, [34] researchers built on evidence that neuronal membranes with high endocytic activity, including in nerve terminals, were less vulnerable to damage due to the rapid endocytosis of bound antibodies [35]. Specifically, anti-ganglioside antibody administered to transgenic mice expressing gangliosides exclusively in neurons (*GalNAcT*$^{-/-}$*Tg*(neuronal) mice) was rapidly cleared by endocytic uptake at nerve terminals. Subsequently, the antibody was transported in retrograde manner to the cell body of the motor neuron in the spinal cord and locally secreted [20]. This might contribute to explain why patients with MFS harboring anti-GQ1b antibodies initially present with peripheral oculomotor neuropathy followed by brainstem encephalitis (‘Bickerstaff encephalitis’) [36].

Novel insight into antibody responses to glycolipids

The finding that anti-glycolipid antibodies are removed from circulation after endocytosis [34] also prompts re-evaluation of the concept of B-cell tolerance to gangliosides. For a long time, immunization of rodents with self-glycolipids to induce antibodies has proven difficult, requiring extensive immunization regimens, or the use of transgenic animals; many of these obstacles have been attributed to B-cell tolerance mechanisms [37, 38]. However, a study demonstrating anti-GD1b antibody- secreting B cells (via ELISPOT) following immunization of wildtype mice with GD1b-containing liposomes [34], indicates that B cells can respond to gangliosides, at least in mice. Also in humans, antibodies against gangliosides can be comprised in the normal immune repertoire, as evidenced by the presence of IgM antibodies against GM1 in healthy individuals [39]. Because B-cell tolerance to gangliosides may not be complete, regulatory mechanisms are likely present that limit the risk of developing autoimmunity to self-glycolipids and this may be impaired in patients with GBS. In support of this, GBS patients were recently found to exhibit defective signaling from the inhibitory receptor Siglec-10 (expressed by B cells);

consequently such impaired signaling could have resulted or contributed to aberrant B-cell responses against gangliosides or sialic-acid bearing molecules such as LOS [40].

Therefore, how are B cells activated to produce anti-glycolipid antibodies in GBS?
The antibody response to glycolipids in GBS does not conform to the standard TD-TI definitions [41], because these glycolipid antibodies recognize repetitive carbohydrates but are dominated by IgG1 and IgG3 subclasses whereas typical anti-carbohydrate antibodies are IgM or IgG2 (Box 3) [42]. However, **somatic hypermutations** reported in the anti-GM1 IgM of a GBS patient suggest that anti-glycolipid antibodies in GBS patients are generated in a TD-manner [43]. Since antibody responses to glycolipids are relatively short-lived in GBS patients (i.e. antibody titers decline within weeks to months), it is possible that class switching is induced by lymphocyte subsets other than T cells. In mice immunized with the haptenated-lipid antigen 4-Hydroxy-3-nitrophenylacetyl (NP)-α-GalCer, invariant NK T cells have been reported that promote TD-associated IgG subclasses in an IL-21 and CD1d-dependent manner; however, in this study, no memory B-cell responses were induced [44]. As memory responses to both TD and TI antigens require a germinal center reaction [45], it remains to be investigated whether a germinal center response to gangliosides is induced in mice and humans.

Recent breakthroughs in understanding molecular mimicry in GBS

C. jejuni strains associated with GBS often produce a mixture of LOS molecules mimicking the saccharide moieties of several gangliosides, such as GM1a and GD1a [25, 46]. The antibodies of GBS patients that recognize ganglioside complexes cross-react with purified LOS of these strains in vitro [47, 48], suggesting that antibodies to heteromeric complexes can also be induced via molecular mimicry. However, purified LOS contains a mixture of molecules which complicates detailed comparisons of antibody specificities and binding strengths. Moreover, the chemical structure of the saccharide component of gangliosides and the oligosaccharide of LOS of *C. jejuni* differ [25]. In particular, the formation of ganglio-oligosaccharides is initiated by a glucose residue followed by galactose, whereas LOS of GBS-associated pathogenic strains contains a heptose moiety attached to galactose (Figure 3) [25]. Furthermore, this LOS heptose is linked to an additional heptose and to ketodeoxyoctonic acid (KDO) moieties which together, form the inner core of LOS, further increasing their dissimilarity with gangliosides [46]. Of note, heptose is only expressed by gram-negative bacteria and its metabolite heptose-1,7-bisphosphate was recently identified as a bacterial **pathogen-associated molecular pattern** (PAMP) [49], demonstrating that human innate immunity has evolved to respond to, rather than tolerate, this oligosaccharide. We hypothesize that this could also be the case for adaptive immunity, in particular for B cells.

To test this hypothesis and develop a more robust platform to diagnose and monitor GBS disease, we exploited advances in chemoenzymatic synthesis to prepare a panel of well-defined and homogeneous oligosaccharides composed of the inner core of the LOS of *C. jejuni* extended by various ganglioside carbohydrate mimics (Figure 4a) [50]. Similar oligosaccharides derived for gangliosides were prepared. All synthetic carbohydrates were equipped with an artificial amino-containing linker which made it possible to

immobilize them on glass slides bearing amino reactive succinamate esters. The resulting glycan microarray provided a convenient platform to examine binding specificities of lectins, anti-ganglioside antibodies, and serum antibodies from GBS patients. Although lectins and monoclonal anti-ganglioside antibodies did not differentiate between ganglio-oligosaccharides and corresponding LOS mimics, GBS patient serum antibodies bound more strongly to particular LOS derived structures than to ganglio-oligosaccharides [50] (Figure 4b). The data suggest that antibodies were elicited against a foreign epitope containing a heptosyl residue which is unique to bacterial LOS, and to an oligosaccharide component mimicking that of gangliosides. The antibodies could cross-react with particular gangliosides because these represent partial epitopes [50]. In summary, these findings suggest that a certain degree of structural dissimilarity is required to break immuno-tolerance and presumably, to develop autoimmunity via molecular mimicry.

Molecular mimicry in other neuroinflammatory diseases

Classic as well as recent work has significantly deepened our understanding of molecular mimicry driving human neurological diseases other than GBS [51–55], notably multiple sclerosis (MS), **neuromyelitis optica spectrum disorder** (NMOSD) and narcolepsy. Table 1 provides a comprehensive selection of putative mimicry epitopes, with variable levels of experimental evidence derived from studies in animal models and patient samples.

Although MS mimicry research mostly focuses on T-cell epitopes, an exciting study from 2022 [54] provides evidence of a human antibody that is cross-reactive between the EBV nuclear antigen 1 (EBNA1) epitope 386–405 and the central nervous system (CNS) protein glial cell adhesion molecule (GlialCAM). It has been suggested that such antibodies might potentially contribute to brain tissue damage in MS; immunization with this specific EBNA1 epitope aggravated symptoms of experimental autoimmune encephalomyelitis (EAE; model for MS) in SJL/J mice, and appeared to be driven by proteolipid protein (PLP) peptide $_{139–151}$ [54].

In MS patients, recent evidence of mimicry at the T-cell level against EBV and *Akkermansia muciniphila* has been reported [53]; *A. muciniphila* is a gut commensal with features that might limit or promote MS and gut inflammation. Epitopes from *A. muciniphila* are presented by HLA-DR15 molecules, and autoreactive T-cell clones cross-react with HLA-DR-derived self-peptides, peptides derived from EBV and *A. muciniphila*, as well as autoantigens [53]. *HLA-II* polymorphisms are considered a major genetic risk factor for developing MS [56]. A similar study with MS patient $CD4^+$ T cells identified a novel candidate autoantigen peptide from RAS Guanyl Releasing Protein 2 (RASGRP2) that is expressed in neurons and B cells [57]. Thus, identifying a putative microbial mimic of RASGRP2 would be most valuable. Another example of molecular mimicry is the presence, in some MS patients, of IgG antibodies binding both the EBV protein EBNA-1 and anoctamin 2, a chloride channel protein on neurons in the CNS [58].

Finally, using the EAE model in C57BL/6 mice immunized with $MOG_{35–55}$ peptide, one study showed that two bacterial species in the gut microbiota jointly activated myelin oligodendrocyte glycoprotein (MOG)-specific $CD4^+$ T cells [51]. Specifically, *Lactobacillus*

reuteri expressed mimicry peptides in its UvrABC system protein A (UvrA) protein, while Erysipelotrichaceae bacteria elicited pro-inflammatory factors IL-23 and serum amyloid A (SAA) which enhanced the effector function of pathogenic Th17 cells, exacerbating EAE [51]. Although in need of confirmation in humans, these findings suggest that two key constituent gut commensal bacteria activate CNS-specific T cells, where one species yields mimicry epitopes (e.g. MOG), and the other elicits inflammatory responses (e.g. IL-23 and SAA).

In NMOSD, there is evidence for an antibody mimicry epitope expressed by *Clostridium perfringens* and a host epitope of aquaporin-4 (AQP4), expressed by astrocytes [59]. Particular Clostridia clusters (IV, XIVa and XVII) promote T-cell regulation [59]. Overabundance of *C. perfringens* appears to generate AQP-specific T and B cells, and plasma cells secrete pathogenic antibodies against AQP4. Moreover, the ABC-TP of *C. perfringens* sequence 204–217 has homology with $AQP4_{63–76}$ [59].

In type 1 narcolepsy, autoantibodies contributing to pathophysiology have not been consistently identified despite large efforts, and the current consensus is that a T-cell mediated response that includes mimicry epitopes is likely contributing to the pathogenic mechanism [60].

In summary, accruing evidence in certain neurological diseases other than GBS suggests that there are key contributions to neural tissue damage that may be mediated by both T- and B-cell mimicry epitopes.

Concluding remarks

By numbers, GBS is a minor disease; however, it provides new insight into basic immunology in terms of TD and TI antigens, antibody and complement functionality, and the fascinating but complex molecular mimicry concept.

The insights and technologies discussed in this Opinion article contribute to the arsenal needed to achieve rapid and updated progress in immunopathogenesis, diagnostics, and innovative treatments for patients suffering from GBS and related diseases in which molecular mimicry is implicated. Indeed, with newly developed innovative technologies such as microarrays of synthetic oligosaccharides, the community can further examine multiplexed carbohydrate antigen-forming antibody epitopes, as well as molecular neighbor and crypto effects on antibody binding. This is important for clarifying the neurotoxic potential of anti-glycolipid antibodies and define the relationship between these antibodies and disease severity and outcomes. Indeed, the international research consortia such as the International GBS Outcome Study (IGOS)[iii] is promoting rapid worldwide diffusion of such insights and technologies, and aims to interpret these in the context of regional variation for GBS clinical phenotypes (e.g. [14]).

Many limitations and questions remain (see outstanding questions), the answer to which can help propel the GBS field forward, and will hopefully also cross-fertilize our understanding, diagnostic approaches, and therapy endeavors for other immune-mediated neurological diseases.

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Glossary

Astrocyte podocytes
end-feet of astrocyte extensions; contribute to the formation and function of the CNS blood-brain barrier.

Axolemma
axon plasma membrane.

Bickerstaff brainstem encephalitis (BBE)
rare variant of GBS presenting as peripheral neuropathy (usually the Miller Fisher syndrome) with weakness of muscles involved in eye movements, but progressing to limb weakness and lowered consciousness due to involvement of the brainstem as part of the CNS. BBE, like MFS, is associated with the presence of anti-ganglioside GQ1b antibodies.

Blood-nerve barrier
cell and tissue structures controlling access of soluble molecules, including pathogenic autoantibodies to the nerve. This barrier does not prevent infiltration of leukocyte subsets since these behave distinctly from soluble molecules. The BNB is less tight at nerve roots, ganglia, and nerve terminals.

C1q complement factor
8-polypeptide chain subcomponent of C1, the first component of the complement protein cascade, with a characteristic six-tulip like shape. The binding of the Fc-components of closely arrayed antibody molecules to C1q initiates the classic pathway of complement activation, including activation of the membrane attack complex (MAC). In humans, C1q can be bound by IgM, IgG1, IgG2 and IgG3, but not by other isotypes.

Gangliosides
sialic acid-containing glycosphingolipids, composed of ceramide and oligosaccharide; enriched in lipid rafts and highly expressed in nervous tissue. Frequent attachment site for microbial toxins.

Guillain-Barré syndrome (GBS)
immune-mediated peripheral neuropathy, usually triggered by a preceding infection; clinically characterized by rapidly progressive bilateral muscle weakness. GBS is clinically diverse and includes the predominant sensorimotor form, pure motor form, Miller Fisher syndrome (MFS), and other forms. The peripheral neuropathy is characterized

by demyelination, axonal degeneration, or both. The specificity of the antibodies to gangliosides is associated with the clinical form and neuropathy subtype.

Lipo-oligosaccharide (LOS)
endotoxin expressed by *C. jejuni*. LOS is chemically distinct from lipopolysaccharide (LPS) since it lacks the repetitive O-antigen. LOS consists of lipid A and an inner and outer core of oligosaccharides. Sialylated LOS strongly activates toll-like receptor TLR4.

Miller Fisher syndrome
Clinical variant of GBS characterized by oculomotor neuropathy and weakness of muscles involved in eye movements causing complaints in double vision; associated with anti-ganglioside GQ1b antibodies.

Molecular mimicry
Formal medical definition used in this Opinion is the structural similarities of host epitopes and pathogen epitopes which elicit autoreactive T and/or B cells driving pathogenesis. Postulates of Koch and Witebsky can be productively applied to validate suspected molecular mimicry.

Neuromyelitis optica spectrum disorder
In the CNS, dominated by inflammation of the optic nerve (optic neuritis) and the spinal cord (myelitis). Previously known as Devic disease or neuromyelitis optica.

Node of Ranvier
Incisures in the myelin sheath allowing saltatory pulse conduction. In the PNS, gangliosides are concentrated and/or more accessible to autoantibodies at the nodes of Ranvier.

Paranodal loops or spirals
At both sides of the node of Ranvier, the myelin sheath is not compacted, and is filled with cytoplasm of the myelinating Schwann cell (PNS) or oligodendrocyte (CNS), spirally wrapped around the axon. Paranodal spirals resemble loops in cross-section.

Pathogen-associated molecular patterns
conserved molecular motifs such as lipopolysaccharides or lipoproteins, present on pathogens and recognized by pattern recognition receptors.

Pure motor form of GBS
Clinical GBS variant; patients have muscle weakness but no sensory deficits; associated with anti-gangliosides GM1 and GD1a antibodies.

Saltatory conduction
occurs along myelinated axons; involves the propagation of electrical pulses from one node of Ranvier to another. Conduction velocity along myelinated fibers is much faster than along non-myelinated fibers (80–120 m/s versus 0.5 to 2.0 m/s).

Sensorimotor form of GBS
Predominant clinical GBS form; patients have sensory deficits in combination with muscle weakness.

Somatic hypermutation
mutations in the variable domains of immunoglobulin genes that occur during germinal center responses.

Schwann cells
cells of the PNS that myelinate one axon, while CNS oligodendrocytes insulate and provide saltatory pulse conduction by wrapping cytoplasmic extensions around up to 70 axons. Schwann cells also surround small-diameter axons that are non-myelinated.

Thymus-dependent (TD) versus thymus-independent (TI) antibody responses
requirements for B-cell activation and induction of antibody responses against antigens, based on classic thymectomy experiments and transgenic mice. As NK cell development also occurs in the thymus, a TD-response can also occur without T cell help.

Witebsky's postulates
a disease can be regarded as autoimmune based on: Direct evidence that it can be transferred by a pathogenic antibody or T cells; Indirect evidence based on the development of autoimmune disease in experimental animals; Conditional evidence from clinical signs and patient symptoms.

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Box 1.

Clinician's corner

- Guillain-Barré syndrome is a highly diverse disorder in terms of clinical presentation, course, and outcomes.
- Preceding infections, cross-reactive anti-glycolipid antibodies, as well as complement activation are the three key factors in the pathogenesis and diversity of GBS.
- Current treatments with immunoglobulins or plasmapheresis/exchange are insufficient for most GBS patients.
- Biomarkers are required to support early GBS diagnosis, and to personalize and monitor patient treatments.
- Advances in immunological and biochemical technologies are allowing the development of combinatorial antigen assays to measure antibody properties for the diagnosis and subtyping of GBS patients.
- We anticipate that new therapies for GBS might include inhibitors that target neuropathogenic antibodies (cleaving enzymes, extracorporeal or in vivo-capturing of antibodies), as well as complement proteins.

Box 2.

Distinguishing features of GBS

GBS is not a typical autoimmune disease

- No predominance in females (male to female ratio 3:2)
- No relapsing-remitting or chronic disease course
- No association with other autoimmune diseases
- No association with *HLA-II* or *HLA-I* alleles [22]
- No clinical improvement by corticosteroid treatment [61]

GBS is a typical post-infectious disease

- More than 95% of patients have a monophasic disease course [62]
- Two-thirds of patients have symptoms of a recent respiratory or gastro-intestinal infection or a vaccination
- Serological evidence for a recent infection is present in 50–60% of patients [5]
- Characteristic clinical features are associated with an eliciting pathogen
- Disease prognosis is associated with an eliciting pathogen

Box 3.

Neural tissue vulnerability to antibody attack

- Neural tissue is particularly sensitive to loss of function, has low resilience and permissiveness to changes in the microenvironment, and has limited repair capabilities.
- The detailed molecular composition of local microenvironments along the soma, extended axon, and synapse of neurons, controls vulnerability to antibodies [29].
- In humans and mice, gangliosides are concentrated and accessible to antibodies at the nodes of Ranvier which are key structures for normal saltatory nerve conduction [18].
- Target-mediated clearance of antibodies by neuronal endocytosis can limit the damage to axon terminals. Mouse ex vivo studies indicate that the highly dynamic nature of the neurological synapse -- with constant vesicle release as well as uptake of antibodies -- is much less vulnerable than the static nature of membranes at the nodes of Ranvier [34, 35]
- Antibodies can access their molecular targets despite the presence of the blood-nerve barrier in the peripheral nervous system (PNS) and blood-brain barrier in the CNS, as the barrier can be leaky at certain locations, e,g, at nerve roots, ganglia, and nerve terminals [18].
- Pathogenic antibodies access the NMOSD self-antigen aquaporin-4 (AQP4) expressed on **astrocyte podocytes** at the blood-brain barrier. Gut microbes are thought to provide cross-reactive mimetics of AQP4 [59].
- In chronic neuropathies such as autoimmune nodopathy, IgG4 binding to Schwann cells can impair the function of the latter in the absence of complement activation; passive transfer of human antibody against neurofascin-155 into mice has resulted in reduced NF155 protein content in nerve roots (via immunoblotting), suggesting depletion of target antigens and interference with the formation of **paranodal loops** [63].
- In MS, autoantibodies do not necessarily need to access the CNS parenchyma for pathogenic action since they can promote antigen presentation in lymph nodes as demonstrated in the EAE mouse model for MS [64]. This is contrary to the common assumption that autoantibodies need to exclusively engage their target in the CNS.

Box 4.

Antibody responses against glycolipids: tolerance, T-cell help, and memory

- Classic distinctions between TI (repetitive carbohydrates, inducing IgM and IgG2 in humans) and TD (protein; inducing IgG1, IgG3, and IgG4 in humans) responses do not fully apply to antibody responses against glycolipids because in GBS, anti-glycolipid antibodies are mainly IgG1 and IgG3 [42, 65].
- TD and TI type 1 and type 2 responses are formally distinguished by using athymic nude mice (*Foxn1* mutation), and CBA/N mice with a mutation in Bruton's tyrosine kinase (*Btk*) [41]. In mice, TD responses are mediated by T cells or NKT cells; the latter give rise to class-switched but short-term antibody responses [44, 66].
- Wildtype mice develop low titers of serum antibodies to self-gangliosides only [37]. However, knockout of the GalNac-transferase ($GalNAcT^{+/-}$) in mice abrogates this 'tolerance' as these mice lack all complex gangliosides such as GM1 or GQ1b. Upon immunization with gangliosides or gangliosides mimicking LOS in mice, antibodies against GM1 or GQ1b undergo class switching to TD-dependent IgG isotypes and B cell memory [37], suggesting that a TD B-cell response to glycolipids can develop in vivo.
- In humans, low titers of IgM antibodies to gangliosides develop only after birth [39], suggesting that antigenic stimulation is required for their induction.
- In GBS patients, a TD response is strongly suggested by the profiles of antibody isotypes and subclasses (IgG1, IgG3, and IgA in addition to IgM). GBS-associated LOS causes strong activation of human dendritic cells, as evidenced by a higher production of cytokines and higher expression of co-stimulatory molecules in its presence; it may also polarize T cells towards a Th2 phenotype [67, 68]
- An IgM-producing hybridoma reactive to GM1 from a GBS patient has provided evidence for **somatic hypermutation** and **affinity maturation** of patient antibodies, similar to what has been observed in chronic neuropathies such as multifocal motor neuropathy, which is mediated by IgM antibodies against GM1 [43, 69, 70]. However, the mutation status of IgG antibodies from GBS patients remains unknown.
- The monophasic nature and rapid decline of antibody titer in GBS patients, mostly within months [21], suggests that there might probably be little to none memory B cell formation, but this remains to be proven.
- Murine studies suggest that anti-glycolipid antibodies originate from an innate type of B cell, since anti-ganglioside antibodies are not mutated, and polyreactive and hybridoma cells express CD5 [71]. Whether this is similar

in humans is unknown. Recent studies suggest that memory B cells and plasmablasts are expanded in the peripheral blood of GBS patients [72, 73].

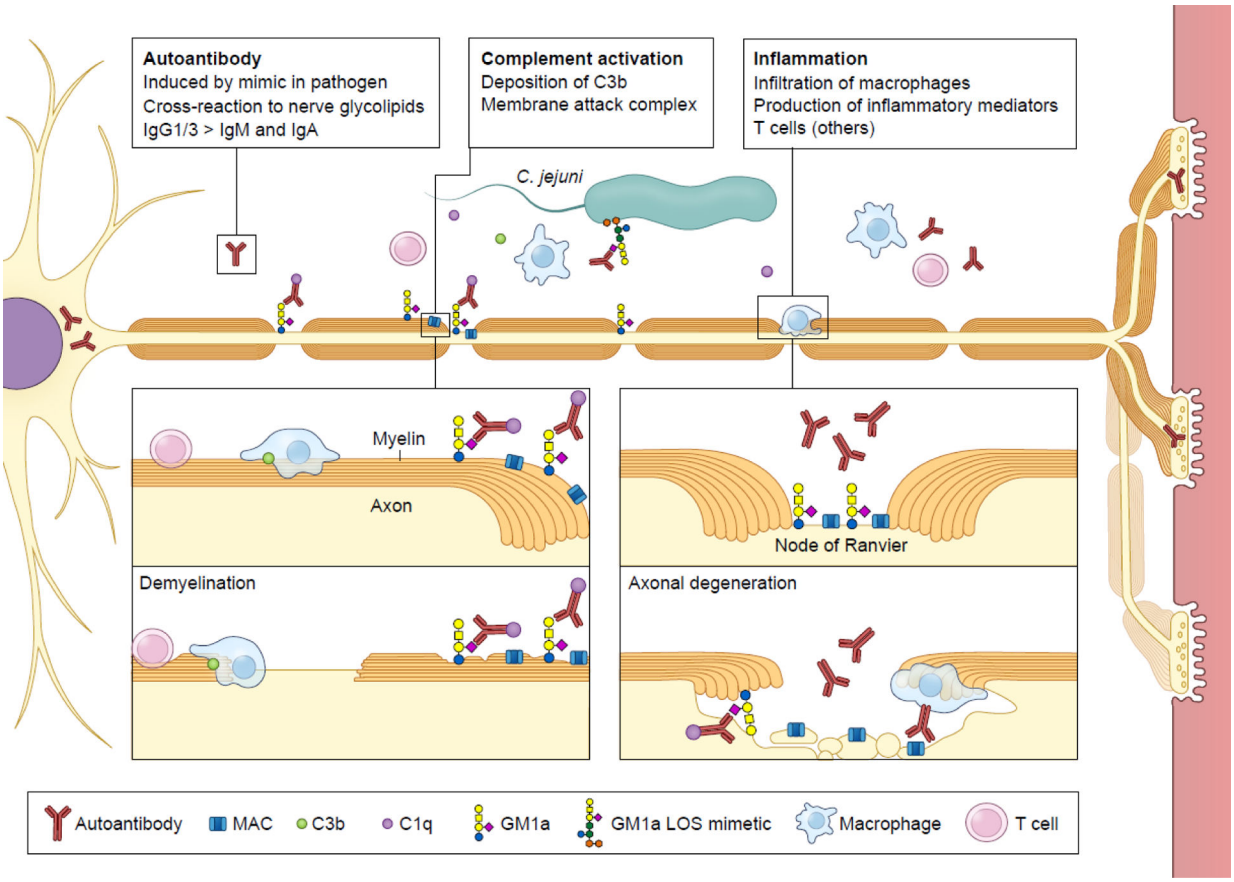
Outstanding questions

- To what extent can controlled measures that can reduce the incidence of infections with *Campylobacter jejuni*, also reduce the incidence of GBS globally? GBS incidence appears to be less frequent in Singapore since the COVID-19 pandemic [74], perhaps due to social distancing with reduced exposure to contaminated food sources. Further identification of new pathogens causing GBS might also contribute to exposure prevention.
- Can novel systems such as oligosaccharide microarrays that are used to detect at high sensitivity and specificity the full spectrum of antibodies against multiplexed carbohydrate antigens be relevant for improving GBS diagnosis, subtyping, and prognostication?
- Which pathogens, other than those currently identified, are associated with GBS and which mimicry epitopes are involved?
- What are the contributions of antibodies against multiplexed antigens and differential functions of IgM versus the four subclasses of IgG in GBS?
- What are the targets of neuropathogenic antibodies (or T cells) in GBS patients not harboring anti-ganglioside antibodies, especially those presenting with the demyelinating form of GBS?
- Which environmental conditions or host factors activate T- and B-cells via mimicry epitopes provided by the gut microbiota and/or infectious pathogens, and which contribute to the pathogenesis of certain neuroimmunological diseases? If so, which specific diseases?
- Which innovative therapies such as B-cell depletion, complement inhibition, or IgG depletion by extracorporeal immune-absorption are safe and effective for GBS patients? For example, pharmacological treatment with *Streptococcus* enzyme (imlifidase) to reduce ganglioside IgG titers is currently being tested in an ongoing open-label, single arm, multi-center Phase II trial (clinicaltrials.gov NCT03943589)[V] in 30 GBS patients receiving standard of care intravenous immunoglobulin (primary endpoints, safety and efficacy).
- Can GBS treatments be optimized (dosing, treatment regimens) using sophisticated antibody monitoring technologies that include sensitive antibody affinity measurements?

[V]Clinical trial (see Outstanding questions Box)

Highlights

- Guillain-Barré syndrome (GBS) following *Campylobacter jejuni* infection is a true case of molecular mimicry-driven disease
- The immunopathogenesis of GBS sheds new light on the multimolecular identities of self-antigens
- The glycan-nature of mimicry epitopes that drives antibody class switching to IgG subclasses challenges rigid concepts of thymus-dependent and -independent B cell responses because anti-glycolipid antibodies are mainly IgG1 and IgG3, albeit short-lasting.
- Combinatorial chemo-enzymatic technologies and arrays allow the development of novel diagnostic and research tools to identify antibodies against mono- and multimeric carbohydrate epitopes
- A certain degree of dissimilarity may be required for the occurrence of molecular mimicry in *Campylobacter jejuni*-associated GBS.



Key Figure, Figure 1. Overview of the pathogenesis of Guillain-Barré syndrome (GBS)

GBS is an acute immune-mediated peripheral neuropathy usually triggered by a preceding infection, the predominant type being *Campylobacter jejuni* (~30%). Antibodies raised against *C. jejuni* lipo-oligosaccharide (LOS) during infection via carbohydrate mimicry may cross-react with various human nerve gangliosides, including GM1a. The specificity of the anti-ganglioside antibodies is associated with the type of clinical GBS variant, reflecting the distribution of the targeted gangliosides in peripheral nerves. GBS is caused by axonal degeneration and/or demyelination of the nerves. Antibody depositions are found in axonal GBS at the nerve axons, especially the nodes of Ranvier, and in demyelinating GBS, at the myelin sheaths. Serum antibodies are usually IgG1 of IgG3 (less frequently IgM or IgA) and the highest titers are found in patients upon hospital admission. Binding of these antibodies results in local deposition of complement factors (including C1q, C3b) and formation of membrane attack complex (MAC). Macrophages infiltrate the nerve at the site of damage and additional T cells may be found in nerves. GBS is a monophasic disorder reflecting transient immune-mediated damage; most patients begin to clinically improve with the disappearance of anti-ganglioside antibodies. Subsequent nerve regeneration is a slow process and is often incomplete, explaining the high proportion of patients with residual disability or complaints.

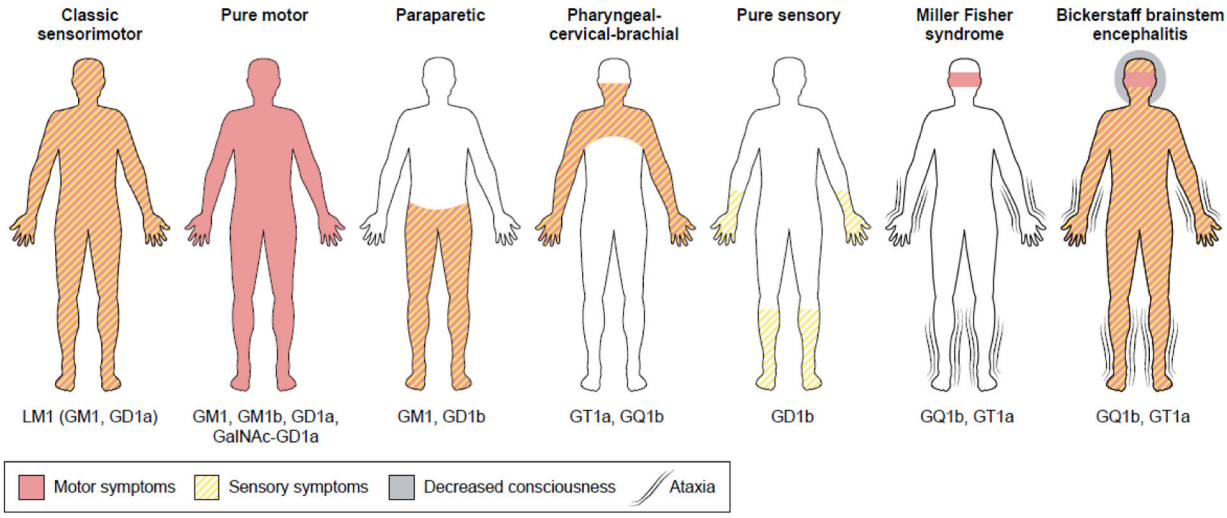


Figure 2. Distinct symptom patterns of GBS variants that have been associated with elicited antibodies

The gangliosides indicated below the clinical variants represent the predominant targets of the antibodies found in the serum of these GBS patients. Antibodies against GM1 and GD1a are associated with the pure motor form of GBS, but also occur in other clinical variants, such as the paraparetic form in which only lower limbs are affected. The pharyngeal-cervical-brachial variant (affecting upper limbs), Miller Fisher syndrome and Bickerstaff brainstem encephalitis are associated with antibodies against GQ1b and GT1a. The figure is modified from [83].

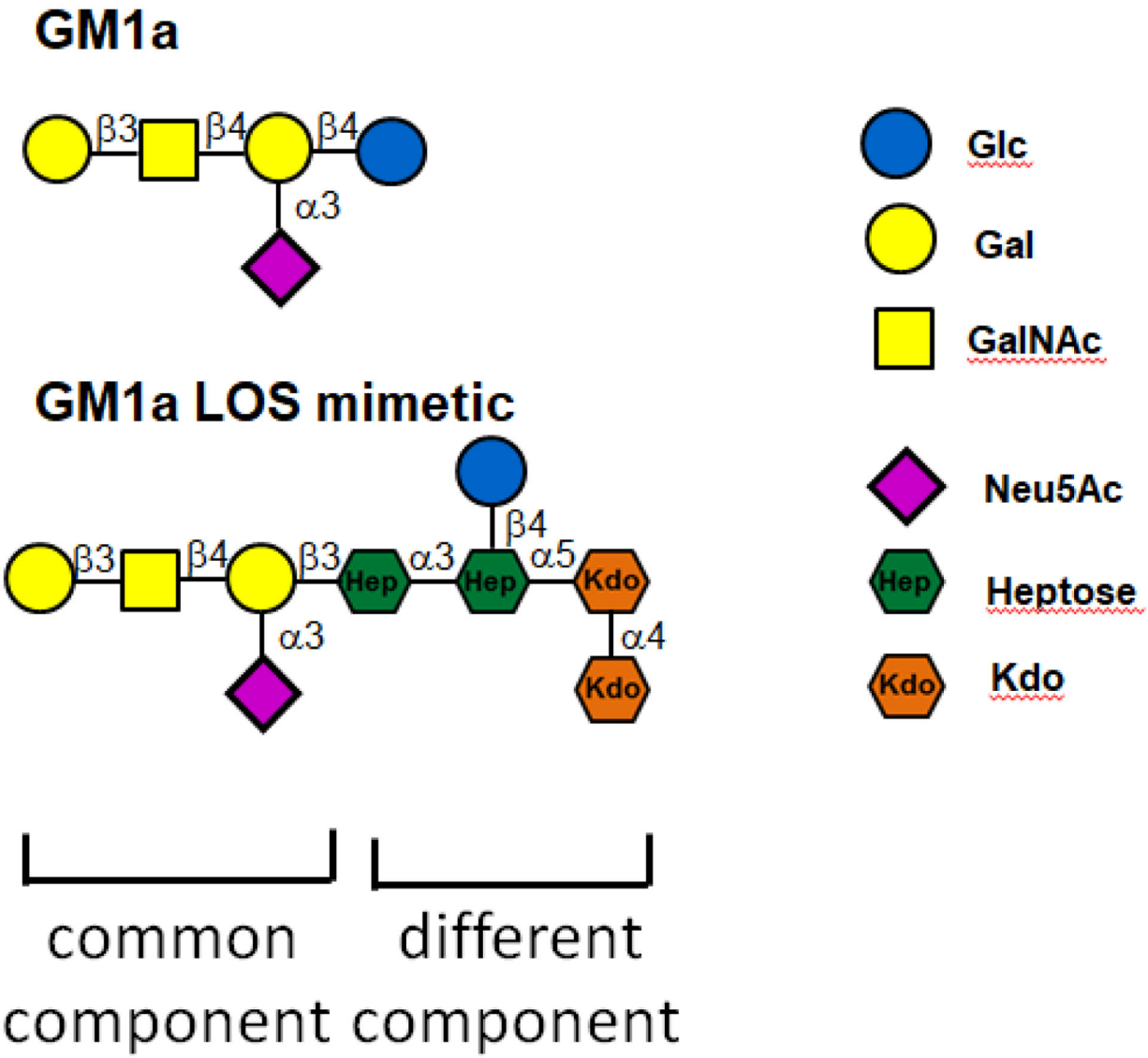


Figure 3. Molecular mimicry of LOS and gangliosides in GBS caused by *Campylobacter jejuni*

The upper part of the figure shows the oligosaccharide moiety of GM1a. The lipo-oligosaccharide (LOS) of *C. jejuni* mimics this structure. The terminal parts of the molecules are shared (common component) whereas LOS also contains a different component representing the inner core. The host ganglio-oligosaccharide has a glucose residue whereas the bacterial LOS has a heptose moiety at the same position. Furthermore, the heptose of LOS is linked to an inner core saccharide composed of additional heptose and ketodeoxyoctonic acid (KDO) moieties [25, 46].

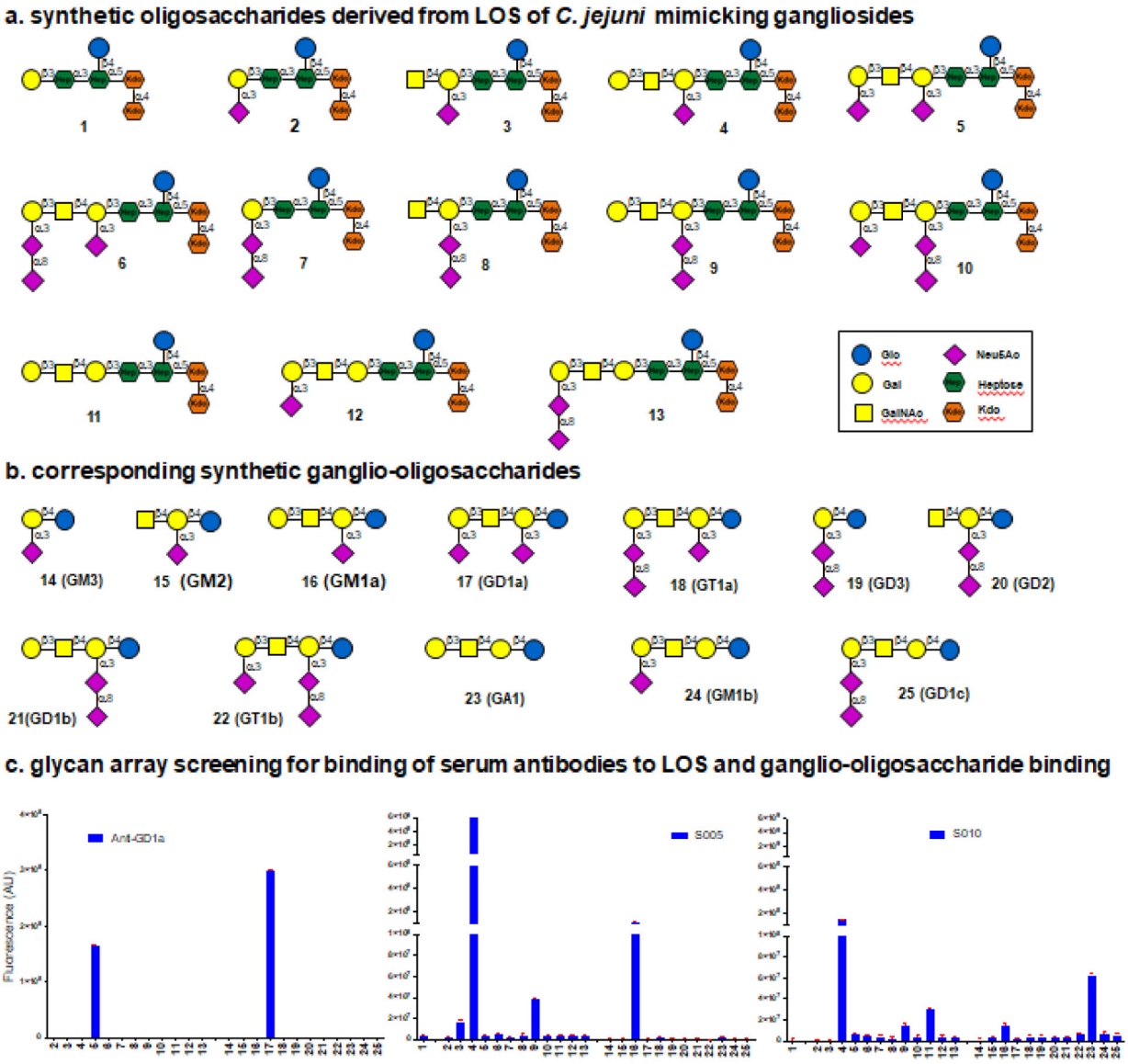


Figure 4. Chemoenzymatic diagnostics for monomeric and heteromeric-multiplexed antigens

Novel combinatorial diagnostics based on immunofluorescence detection are being developed to finely discriminate antibodies cross-reacting between host structures and pathogen mimetics. Panel a and b show examples of various oligosaccharides that have been synthesized [50]. Panel c shows examples of probed oligosaccharide arrays with a monoclonal antibody against GD1a, and the sera of two GBS patients (S005 and S010). Bound antibodies were visualized using fluorescently-conjugated anti-human IgG secondary antibodies and fluorescence intensities were quantified using a microarray scanner [50]. The numbers on the x-axis correspond to the structures displayed in a and b. These diagnostic systems can also be used to identify antibodies directed against epitopes being formed by heteromeric-multiplexed gangliosides. The figure is modified from [50].

Table 1.

Neurological autoimmune diseases and molecular mimicry

Disease	Pathogen	Pathogen epitope	Host epitope	Host	References
MS	Epstein-Barr virus	EBNA1 (386–405) SQSSSSG**SPPR**R**P**PPGR**R**PF	Glial Cellular Adhesion Molecule (Glial)CAM (370–389) ATGRTHS**SPPR**A**P**SSPG**R**SR	Human	[54]
MS	Epstein-Barr virus	EBNA1 (431–440) **PG**A**IE**Q**GP**A**D**	Anoctamin 2 (140–147) **PG**D**IE**L**GP**L**D**	Human	[58]
MS – EAE	Epstein-Barr virus	FARQAVWLRE	RASGRP2 (78–87)	Human	[53]
MS	Human herpesvirus 6	U24(1–13) MDP**PRTPPPS**YSE	MBP (92–104) IVT**PRTPPPS**QGK	Human	[75]
MS – EAE	Cytomegalovirus	UL86; mcp (986–993) **W**L**RSPFSR**	MOG (39–46) WYRPPFSR	Rhesus monkey	[76]
MS – EAE	*Akkermansia muciniphila*	LSVGWISGQY	RASGRP2 (78–87)	Human	[53]
MS – EAE	*Chlamydia pneumoniae*	Cpn0483 gene **YG**C**L**LPRNP**RT**E**D**Q**N**	MBP (68–86) **YGSL**PQKSQ**RT**Q**DEN**	Lewis rat	[77]
MS	*Chlamydia pneumoniae*	Cpn0442 gene **KN**LFP**P**YE**PPP**	MBP (84–102) **KN**IVT**P**RT**PPP**	Lewis rat	[77]
MS	*Acinetobacter calcoaceticus*	3-oxoadipate COA-transferase subunit A (83–97) DSYVFDE**LYRAGK**IE	MOG (43–57) PFSRVVH**LYR**N**GK**DQ	Human	[78]
MS	*Acinetobacter calcoaceticus*	4-carboxy-muconolactone decarboxylase (38–52) QNFI**SRF**A**WG**EVNSR	MBP (110–124) GLSL**SRFSWG**AEGQR	Human	[78]
MS	*Pneumocystis aeruginosa*	γ-carboxy-muconolactone decarboxylase (38–52) QEMIT**R**HA**WG**DIWTR	MBP(110–124) GLSL**SRFSWG**AEGQR	Human	[78]
MS – EAE	*Lactobacillus reuteri*	UvrA peptide TIKREGFVRVQVD	MOG(38–50) GWY**R**SP**FSRV**VHL	Mouse C57Bl/6	[51]
NMOSD	*Clostridium perfringens*	ABC transporter permease(204–217) FII**LPV**S**MVLISL**V	AQP4 (63–76) EKP**L**PVD**MVLISL**C	Human	[79]
NMOSD	Human T-lymphotropic virus	TAX1BP1 protein (219–233) **EFK**K**RF**SD**ATSKA**H**Q**	AQP4 (6–20) **EFKRRF**KE**A**F**SKA**A**Q**	Human	[80]
Narcolepsy	Influenza virus H1N1	pHA273–287 AMERNAGSGIIISDT	Hypocretin/orexin 54–66 HGAGNHAAGILTL	Human	[81, 82]

Bold letters indicate amino acids identical in both epitopes.

MS: multiple sclerosis, NMOSD: neuromyelitis optica spectrum disorder, GBS: Guillain-Barré syndrome, HCRT: hypocretin/orexin