REVIEW

For reprint orders, please contact: reprints@futuremedicine.com

Human-derived natural antibodies: biomarkers and potential therapeutics

Xiaohua Xu¹, Sher May Ng², Eamonn Hassouna³, Arthur Warrington¹, Sang-Hyun Oh^{4,5} & Moses Rodriguez^{*,1,6}

ABSTRACT The immune system generates antibodies and antigen-specific T-cells as basic elements of the immune networks that differentiate self from non-self in a finely tuned manner. The antigen-specific nature of immune responses ensures that normal immune activation contains non-self when tolerating self. Here we review the B-1 subset of lymphocytes which produce self-reactive antibodies. By analyzing the IgM class of natural antibodies that recognize antigens from the nervous system, we emphasize that natural antibodies are biomarkers of how the immune system monitors the host. The immune response activated against self can be detrimental when triggered in an autoimmune genetic background. In contrast, tuning immune activity with natural antibodies is a potential therapeutic strategy.

Antibodies reactive to self-antigens play a key role in both healthy individuals and patients with autoimmune disorders. To avoid autoimmunity, the development of self-reactive B-cell clones and generation of antibodies reactive to the self-antigens should be closely monitored and put under control. The identification of B-1 cells in mice that produce many self-reactive antibodies has indicated how the immune system interacts with self- molecules and cells [1,2]. Studies in this field have revealed clues to developing therapeutics to treat human diseases [3]. As a result, human-derived antibodies that react to self-antigens have been developed and tested in disease models, and clinical trials are being carried out in patients to evaluate their efficacy [4]. Here we review the generation of self-reactive antibodies, the nature of the self-antigens and the development of human natural antibodies as therapeutics for human diseases. We focus on the IgM class of human natural antibodies that recognize the surface antigens on neural cells.

Although self-reactive antibodies from patients with autoimmune diseases can cause nerve damage [5], some antibodies from healthy individuals that recognize self-antigens on neural cells play critical roles in physiology and maintenance of health [4]. Approximately 2–3% of healthy people carry antibodies that recognize self-antigens in the nervous system. These antibodies can enter the brain tissue but do not contribute to neuropathology [6]. Here, we refer to the self-antibodies identified in autoimmune diseases as auto-antibody (AutoAb), whereas antibodies from healthy individuals without any previous known exposure to the antigens recognized by the antibodies will be known as natural antibody (NAb). Nevertheless, the repertoire of antibodies binding to self-antigens on

KEYWORDS

• autoimmunity • B-1 cell
• CNS inflammation • natural auto-antibody • T-cell activation

¹Department of Neurology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA

²School of Clinical Medicine, University Of Cambridge, Hills Rd, Cambridge CB2 0SP, UK

³Department of General Medicine, Charles University Hradec Kralove Faculty, Prague, Czech Republic

⁴Laboratory of Nanostructures & Biosensing, Department of Electrical & Computer Engineering, University of Minnesota, Minneapolis, MN 55455, USA

⁵Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455, USA

⁶Department of Immunology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA

*Author for correspondence: rodriguez.moses@mayo.edu

neural cells can serve as biomarkers indicating functional changes of the nervous system and may lend themselves as potential therapeutics.

AutoAbs that target the nervous system arise in two situations: AutoAb identified in autoimmune disease; or AutoAb arising in cancer patients. Autoantibodies against exogenous antigens can cross-react with self-antigens of the nervous system. The immune responses triggering the generation of these AutoAbs may be initiated by self-antigens or microbial, food and other exogenous antigens. In the autoimmune host, the developed immunological memory continues after the exogenous antigen is cleared. For example, anti-DNA [7,8] and ribosomal P [9] AutoAbs can cross-react with neuronal antigens. Additionally, the immune system can generate AutoAbs that target a specific neural-antigen. In patients with neuromyelitis optica, a pathogenic antibody against astrocytic aquaporin-4 water channel protein was identified [10]. Tumor antigens can induce the secretion of antibodies that cross-react with structurally similar patterns in the nervous system [11,12]. In general, dysfunctional immune tolerance facilitates AutoAb production, which may be a sustained process or have a relapsing/remitting course. Whether clinical symptoms develop depends on the reversibility of the pathologic process initiated by the antibodies. A full restoration of neural function is possible when the AutoAbs are cleared and no permanent neurological deficits develop. However, when components of the neural circuits are damaged irreversibly, the functional loss can be permanent and may require rehabilitation therapies [6]. The genetic susceptibility to these autoimmune diseases is also an issue that needs further investigation.

NABs are generated without prior immune activation. It has been known that B cells can proliferate and secrete antibodies in response to lipopolysaccharide (LPS) from the Gram-negative bacterial cell membrane independent to the specific B-cell receptor (BCR) [13]. B-1 cells in mice are positively selected on the basis of self-reactivity and proliferate to form a pool of self-renewing B cells that produce most of the circulating natural IgM antibodies [14]. In laboratory mice, B-1 cells mainly reside in the peritoneal and pleural cavities. B-1 cells are CD5 positive and are rare in lymph nodes [1]. B-1 cells expressing CD5 are known as B-1a cells, whereas CD5-negative B-1 cells are known as B-1b cells [15] (see review [16,17]).

The self-reactive BCR repertoire enables B-1 cells to carry out their unique functions in regulating immunity. Additionally, NABs can cross-react with pathogen epitopes, which ensures the early and continuous protection to the host independent of the previous invasion of a specific pathogen. However, it is unknown how the self-antigens shape the BCR repertoire of B-1 cells, which may function as templates for the selection of antibody specificities. Nevertheless, regulation of the activation and NAB production of this auto-reactive B-cell subset is under control of the immune system.

Self-reactive B lymphocytes & production of NABs

NABs in mice are mainly produced by B-1 cells. The trajectory of NAB generation illustrates how NAB maintains tissue homeostasis and immune defense against infection. Although B-1 cells are the main residential B-cell subpopulation in the pleural and peritoneal cavity, the spleen and bone marrow derived B-1 cells secrete a higher proportion of circulating NABs. In contrast, B-1 cells in the coelomic cavity produce only a small amount of antibodies at steady state. B-1 cells express a BCR repertoire that binds to both self and microbial antigens, which are involved in T cell-independent regulation and function differently from the conventional B-2 cells. Thus a distinct developmental program underlies B-1 cell development [14,17].

• Genesis of B-1 cells

B-1a cells are generated in both the embryonic and early postnatal periods. B-1a precursors have been identified as early in embryonic day 8.5 (E8.5) mice [18]. The fetal liver, but not the adult bone marrow, can efficiently generate B-1a cells [19], whereas B-1b cells can be reconstituted from both fetal liver and adult bone marrow [20]. It seems that B-1 cells are maintained by a self-renewal process, that is, they undergo limited proliferation to replace dying cells and sustain a stable population over time. Once populated in various tissue locations, the *de novo* renewal of B-1 cells during adulthood is restricted under steady state conditions [21]. After introduction into the peritoneal cavity of neonates with short-term B-cell depletion, B-1 cells can completely reconstitute in all tissue [22]. No more than 20% of B-1 cells are derived from host bone marrow at 8 months after donor-cell transfer [23]. The feedback inhibition of *de novo* B-cell development

is mediated by secreted IgMs, as mice deficient in secreted IgM have an enlarged B-1 cell compartment [24,25]. After altering the steady state renewal by irradiation or chemical ablation, B-1 cells can be reintroduced into the periphery from bone-marrow precursors [26,27]. Antigen specificity is crucial to B-1 cell development and function, as B-1 cells are selected based on their self-reactive BCR signaling. For example, Thy1 knockout mice, which do not express the Thy1 antigen, do not develop Thy1-specific B-1 cells and secrete antibodies against Thy1 antigen [28]. The number of B-1 cells was markedly decreased in BCR-transgenic mice generated with immunoglobulin genes encoding a known BCR specificity of B-1 cells replaced with the B-2 heavy chain gene [29]. Therefore, it seems that self-antigens, B-1 cells and secreted IgMs interact with each other to form a feed-back loop [30].

• Activation of B-1 cells & the generation of NABs

Once generated, the innate state activation of B-1 cells is BCR-independent [31]. The identification of Toll-like receptor 4 (TLR4) as the recognition receptor for LPS and subsequent studies of other pattern recognition receptors have shown that B cells express numerous innate immune receptors, which mediate BCR-independent activation of B-1 cells [32–34]. Stimuli such as IL-15, IL-10, TLR agonists or whole bacteria can induce activation of B-1 cells. The activated B-1 cells in the peritoneal cavity rapidly accumulate in the omentum and migrate to the spleen, regional lymph nodes or intestinal lamina propria. B-1 cells commonly make translocations from the body cavity in various diseases. Following their rapid redistribution from the body cavities, B-1 cells differentiate and secrete large amounts of IgM and/or IgA [35]. The partial differentiation and ability to respond quickly are essential for B-1 cells to produce antibodies to protect the host from pathogens entering through mucosal surfaces and the blood. The steady state production of natural IgM by B-1 cells provides a crucial barrier against pathogen replication before the establishment of specific immune response. These pre-existing IgM antibodies can directly neutralize and/or inhibit early pathogen replication through complement binding. B-1 cell-derived antibodies have specificity for pathogen-expressed molecules including phosphorylcholine of Gram-positive bacteria, LPS

of Gram-negative bacteria and various viral and parasite-expressed antigens [36].

• B-1 cell responses to tissue injury: regulate inflammation & homeostasis

B-1 cells secrete NABs in response to self-antigen activation and maintain homeostasis. The NABs generated by B-1 cells bind to self-antigens such as oxidized lipids [37,38] and new antigens on apoptotic cells [39]. An increased incidence of autoimmune disorder occurs in gene-targeted mice that lack secreted IgMs. Those mice have accelerated production of self-reactive IgG and increased disease progression [40]. Secreted IgM antibodies specific for apoptotic cells promote phagocytosis by immature dendritic cells *in vitro* [41]. NABs against oxidized low-density lipoproteins can decrease the development of atherosclerosis [38,42]. The housekeeping functions of B-1 cells and NABs to inhibit the induction of inflammatory responses are analogous to the role of macrophages in scavenging apoptotic cells [43]. B-1 cells can also secrete IL-10, which further prevents inflammation [44]. Taken together, the crucial roles for B-1 cells and NABs in regulating homeostasis are appreciated; therefore, depleting B cells in certain patients with autoimmune disease exacerbates the diseases [45]. It should be noted that natural IgMs can induce ischemia-reperfusion (I/R) injury during the process to re-establish homeostasis after hypoxic stress. Sudden re-perfusion and binding of IgMs to apoptotic cells and altered tissue antigens mediate widespread inflammation by activating the complement cascade [46].

• B-1 cells regulate immune responses

B-1 cells accumulate in regional lymphoid tissue and regulate the adaptive immune response [47]. B-1 cells generate IgMs that promote B-2 cells to produce IgGs [25,48]. Additionally, a facet of the innate nature of the B-1 cell is shown in its independence of BCR specificity. The majority of B-1 cells accumulating in the lymphoid tissue of the respiratory track secrete IgM in response to influenza virus infection but are not specific to the influenza virus [48,49]. The secretion of a broad repertoire of poly-reactive IgMs can prevent the potential secondary infections in an already injured mucosal epithelium. By promoting removal of the dead and dying cells, B-1 cells suppress self-reactive T-cell responses. For example, a subset of peritoneal B cells can inhibit T-cell activation in response to antigenic

stimuli [50–52]. The accumulating evidence supports the concept that B-1 cells respond more to self-antigens of the host than to exogenous antigens from the pathogens during an infectious process. Accordingly, B-1 cell responses are more regulated through a self-selection process that shape the self-reactive B-1 cell repertoire, rather than through clonal expansion. Thus B-1 cells can function as both effectors and regulators of the adaptive immune response.

• B-1 cells in humans

A subset of human B cell clones having a phenotype of CD19^{hi}CD21^{low}CD23⁺CD86^{hi}IgM^{hi} was isolated and expanded from the peripheral blood of a patient with common variable immunodeficiency. Those CD21^{low} B cells showed many functional characteristics similar to the mouse B-1 cells, that is, they have few somatic mutations in their immunoglobulin genes and differentiate into IgM-producing cells [53]. Recently, the functional criteria for human B-1 cells were established. B-1 cells carrying the phenotype profile, CD20⁺CD27⁺CD43⁺CD70⁺, were identified from both umbilical cord and adult peripheral blood, which can develop into B-1 cells [54]. B-1 cells could be a target for therapy; and B-1 cell-derived NABs are attractive natural molecules in immune regulation [55].

Neural reactive natural IgMs & the nature of neural surface antigens

How B cells are activated to secrete antibodies that recognize self-antigens of the nervous system remains unknown. The identification of human natural IgMs that recognize surface molecules of neural cells indicates that there exists a subset of B lymphocytes selected in response to self-antigens from the nervous system. As revealed by the specific self-antigens, the selection process may develop at the early embryonic stage and extend into adulthood.

• Identification of human natural IgMs: the potential therapeutics to treat human diseases

A repertoire of human IgM antibodies was identified based on a screening protocol that selects human IgMs binding to fresh brain slices [56]. By screening a serum bank originally established by Robert Kyle at Mayo Clinic, investigators discovered a group of natural human IgMs that bind to neural surface antigens. Two serum-derived human monoclonal IgMs (sHIgM) were

isolated and tested in animal models mimicking distinctive neurological diseases. Serum-derived human IgM 22 (sHIgM22) that binds to mature oligodendrocytes promotes remyelination in the Theiler's Murine Encephalomyelitis Virus (TMEV) model of multiple sclerosis (MS) [4]; sHIgM12 binding to neurons supports neurite extension *in vitro* [57], and protects neurons when administrated *in vivo* either in the TMEV model of MS [4] or in models of amyotrophic lateral sclerosis induced by expressing the genetic mutants of human superoxide dismutase 1 (SOD1) (Rodriguez laboratory unpublished observation). Recombinant forms of sHIgM22 and sHIgM12 (rHIgM22 and rHIgM12) manufactured in GMP quality have confirmed efficacy in promoting remyelination in animal models of MS [58] (rHIgM22); supporting axonal outgrowth in neuronal cultures [59] (rHIgM12); and improving neurological functions in mouse models of MS [58] (rHIgM22 or rHIgM12) and amyotrophic lateral sclerosis (rHIgM12). A Phase I clinical trial of rHIgM22 in patients with MS is ongoing [60]. NABs binding to neural cells apparently function through a lipid-raft mechanism [59]. Indeed, accumulating evidence has demonstrated that the IgM type of NABs recognize lipid components of neural membranes.

• Myelin-derived antigens & myelin-reactive antibodies

Myelin components can trigger autoimmunity against the nervous system [61,62]. Experimental autoimmune encephalomyelitis (EAE) can be induced by immunizing susceptible animal strains with myelin molecules such as myelin oligodendrocyte glycoprotein or myelin proteolipid protein [63]. On the other hand, earlier studies in the Rodriguez laboratory have demonstrated that immune responses raised against self-antigens of CNS can induce repair of the demyelinated lesions. For example, immunizing mice with spinal-cord homogenates and incomplete Freund's adjuvant or adoptive transfer of the anti-sera from the immunized animals promoted remyelination in the recipient animals [64]. Further studies to address the autoimmunity to antigens of the CNS have provided clues to solve the contradictions. A major milestone event was the demonstration of antigen-specific T cells in EAE pathogenesis [65,66]. The finding provided strong evidence that the CNS auto-reactive T cells in the normal immune system form the basis of the

autoimmune reaction. T-lymphocytes specific for myelin antigens start an inflammatory reaction in CNS, which ultimately leads to demyelination and subsequent axonal injury. These T cells need to be re-activated by their antigens in the context of major histocompatibility complex class II-bearing antigen-presenting cells (APCs) in order to recognize their target. This re-stimulation of T cells may occur in vessel-associated macrophages or dendritic cells within the brain [67]. However, these APCs do not produce myelin-associated self-antigens *per se*. The myelin molecules have to be released (by injury to the myelin) and transferred to the APCs (an inflammation that facilitates antigen presentation). Thus the myelin-derived self-antigen is basically the bio-marker of myelin itself, which ensures myelin-specific immune activation.

However, the triggering factors leading to autoimmunity remain obscure. Both genetic and environmental factors contribute to autoimmune diseases. Factors triggering the disease may not be identical to the elements that influence the severity or progression of the disorder. Human monozygotic twin studies and transgenic models demonstrate that genetic influences strongly determine whether one will develop autoimmunity [68,69]. It is regarded that environmental factors function on a specific genetic background to disrupt the immune tolerance and trigger autoimmune reactivity. Genetic changes in the human genome which explain the locus of autoimmunity are scarce. An increasing number of environmental components may be involved in autoimmunity in a world where new technologies are developed in agriculture, industry and pharmacology to generate chemicals used in foods, drugs and diets. The toxic factors released have had widespread impact on human health. Researches in clinical and experimental biology have shown that autoimmunity may be induced by chronic exposure to various chemicals. Organic compounds such as toluene diisocyanate, trimellitic anhydride, phthalic anhydride, bezoquinone, formaldehyde, ethylene oxide, dinitrochlorobenzene, picryl chloride, penicillins and D-penicillinamine have been shown to induce autoimmune responses [70]. The reactive organic compounds can bind covalently to protein nucleophilic groups such as thiol, amino, and hydroxyl groups and mediate functional changes. Sensitizing metal ions can oxidize proteins or lipids to form stable metal chelate complexes [71]. The dietary components

also play a role in autoimmunity. For example, there is a link between gluten ingestion and gluten sensitive enteropathies [72]. Low levels of vitamin D are related to MS and other autoimmune disorders [73]. Stress has also been linked to autoimmunity [74]. The toxic molecules may induce aberrant cell death making the hidden molecules exposed to and processed by antigen presenting cells thus forming neoantigens by converting self- into non-self molecules. In addition to the environmental factors, microbes including viruses, bacteria and eukaryotic parasites are also involved in host immune reaction. Millions of years of coevolution has forged pervasive host–microbiota interconnections, which are particularly apparent in immune responses to microbiota invasion. The abundant microbiota residence on the body surfaces like the digestive, respiratory and urogenital tracts poses immense health challenges. Opportunistic infection can induce inflammation and sepsis. The immune system has thus evolved adaptations to contain the microbiota and foster complex microbial communities for their metabolic benefits and avoid triggering autoimmunity [75,76].

Therefore, we propose that exposure to toxins from the environment, infectious agents and/or idiopathic cellular injuries in genetically predisposed individuals activates antigen-specific T cells that cross the blood–brain barrier (BBB). Reactivation of the T cells by CNS-resident APCs, taking the example of MS in which the myelin antigens are presented, recruits innate immune cells leading to further damage. During the process, immune cells infiltrate the CNS. The original triggers that initiate disease may be resolved and therefore are difficult to connect to the disease.

Furthermore, the T-cell response is necessary but insufficient to initiate an ‘MS’ pathology in the EAE model. The formation of large demyelinating lesions depends on the generation of myelin-specific antibodies by B lymphocytes. In MS patients, the identity of the antigens targeted by myelin-reactive antibodies has not been identified. There are only three known antigens, galactosyl ceramide [77], sulfatide [78] and myelin oligodendrocyte glycoprotein [79], which can initiate a demyelinating AutoAb response in EAE, and the complete antigenic profile on the myelin surface is yet to be determined. The demyelination in response to the AutoAb binding is mediated by complement activation and subsequent inflammatory response that further stimulates

and recruits effector cells into the lesions. The AutoAbs in normal healthy animals are usually non-pathogenic. More intriguingly, we have shown that O4, a mouse IgM that targets sulfatide, induces myelin repair in the TMEV model of MS [80], an effect similar to the human natural IgM, HIgM22. Therefore, the presence of CNS-reactive antibodies is generally harmless [81] but, in certain situations, may induce demyelination [10] or in contrast remyelination [4].

Although failure to sense auto-reactive B cells through central and peripheral tolerance mechanisms exists, the exchange of molecules and cells between CNS and other organs is highly restricted due to multiple barrier mechanisms including the BBB. Because of the relatively 'isolated' status, cells in CNS can express a large number of antigens or the neural-specific isoforms and/or novel post-translational modification(s) of a more widely expressed antigen can be induced in response to changes in the milieu. Only when a neural-specific immune memory develops and the BBB integrity is compromised can autoimmune-mediated neural pathology ensue.

• Myelin surface antigens recognized by HIgM22

Surface antigens of oligodendrocyte are markers that indicate their developmental status. For example, oligodendrocyte marker O4 is an antigen expressed on the surface of oligodendrocytes [82]. The mouse monoclonal IgM antibody O4 binds to the O4 epitope carried by the myelin glycolipid, sulfatide, which is the earlier marker specific for oligodendrocyte lineage. The anti-A2B5 and -O1 antibodies are frequently used in combination with anti-O4 to define distinct stages of oligodendrocyte development [83]. Generally, A2B5⁺/O4⁺ markers are expressed on the earlier stage oligodendrocytes; O4⁺/O1⁺ cells are intermediate; and O1⁺ defines the more mature stage oligodendrocytes. The human natural IgMs corresponding to the mouse A2B5, O1 and O4 have not been identified. We have shown that HIgM22 recognizes the surface antigen(s) on mature oligodendrocytes. Studies in cerebroside sulfotransferase (CST) knockout mice suggest that HIgM22 binds to a sulfated molecule(s), synthesized by CST enzyme activity, which catalyzes the 3'-sulfation of galactose residues in several glycolipids [84]. The major product of CST in the mammalian nervous system is sulfatide, an essential component of myelin [85].

The fact that HIgM22 recognizes surface antigen(s) of mature oligodendrocytes/myelin suggests that the human immune system can react to the antigenic stimuli (a sulfated molecule) of the mature myelin. How HIgM22 is generated remains obscure. However, it is clear that the antigen(s) of IgM22 can be presented to activate natural IgM production without inducing autoimmunity. As previously mentioned, HIgM22 can mediate signaling to promote oligodendrocyte precursor cell proliferation in cultured mixed glia [86] and accelerate myelin repair *in vivo* [4]. This finding has provided evidence that, in addition to regulating the immune system, natural IgMs signal the neural cells directly and/or indirectly to mediate a novel CNS function distinctive to the traditional view of natural IgMs in maintaining homeostasis. This concept is further confirmed by the identification of HIgM12, another human natural IgM that recognizes antigens on neurons.

• Neuron surface antigens recognized by HIgM12

Cell-surface antigens are frequently glycosylated proteins or lipids or complex glycans [87]. In contrast to the antigens of HIgM22 on myelin, the surface antigens to which HIgM12 binds are developmentally conserved molecules that can be traced from neural stem cells to mature neurons (Figure 1). During differentiation, HIgM12-positive neural stem cells tend to develop into the neuronal lineage (Figure 2). In primary cultured neurons, HIgM12 binding to neurons is sensitive to neuraminidase treatment, suggesting that the antigens recognized by HIgM12 are sialic acid-modified molecule(s) [57]. New evidence indicates that HIgM12 binds to antigens of both glycoprotein and glycolipid that are decorated with sialic acids. The glycolipid components are actually a group of glycosphingolipids, called complex gangliosides (Figure 3) [59]. Therefore, it is likely that the sialic acids participate to form the epitope(s) for HIgM12 binding. Based on their binding specificity, natural human IgMs and pathogens such as influenza virus may bind to similar cell surface glycans [88]. Therefore, it is likely that the neural surface glycans recognized by HIgM12 can potentially affect the host immune system.

The sialo-glycan moieties contribute important functional roles to the sialic-acid-bearing molecules [93]. The sialic-acid epitopes are evolutionarily conserved cell surface structures

Figure 1. HlgM12 recognizes antigen(s) on both neural stem cells and mature neurons. The neural stem cells were isolated and propagated from the cortex of E15 mice. The neurospheres in culture were harvested by quick spin, washed and stored in phosphate-buffered saline, pH 7.4 on ice. Both the neurospheres and the dissociated neural stem cells from the sphere were plated on poly-D-lysine/laminin coated coverslips and cultured in the CO₂ incubator at 37°C in the presence of EGF and bFGF overnight. The adult hippocampal neurons were isolated from the brains of 1–2-month-old mice by Percoll gradient centrifugation and cultured in neurobasal media containing 2% B27 supplement. To stain surface molecules, live cells were kept on ice-bath and incubated with PBS containing 1 μg/ml of HlgM12. Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline, permeabilized with 0.1% Triton X-100, and stained with the corresponding antibodies for intracellular molecules. HlgM12 binds to (A) neurospheres, (B) dissociated neural stem cells and (C) adult neurons. (A1–C1) HlgM12, green; (A2 & B2) nestin, red; (C2) β3-tubulin, red; (A3–C3) merged picture of (A1–C1). The stem cells were also double stained with antibodies to nestin and glial fibrillary acidic protein and were positive to both markers (not shown here) [89]. Scale bar: 100 μm in (A & B); 200 μm in (C).

containing a five-carbon core, an exocyclic glycerol side chain (C-7, C-8, C-9) and an N-acetyl or glycolyl moiety. Variations at C-5 determine the core sialic-acid structures; *O*-substitutions at C-4, C-7, C-8 and/or C-9 (including acetyl, methyl, lactyl, sulfate and phosphate groups) generate further diversity to sialic-acid structures,

and the anionic C-1 carboxylate can form lactones and lactams that neutralize the charges [94]. Some Gram-negative bacteria associated with human and/or animal hosts carry sialic acids and can cause serious illness. Neuroinvasive bacteria such as *Escherichia coli* k1 and *Neisseria meningitidis* sero-group B express α2–8 and/or α2–9-linked

Figure 2. HlgM12 binds to the neuronal lineage antigen(s). Neural stem cells were isolated and propagated from the cortex of one to two month old mice [89,90]. Neurospheres and neural stem cells were cultured and maintained as described in Figure 1. Dissociated neural stem cells were plated on PDL-LM-coated coverslips and differentiated in neurobasal media that contained retinoic acid and 2% B-27. The cells were collected at multiple time points and stained with HlgM12 ([A1–C1] green), anti-MAP2, a neuronal marker ([A2–C2] blue), and anti-GFAP, the astrocyte marker ([A3–C3] red). (A) Neural stem cells differentiated for 24 h. HlgM12 binds to the surface of cells that express high levels of GFAP, but low MAP2. (B) Neural stem cells differentiated for 48 h. Note that some cells that were mostly spindle in shape began to become polarized and presented with a round cell body bearing two processes. (C) At 1 week after plating in the differentiation media, many polarized cells were generated that were similar, but different to the ones observed in (B). Those cells expressed high levels of both HlgM12 epitopes and MAP2, but almost no expression of GFAP, which were designated as ‘HlgM12⁺MAP2⁺GFAP⁻ cells’. It is not known whether those ‘HlgM12⁺MAP2⁺GFAP⁻ cells’ were differentiated from the neural stem cells originally plated or were actually propagated from a different repertoire of neural stem cells formed in the GFAP-positive cell niche, as the GFAP-positive cells carry a morphology different from that in (A) or (B).

Figure 2. HIgM12 binds to the neuronal lineage antigen(s) (cont.). The MAP2⁺GFAP⁻ phenotype indicated that the newly formed 'HIgM12⁺MAP2⁺GFAP⁻ cells' tended to develop into the neuronal lineage. Those 'HIgM12⁺MAP2⁺GFAP⁻ cells' stayed close with each other and had similar size, morphology and markers, suggesting that they are from the same ancestor(s). However, the possibility that the cells migrated to the current position in response to chemokine(s) cannot be ruled out. Scale bar: 50 μ m.

polysialic acid [95]. The size and charge of polysialic acid could facilitate bacterial dissemination and/or hindering host immune responses [96,97]. The pathogenic bacteria, *campylobacter jejuni*, express sialo-glycans that attach to their lipooligosaccharides and are implicated to induce autoimmune neuropathies [98]. The influenza C virus hemagglutinin binds specifically to the 9-*O*-acetyl bearing sialic acid [99]; and the budding viruses use a Sialo-specific *O*-acetyl esterase to release from the host cells [100].

• HIgM12 regulates neuronal functions

When administrated *in vivo*, HIgM12 can mediate neuronal functions by directly targeting neurons/axons, which is distinct from the roles through regulating the immune system. The cell-surface sialic acids function by modifying the structures of oligosaccharides, larger glycans and multiglycan complexes to affect the protein or lipid cores carrying the sugar chains. At the cell membrane, glycolipids and glycoproteins organize into microdomains called lipid rafts. Sphingolipids, including gangliosides, have long, saturated carbohydrate chains on their ceramide moieties that associate with cholesterol and/or with selected membrane proteins to create the dynamic microdomains on cell surfaces [101]. The sialic-acid-decorated molecules extend into the extracellular milieu and function as the antenna to interact with other cells and/or extracellular matrices. Accordingly, HIgM12 was shown to associate with lipid raft and participates in dynamic regulations of the raft microdomains [59]. An immobilized layer of HIgM12 supports axonal differentiation and growth [102] and can override the myelin-mediated neurite outgrowth inhibition [57], whereas the free HIgM12 molecules applied to cultured neurons regulate the cytoskeletal signaling cascade [59].

It remains to be determined whether HIgM12 induces the same or similar signaling cascade when administrated *in vivo*. Gangliosides function as receptors in axon–myelin interactions [103]. Myelin-associated glycoprotein (MAG) is a transmembrane protein expressed by myelinating cells: oligodendrocytes in the CNS and

Schwann cells in the PNS [104]. MAG distributes on the innermost wrap of myelin, which directly contacts the axonal surface. MAG binds preferentially to the 'NeuAc(α 2–3)-Gal(β 1–3)-GalNAc' sugar chain, which is the shared terminal sequence of gangliosides GD1a and GT1b [105,106]. The phenotypes of complex ganglioside-null mice support the roles for GD1a and GT1b in the function of MAG. Mice lacking complex gangliosides (B4galnt1-null) display the similar phenotypes as MAG-null mice including myelin anomalies, progressive axonal degeneration in the central and peripheral nervous systems, reduced neurofilament spacing, reduced axon diameters of myelinated axons and disrupted nodes of Ranvier. Behaviorally, both MAG-null and B4galnt1-null mice showed disruptions in hind limb reflexes and coordination as well as similar patterns of whole-body tremor and hyperactivity [107]. The double-null mice all have the defects observed in each single-null strain (see review [108]). Given that HIgM12 beneficially affects distinctive models of neurological diseases, it turns out that HIgM12 likely functions by inducing signal cascades through gangliosides and mediate glia–neuron interactions. Indeed, the signaling cross-talk(s) between HIgM12 and MAG were observed in cultured neurons when extending their neurites (unpublished observations). This supports a role for complex gangliosides in mediating protective signaling initiated either by MAG or HIgM12. The fact that HIgM12 also recognizes sialic acid-modified protein(s) may provide a redundant function and adds further complexity to HIgM12-induced signaling.

Conclusion: self-defense versus autoimmunity

The critical function of the immune system is to discriminate self- from non-self and maintain tolerance against self-antigens. Therefore, the immune system must deplete the self-reactive lymphocytes that produce AutoAb. B cells develop from hematopoietic stem cells. B cell maturation involves the rearrangement of the immunoglobulin (Ig) heavy chain, which is independent of the antigen and requires

Figure 3. HlgM12 recognizes the epitope(s) on both proteins and lipids. (A) The hippocampal neurons isolated from E15 mice were cultured for 3 days *in vitro* (DIV3). For labeling neuronal surface sialic acids, the live neurons were first washed with PBS, pH 7.4 at 4°C and then treated with 2 mM of sodium periodate (NaIO₄) for 15 min at 4°C, which created C7 aldehydes on the side chain of sialic acid. Finally, aminooxy-biotin with a final concentration of 250 μ M was added to the neuronal cultures and incubated at room temperature for 2 h [91]. Neurons were rinsed with PBS and lysed in RIPA buffer. The lysates from both control and treated neurons were separated on SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were then cut into several pieces, with one incubated in 1% NaIO₄, and another in 2 M acetic acids. Proteins separated were probed with HlgM12, anti- β 3-tubulin or avidin, respectively. HlgM12 binds to the protein band(s) at about 250 kilodalton, whereas treatment with 1% NaIO₄ or 2 M acetic acid, which cleaved either the total carbohydrate or sialic acids moieties, prevented HlgM12 binding. Probing with anti- β 3-tubulin indicated that the treatment only destroyed the carbohydrate moieties, and the amino acid chains were not affected. The avidin blot confirmed that HlgM12 likely binds to the sialic acid epitope(s) that were cleaved by 2 M acetic acids. **(B)** Gangliosides GM1, GD1a and GT1b from bovine brain were separated on high performance thin layer chromatography plate and immune-blotted with HlgM12, which mainly recognized GD1a and trace of GT1b [92]. Ctrl: Control.

interactions with bone marrow stromal cells leading to the generation of the pre-BCR (central tolerance). The BCR complexes regulate the continuous B-cell development and define its reactivity. BCR cross-linking by the self-antigens initiates BCR editing, which rearranges the Ig light chain genes to substitute the self-reactive receptors with the non-self-reactive receptors. The B cells that display auto-reactive Igs after BCR editing are removed by apoptosis, and the surviving B cells migrate to the periphery and mature to become immune-competent naïve B cells. However, the selection involves only the antigens expressed in the central lymphoid organs. The auto-reactive B cells that encounter self-antigens in the periphery must also be removed (peripheral tolerance). Nevertheless, a negative selection mechanism exists to delete auto-reactive B cell clones. Unless a B cell engaged with a self-antigen interacts with a T helper cell, it will be led to apoptosis. This indicates that auto-reactive T helper cells are critically involved in the breakdown of B cell tolerance, leading to the production of AutoAb. Thus B-cell proliferation in response to self-antigen depends on the cooperation of both B and T cells. Secretion of AutoAbs indicates the breakdown of tolerance in which both B and T cells escape the sensing process.

The peripheral mechanisms that maintain immune tolerance are not completely understood. Failure of both central and peripheral tolerance results in self-reactive B cells and generation of AutoAbs, favoring the development of autoimmune diseases. Efficient clearance of apoptotic cells is crucial to avoid generation of autoimmune cells and maintain tolerance, which requires a large spectrum of NABs. To generate this NAB diversity, Ig genes undergo recombinations in which a huge number of intact Ig genes are assembled by assorting a relatively small number of gene segments. On the other hand, NABs can still potentially contribute to harmful immune reactions, though the presence of NAB alone is not enough to lead to autoimmunity. The switches between self-defense and autoimmune response remain to be clarified.

The CNS is relatively isolated by several barrier systems including the BBB, which adds additional features to immune responses that target self-antigens of the CNS. The cloning of monoclonal natural human IgMs that recognize neural self-antigens provides powerful tools for understanding the generation of immunity against the CNS, and will benefit both research and therapeutics.

Future perspective

In the next 5–10 years, an increasing number of patients will be diagnosed to suffer from autoimmune diseases in a world that is ‘modernized’. We expect fundamental progress in the following areas which will help address the mechanisms of autoimmunity: How do environmental factors function on a specific genetic background to trigger autoimmune diseases? Genomics driven by rapidly advancing technologies such as DNA/RNA microarray and deep sequencing will provide insight on how the environmental milieu is associated with genetics that includes both coding and non-coding sequences. Evaluation of the genomes from autoimmune families will provide genomic associations that will define the genetic signature of the autoimmune phenotype. High throughput studies of serial, neuropathologic, biofluid and medical imaging banks will provide samples to identify bio-mechanism of disease. How are CNS antigens presented and antigen-specific T cells activated? Detailed studies are expected to reveal the nature of self-antigens in the CNS. The mechanisms of how antigen specific B- and T-cell responses are coordinated during autoimmune activation will be understood. How do the immune cells and AutoAb cross the BBB to infiltrate the CNS tissue? The precise mechanisms of how B and T cells infiltrate the CNS will be discovered. This will allow for new therapies to prevent the entry of specific phenotype of cells. How do we modulate immune activity to minimize autoimmune activation and facilitate repair? Molecules important in disease activation will be inhibited and molecules important in repair mechanism will be enhanced. We already know that the same molecule in different milieu can have paradoxical and opposite effects. Therefore, the environment surrounding the target of interest will be paramount for effective therapeutics. What will be the impact of individualized medicine? The employment of individualized medicine and information technology will bring a revolution in every element of medicine from collecting and analyzing medical information to design of personalized therapies.

Breakthroughs in new technologies will boost novel therapeutics in treating autoimmune disorders. Advances in imaging technology will visualize not only pathology but also CNS physiology. We expect to witness advances in monoclonal antibody therapy and reprogramed B- and T-cell transplantation that will finely tune immune tolerance.

Acknowledgements

The authors thank Lea Dacy for proofreading and editorial assistance.

Financial & competing interests disclosure

This work was supported by grants from the NIH (R01 GM092993, R01 NS048357 and R21 NS073684) and the National Multiple Sclerosis Society (CA 1060A). This work was also supported by a High-Impact Pilot and Feasibility Award (HIPFA) and Novel Methodology Award (NMDA) from the Mayo Clinic Center for Translational Science Activities (CTSA) and Mayo Clinic CTSA grant number UL1 TR000135 from the National Center for Advancing Translational Science (NCATS), a component of the NIH. We also acknowledge with thanks

support from the Applebaum, Hilton, Peterson and Sanford Foundations, the Minnesota Partnership Award for Biotechnology and Medical Genomics, the Moon and Marilyn Park Directorship Fund and the McNeilus family. SOD1 G93A mice were donated from the Prize4Life colony at The Jackson Laboratory. Patents for antibodies that promote remyelination and CNS repair are issued and owned by Mayo Foundation. Therefore, the authors have a potential future conflict of interest. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

EXECUTIVE SUMMARY

- Self-reactive antibodies play critical roles in maintaining health but may also mediate autoimmune disease. To avoid autoimmunity, the immune system closely monitors the generation of self-reactive B-cell clones. There are healthy individuals who carry antibodies that recognize antigens on the surface of neural cells. Of note, these antibodies can enter the CNS but do not induce pathology. To differentiate the auto-antibodies involved in autoimmune diseases, self-antibodies identified in healthy individuals without previous exposure to self-antigens are designated as natural antibodies. B-1 cells in mice are positively selected on the basis of self-reactivity and proliferate to form a self-renewing repertoire of lymphocytes that produce natural antibodies. Those B-1 cells expressing CD5 are B-1a cells, whereas B-1b cells are CD5 negative.
- Antigen specificity is crucial to B-1 cell development and function. B-1 cells are selected based on their self-reactive B-cell receptor (BCR) signaling. However, once generated, the innate state activation of B-1 cells is BCR-independent. B cells express numerous innate immune receptors/patterns that mediate BCR-independent B-1 cell activation.
- Activated B-1 cells differentiate and secrete natural IgM/IgA antibodies that protect pathogen invasion.
- Natural antibodies bind to self-antigens such as oxidized lipids and new antigens to inhibit inflammation and maintain homeostasis.
- B-1 cells mainly respond to self-antigens in immune activation. B-1 cells generate polyreactive IgMs that promote B-2 cells to produce high-affinity IgGs.
- The immune system can generate a subset of B cells in response to self-antigens from the nervous system. The selection process may begin at the early embryonic stage and extend into adulthood.
- Natural IgM antibodies that bind to myelin promote remyelination, which is paradoxical to the finding in experimental autoimmune encephalomyelitis where immune activation to myelin antigens in the presence of adjuvants induces injury leading to demyelination and axonal degeneration.
- Environmental triggers, either toxins from pollution, infectious agents and/or idiopathic cellular injuries in the genetically predisposed individual, activate antigen-specific T cells. Reactivation of T cells further recruits innate immune cells and leads to inflammatory cellular damage. During the process, the original triggers that initiate the disease may be resolved and are difficult to connect to the trigger of the disease.
- T- and B-cell responses coordinate to induce the demyelinating lesions that mediate multiple sclerosis.
- In addition to regulating immune activity, natural IgMs that bind to neural surface antigens can signal nerve cells and mediate remyelination and axonal growth.
- Natural IgMs that label surface antigens recognize the carbohydrate epitopes on glycoproteins and/or glycolipids.
- Natural IgMs are potential therapeutics. Clinical trials to treat multiple sclerosis with human natural IgM are underway.

References

- Hayakawa K, Hardy RR, Herzenberg LA. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J. Exp. Med.* 161(6), 1554–1568 (1985).
- Hayakawa K, Hardy RR, Parks DR, Herzenberg LA. The 'Ly-1 B' cell subpopulation in normal immunodeficient, and autoimmune mice. *J. Exp. Med.* 157(1), 202–218 (1983).
- Sauerborn M, Schellekens H. B-1 cells and naturally occurring antibodies: influencing the immunogenicity of recombinant human therapeutic proteins? *Curr. Opin. Biotechnol.* 20(6), 715–721 (2009).
- Rodriguez M, Warrington AE, Pease LR. Invited article: human natural autoantibodies in the treatment of neurologic disease. *Neurology* 72(14), 1269–1276 (2009).
- Iorio R, Lennon VA. Neural antigen-specific autoimmune disorders. *Immunol. Rev.* 248(1), 104–121 (2012).
- Diamond B, Honig G, Mader S, Brimberg L, Volpe BT. Brain-reactive antibodies and disease. *Annu. Rev. Immunol.* 31, 345–385 (2013).
- Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science* 239(4837), 290–292 (1988).
- Greter M, Heppner FL, Lemos MP *et al* Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat. Med.* 11(3), 328–334 (2005).
- Hay EM, Isenberg DA. Autoantibodies in central nervous system lupus. *Br. J. Rheumatol.* 32(4), 329–332 (1993).
- Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J. Exp. Med.* 202(4), 473–477 (2005).
- Panzer J, Dalmau J. Movement disorders in paraneoplastic and autoimmune disease. *Curr. Opin. Neurol.* 24(4), 346–353 (2011).
- Blaes F, Tschernatsch M. Paraneoplastic neurological disorders. *Expert Rev. Neurother.* 10(10), 1559–1568 (2010).
- Andersson J, Sjöberg O, Møller G. Induction of immunoglobulin and antibody synthesis in vitro by lipopolysaccharides. *Eur. J. Immunol.* 2(4), 349–353 (1972).
- Baumgarth N. A two-phase model of B-cell activation. *Immunol. Rev.* 176, 171–180 (2000).
- Kantor AB. The development and repertoire of B-1 cells (CD5 B cells). *Immunol. Today* 12(11), 389–391 (1991).
- Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective effector functions. *Nat. Rev. Immunol.* 11(1), 34–46 (2011).
- Hardy RR, Hayakawa K. B cell development pathways. *Annu. Rev. Immunol.* 19, 595–621 (2001).
- Godin IE, Garcia-Porrero JA, Coutinho A, Dieterlen-Lievre F, Marcos MA. Para-aortic splanchnopleura from early mouse embryos contains B1a cell progenitors. *Nature* 364(6432), 67–70 (1993).
- Herzenberg LA. B-1 cells: the lineage question revisited. *Immunol. Rev.* 175, 9–22 (2000).
- Dorshkind K, Montecino-Rodriguez E. Fetal B-cell lymphopoiesis and the emergence of B-1-cell potential. *Nat. Rev. Immunol.* 7(3), 213–219 (2007).
- Solvason N, Wu WW, Parry D *et al* Cyclin D2 is essential for BCR-mediated proliferation and CD5 B cell development. *Int. Immunol.* 12(5), 631–638 (2000).
- Lalor PA, Herzenberg LA, Adams S, Stall AM. Feedback regulation of murine Ly-1 B cell development. *Eur. J. Immunol.* 19(3), 507–513 (1989).
- Baumgarth N, Herman OC, Jäger GC, Brown L, Herzenberg LA. Innate and acquired humoral immunities to influenza virus are mediated by distinct arms of the immune system. *Proc. Natl Acad. Sci. USA* 96(5), 2250–2255 (1999).
- Deenen GJ, Kroese FG. Murine peritoneal Ly-1 B cells do not turn over rapidly. *Ann. N Y Acad. Sci.* 651, 70–71 (1992).
- Boes M, Esau C, Fischer MB, Schmidt T, Carroll M, Chen J. Enhanced B-1 cell development, but impaired IgG antibody responses in mice deficient in secreted IgM. *J. Immunol.* 160(10), 4776–4787 (1998).
- Duber S, Hafner M, Krey M *et al* Induction of B-cell development in adult mice reveals the ability of bone marrow to produce B-1a cells. *Blood* 114(24), 4960–4967 (2009).
- Holodick NE, Repetny K, Zhong X, Rothstein TL. Adult BM generates CD5+ B1 cells containing abundant N-region additions. *Eur. J. Immunol.* 39(9), 2383–2394 (2009).
- Hayakawa K, Asano M, Shinton SA *et al* Positive selection of natural autoreactive B cells. *Science* 285(5424), 113–116 (1999).
- Lam KP, Rajewsky K. B cell antigen receptor specificity and surface density together determine B-1 versus B-2 cell development. *J. Exp. Med.* 190(4), 471–477 (1999).
- Hardy RR, Hayakawa K. Positive and negative selection of natural autoreactive B cells. *Adv. Exp. Med. Biol.* 750, 227–238 (2012).
- Baumgarth N. Innate-like B cells and their rules of engagement. *Adv. Exp. Med. Biol.* 785, 57–66 (2013).
- Meyer-Bahlburg A, Rawlings DJ. B cell autonomous TLR signaling and autoimmunity. *Autoimmun. Rev.* 7(4), 313–316 (2008).
- Bekeredjian-Ding I, Jegou G. Toll-like receptors—sentinels in the B-cell response. *Immunology* 128(3), 311–323 (2009).
- Crampton SP, Voynova E, Bolland S. Innate pathways to B-cell activation and tolerance. *Ann. N Y Acad. Sci.* 1183, 58–68 (2010).
- Nisitani S, Tsubata T, Murakami M, Honjo T. Administration of interleukin-5 or -10 activates peritoneal B-1 cells and induces autoimmune hemolytic anemia in anti-erythrocyte autoantibody-transgenic mice. *Eur. J. Immunol.* 25(11), 3047–3052 (1995).
- Racine R, Winslow GM. IgM in microbial infections: taken for granted? *Immunol. Lett.* 125(2), 79–85 (2009).
- Binder CJ. Natural IgM antibodies against oxidation-specific epitopes. *J. Clin. Immunol.* 30(Suppl. 1), S56–S60 (2010).
- Chou MY, Fogelstrand L, Hartvigsen K *et al* Oxidation-specific epitopes are dominant targets of innate natural antibodies in mice and humans. *J. Clin. Invest.* 119(5), 1335–1349 (2009).
- Gronwall C, Vas J, Silverman GJ. Protective roles of natural IgM antibodies. *Front. Immunol.* 3, 56–66 (2012).
- Boes M, Schmidt T, Linkemann K, Beaudette BC, Marshak-Rothstein A, Chen J. Accelerated development of IgG autoantibodies and autoimmune disease in the absence of secreted IgM. *Proc. Natl Acad. Sci. USA* 97(3), 1184–1189 (2000).
- Chen Y, Park YB, Patel E, Silverman GJ. IgM antibodies to apoptosis-associated determinants recruit C1q and enhance dendritic cell phagocytosis of apoptotic cells. *J. Immunol.* 182(10), 6031–6043 (2009).
- Shaw PX, Horkko S, Chang MK *et al* Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. *J. Clin. Invest.* 105(12), 1731–1740 (2000).
- Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* 23, 901–944 (2005).

- 44 O'Garra A, Stapleton G, Dhar V *et al* Production of cytokines by mouse B cells: B lymphomas and normal B cells produce interleukin 10. *Int. Immunol.* 2(9), 821–832 (1990).
- 45 Lampropoulou V, Calderon-Gomez E, Roch T *et al* Suppressive functions of activated B cells in autoimmune diseases reveal the dual roles of Toll-like receptors in immunity. *Immunol. Rev.* 233(1), 146–161 (2010).
- 46 Zhang M, Carroll MC. Natural antibody mediated innate autoimmune response. *Mol. Immunol.* 44(1–3), 103–110 (2007).
- 47 Bouaziz JD, Yanaba K, Tedder TF. Regulatory B cells as inhibitors of immune responses and inflammation. *Immunol. Rev.* 224, 201–214 (2008).
- 48 Baumgarth N, Herman OC, Jager GC, Brown LE, Herzenberg LA, Chen J. B-1 and B-2 cell-derived immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection. *J. Exp. Med.* 192(2), 271–280 (2000).
- 49 Choi YS, Baumgarth N. Dual role for B-1a cells in immunity to influenza virus infection. *J. Exp. Med.* 205(13), 3053–3064 (2008).
- 50 Alam C, Valkonen S, Ohls S, Tornqvist K, Hanninen A. Enhanced trafficking to the pancreatic lymph nodes and auto-antigen presentation capacity distinguishes peritoneal B lymphocytes in non-obese diabetic mice. *Diabetologia* 53(2), 346–355 (2010).
- 51 Riggs JE, Howell KF, Taylor J *et al* Mls presentation by peritoneal cavity B cells. *Immunobiology* 209(3), 255–264 (2004).
- 52 Ryan GA, Wang CJ, Chamberlain JL *et al* B1 cells promote pancreas infiltration by autoreactive T cells. *J. Immunol.* 185(5), 2800–2807 (2010).
- 53 Rakhmanov M, Keller B, Gutenberger S *et al* Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. *Proc. Natl Acad. Sci. USA* 106(32), 13451–13456 (2009).
- 54 Covens K, Verbinen B, Geukens N *et al* Characterization of proposed human B-1 cells reveals pre-plasmablast phenotype. *Blood* 121(26), 5176–5183 (2013).
- 55 Rothstein TL, Griffin DO, Holodick NE, Quach TD, Kaku H. Human B-1 cells take the stage. *Ann. NY Acad. Sci.* 1285, 97–114 (2013).
- 56 Warrington AE, Rodriguez M. Method of identifying natural antibodies for remyelination. *J. Clin. Immunol.* 30(Suppl. 1), S50–S55 (2010).
- 57 Warrington AE, Bieber AJ, Van Keulen V, Ciric B, Pease LR, Rodriguez M. Neuron-binding human monoclonal antibodies support central nervous system neurite extension. *J. Neuropathol. Exp. Neurol.* 63(5), 461–473 (2004).
- 58 Warrington AE, Bieber AJ, Ciric B, Pease LR, Van Keulen V, Rodriguez M. A recombinant human IgM promotes myelin repair after a single, very low dose. *J. Neurosci. Res.* 85(5), 967–976 (2007).
- 59 Xu X, Warrington AE, Wright BR *et al* A human IgM signals axon outgrowth: coupling lipid raft to microtubules. *J. Neurochem.* 119(1), 100–112 (2011).
- 60 <http://clinicaltrials.gov/show/NCT01803867>
- 61 Nilsson O. Immunological aspects on demyelinating diseases. *Acta Neurol. Scand. Suppl.* 51, 321–336 (1972).
- 62 Martin R, McFarland HF, McFarlin DE. Immunological aspects of demyelinating diseases. *Annu. Rev. Immunol.* 10, 153–187 (1992).
- 63 Wekerle H, Kojima K, Lannes-Vieira J, Lassmann H, Linington C. Animal models. *Ann. Neurol.* 36(Suppl.), S47–S53 (1994).
- 64 Rodriguez M, Lennon VA. Immunoglobulins promote remyelination in the central nervous system. *Ann. Neurol.* 27(1), 12–17 (1990).
- 65 Ben-Nun A, Wekerle H, Cohen IR. The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur. J. Immunol.* 11(3), 195–199 (1981).
- 66 Schluesener HJ, Wekerle H. Autoaggressive T lymphocyte lines recognizing the encephalitogenic region of myelin basic protein: *in vitro* selection from unprimed rat T lymphocyte populations. *J. Immunol.* 135(5), 3128–3133 (1985).
- 67 Sosa RA, Murphey C, Ji N, Cardona AE, Forsthuber TG. The kinetics of myelin antigen uptake by myeloid cells in the central nervous system during experimental autoimmune encephalomyelitis. *J. Immunol.* 191(12), 5848–5857 (2013).
- 68 Bogdanos DP, Smyk DS, Rigopoulou EI *et al* Twin studies in autoimmune disease: genetics, gender and environment. *J. Autoimmun.* 38(2–3), J156–J169 (2012).
- 69 Ben-Nun A, Kaushansky N, Kawakami N *et al* From classic to spontaneous and humanized models of multiple sclerosis: Impact on understanding pathogenesis and drug development. *J. Autoimmun.* (2014).
- 70 Pollard KM, Hultman P, Kono DH. Toxicology of autoimmune diseases. *Chem. Res. Toxicol.* 23(3), 455–466 (2010).
- 71 Pollard KM. Gender differences in autoimmunity associated with exposure to environmental factors. *J. Autoimmun.* 38(2–3), J177–186 (2012).
- 72 Vojdani A. The characterization of the repertoire of wheat antigens and peptides involved in the humoral immune responses in patients with gluten sensitivity and Crohn's disease. *ISRN Allergy.* 950104 (2011).
- 73 Miller FW, Pollard KM, Parks CG *et al* Criteria for environmentally associated autoimmune diseases. *J. Autoimmun.* 39(4), 253–258 (2012).
- 74 Temajo NO, Howard N. The mosaic of environment involvement in autoimmunity: the abrogation of viral latency by stress, a non-infectious environmental agent, is an intrinsic prerequisite prelude before viruses can rank as infectious environmental agents that trigger autoimmune diseases. *Autoimmun. Rev.* 13(6), 635–640 (2014).
- 75 Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science* 336(6086), 1268–1273 (2012).
- 76 Munz C, Lunemann JD, Getts MT, Miller SD. Antiviral immune responses: triggers of or triggered by autoimmunity? *Nat. Rev. Immunol.* 9(4), 246–258 (2009).
- 77 Saida K, Saida T, Brown MJ, Silberberg DH. In vivo demyelination induced by intraneural injection of anti-galactocerebroside serum: a morphologic study. *Am. J. Pathol.* 95(1), 99–116 (1979).
- 78 Roth GA, Monferran CG, Maggio B, Cumar FA. Central nervous system lipid alterations in rats with experimental allergic encephalomyelitis and its suppression by immunosuppressive drugs. *Life Sci.* 30(10), 859–866 (1982).
- 79 Lassmann H, Brunner C, Bradl M, Linington C. Experimental allergic encephalomyelitis: the balance between encephalitogenic T lymphocytes and demyelinating antibodies determines size and structure of demyelinated lesions. *Acta Neuropathol.* 75(6), 566–576 (1988).
- 80 Asakura K, Miller DJ, Pease LR, Rodriguez M. Targeting of IgMkappa antibodies to oligodendrocytes promotes CNS remyelination. *J. Neurosci.* 18(19), 7700–7708 (1998).
- 81 Gold R, Linington C, Lassmann H. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain* 129(Pt 8), 1953–1971 (2006).

- 82 Sommer I, Schachner M. Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. *Dev. Biol.* 83(2), 311–327 (1981).
- 83 Rivkin MJ, Flax J, Mozell R, Osathanondh R, Volpe JJ, Villa-Komaroff L. Oligodendroglial development in human fetal cerebrum. *Ann. Neurol.* 38(1), 92–101 (1995).
- 84 Wright BR, Warrington AE, Edberg DD, Rodriguez M. Cellular mechanisms of central nervous system repair by natural autoreactive monoclonal antibodies. *Arch. Neurol.* 66(12), 1456–1459 (2009).
- 85 Honke K. Biosynthesis and biological function of sulfoglycolipids. *Proc. Jpn Acad. Ser. B Phys. Biol. Sci.* 89(4), 129–138 (2013).
- 86 Watzlawik J, Holicky E, Edberg DD *et al* Human remyelination promoting antibody inhibits apoptotic signaling and differentiation through Lyn kinase in primary rat oligodendrocytes. *Glia* 58(15), 1782–1793 (2010).
- 87 Furukawa K, Ohkawa Y, Yamauchi Y, Hamamura K, Ohmi Y. Fine tuning of cell signals by glycosylation. *J. Biochem.* 151(6), 573–578 (2012).
- 88 Rogers GN, Paulson JC, Daniels RS, Skehel JJ, Wilson IA, Wiley DC. Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity. *Nature* 304(5921), 76–78 (1983).
- 89 Brewer GJ, Torricelli JR. Isolation and culture of adult neurons and neurospheres. *Nat. Protoc.* 2(6), 1490–1498 (2007).
- 90 Fischer J, Beckervordersandforth R, Tripathi P, Steiner-Mezzadri A, Ninkovic J, Gotz M. Prospective isolation of adult neural stem cells from the mouse subependymal zone. *Nat. Protoc.* 6(12), 1981–1989 (2011).
- 91 Zeng Y, Ramya TN, Dirksen A, Dawson PE, Paulson JC. High-efficiency labeling of sialylated glycoproteins on living cells. *Nat. Methods* 6(3), 207–209 (2009).
- 92 Saito M, Kitamura H, Sugiyama K. The specificity of monoclonal antibody A2B5 to c-series gangliosides. *J. Neurochem.* 78(1), 64–74 (2001).
- 93 Varki A, Gagneux P. Multifarious roles of sialic acids in immunity. *Ann. N Y Acad. Sci.* 1253, 16–36 (2012).
- 94 Varki A. Selectins and other mammalian sialic acid-binding lectins. *Curr. Opin. Cell Biol.* 4(2), 257–266 (1992).
- 95 Vimr ER, Kalivoda KA, Deszo EL, Steenbergen SM. Diversity of microbial sialic acid metabolism. *Microbiol. Mol. Biol. Rev.* 68(1), 132–153 (2004).
- 96 Allen PM, Roberts I, Boulnois GJ, Saunders JR, Hart CA. Contribution of capsular polysaccharide and surface properties to virulence of *Escherichia coli* K1. *Infect. Immun.* 55(11), 2662–2668 (1987).
- 97 Ziak M, Qu B, Zuo X *et al* Occurrence of poly(alpha2,8-deaminoneuraminic acid) in mammalian tissues: widespread and developmentally regulated but highly selective expression on glycoproteins. *Proc. Natl Acad. Sci. USA* 93(7), 2759–2763 (1996).
- 98 Yuki N. Ganglioside mimicry and peripheral nerve disease. *Muscle Nerve* 35(6), 691–711 (2007).
- 99 Rogers GN, Herrler G, Paulson JC, Klenk HD. Influenza C virus uses 9-O-acetyl-N-acetylneuraminic acid as a high affinity receptor determinant for attachment to cells. *J. Biol. Chem.* 261(13), 5947–5951 (1986).
- 100 Herrler G, Rott R, Klenk HD, Muller HP, Shukla AK, Schauer R. The receptor-destroying enzyme of influenza C virus is neuraminidase-O-acetyltransferase. *EMBO J.* 4(6), 1503–1506 (1985).
- 101 Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science* 327(5961), 46–50 (2010).
- 102 Xu X, Wittenberg NJ, Jordan LR *et al* A patterned recombinant human IgM guides neurite outgrowth of CNS neurons. *Sci. Rep.* 3, 2267 (2013).
- 103 McKerracher L. Ganglioside rafts as MAG receptors that mediate blockade of axon growth. *Proc. Natl Acad. Sci. USA* 99(12), 7811–7813 (2002).
- 104 Quarles RH. Myelin-associated glycoprotein in development and disease. *Dev. Neurosci.* 6(6), 285–303 (1983).
- 105 Schnaar RL, Collins BE, Wright LP *et al* Myelin-associated glycoprotein binding to gangliosides. Structural specificity and functional implications. *Ann. N Y Acad. Sci.* 845, 92–105 (1998).
- 106 Yang LJ, Zeller CB, Shaper NL *et al* Gangliosides are neuronal ligands for myelin-associated glycoprotein. *Proc. Natl Acad. Sci. USA* 93(2), 814–818 (1996).
- 107 Sheikh KA, Sun J, Liu Y *et al* Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. *Proc. Natl Acad. Sci. USA* 96(13), 7532–7537 (1999).
- 108 Schnaar RL, Gerardy-Schahn R, Hildebrandt H. Sialic acids in the brain: gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. *Physiol. Rev.* 94(2), 461–518 (2014).