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Mini review

Integration of lectin–glycan recognition systems and immune cell networks in CNS inflammation

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ABSTRACT

Multiple sclerosis (MS) is a progressive degenerative disorder of the central nervous system (CNS), characterized by inflammation, demyelination and axonal loss. While the majority of MS patients experience relapsing-remitting symptoms followed by a secondary progressive phase, about 10–15% patients exhibit a primary progressive disease involving continuous progression from its onset. Here we review the role of lectin–glycan recognition systems, including those concerning siglecs, C-type lectins and galectins in the pathogenesis of MS and experimental autoimmune encephalomyelitis. Particularly, we will focus on the role of galectins in the fate of T cells, dendritic cells and CNS cell populations. Understanding the regulatory circuits governed by lectin–glycan interactions and their association with disease-associated cytokine networks will contribute to develop novel therapeutic strategies in MS.

1. Introduction

1.1. Chronic inflammation in the era of glycomics

Multiple sclerosis (MS) is an inflammatory demyelinating and degenerative disease of the central nervous system (CNS), which affects approximately 2,000,000 people worldwide. While the majority of MS patients (85%) experience relapsing-remitting (RR) symptoms followed by a secondary progressive (SP) phase, about 10–15% patients exhibit a primary progressive disease (PP-MS) which involves continuous progression from its onset [1]. Current therapies partially alter disease course by decreasing relapses; yet chronic disease remains resistant to such treatments [2].

Most effective therapies for chronic inflammatory disorders are typically based on targeting molecular differences between healthy and inflamed tissues. In general, these differences have been appreciated as a result of exploratory strategies based on genomics, proteomics and lipidomics approaches. Interestingly, in the postgenomic era, the study of the glycome has facilitated the association of specific glycan structures with the development and course of various inflammatory conditions [3,4]. Carbohydrates are, because of their distinctive chemical properties, ideal for generating compact units of high coding capacity that contain specific biological information. Their structural variability is illustrated not only by their diverse sequences but also by their spatial distribution: glycans are capable of branching and twisting, with a flexibility that influences their three-dimensional structure, enabling their performance as hierarchical biochemical signals. The cellular glycome is anything but static: its exquisite

Abbreviations: BBB, blood–brain barrier; CLR, C-type lectin receptors; CNS, central nervous system; CRD, carbohydrate recognition domain; CTLA-4, cytotoxic T lymphocyte antigen-4; DCs, dendritic cells; EAE, experimental autoimmune encephalomyelitis; MGAT5, β1,6 N-acetylglucosaminyltransferase 5; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; PP-MS, primary progressive multiple sclerosis; RR-MS, relapsing remitting multiple sclerosis; S1P, sphingosine 1-phosphate; Sn, sialoadhesin; SP-MS, secondary progressive multiple sclerosis; TCR, T cell receptor; TNF, tumor necrosis factor; TGF-βR, transforming growth factor-β receptor; Th, T helper cells; Treg cells, T regulatory cells.

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dependency on different variables (*i.e.* the nutritional state, differentiation and activation status of the cells) makes glycan remodeling *via* the combined action of glycosyltransferases and glycosidases, a common process that controls critical biological processes [6,7]. This biochemical complexity, fascinating from biological and evolutionary perspectives, makes functional and molecular glycomics one of the most challenging research fields at present. The development of new technologies capable of expanding our knowledge of the glycome is growing apace: new methodologies such as glyco-gene arrays, frontal affinity chromatography and the generation of relevant transgenic and knockout mice [8–10] are rapidly changing the scene and creating novel opportunities for significant progress.

The information stored in glycan structures can be specifically deciphered by an array of endogenous glycan-binding proteins or lectins which expression and function are highly regulated at sites of inflammation [11]. These proteins are classified into diverse lectin families, most of them with essential roles in immunological and neurological networks, including those operating during the course of MS. Whereas all glycan-binding proteins exhibit an affinity site that is complementary to different carbohydrate structures (glyco-epitopes), they are capable of detecting and decoding subtle differences in sequence motifs and to generate high avidity interactions with glycans due to their multivalent nature [12].

In this article we review the emerging roles of lectin–glycan recognition systems, including those mediated by siglecs, C-type lectins and galectins, in the development, severity and resolution of MS and in experimental autoimmune encephalomyelitis (EAE), a prototypic animal model established in different species and strains that recapitulates some of the features of the disease. Particularly, we will emphasize on recent findings identifying a role for galectins in regulating the fate and signaling of T cells, dendritic cells and CNS immune cell populations including macrophages, astrocytes and oligodendrocytes. Understanding the complexity of circuits triggered by lectin–glycan interactions and their interplay with cytokine networks will contribute to develop novel therapeutic strategies that could benefit a wider population of MS patients.

1.2. The experimental autoimmune encephalomyelitis (EAE) model

Dissecting the pathogenesis of a disease as complex as MS in humans faces several problems, particularly those associated with clinical and genetic heterogeneity. Because brain and spinal cord tissue cannot easily be sampled in MS patients, a number of animal models have been generated to provide insights into the underlying pathology, as well as to identify surrogate biomarkers and therapeutic targets. The basic protocol for inducing EAE involves immunizing animal strains and species using either foreign or self-proteins from the white matter of the CNS, usually myelin basic protein (MBP), proteolipid protein (PLP) or myelin oligodendrocyte glycoprotein (MOG) [13]. Immunization leads to the development of a disease state in the animals which partially recapitulates some neuropathologic similarities of human MS. Clinical course of the disease varies from acute monophasic episodes of paralysis in some models to chronic-relapsing neurological episodes and progressive disability in others [14]. These episodes correlate with perivascular mononuclear cell infiltrates in the CNS and, in some species and strains, with extensive myelin destruction and axonal loss [15]. After an initial period of experimentation, active sensitization in murine models of chronic demyelination progressed to adoptive transfer technologies, whereby CNS antigen-specific or bulk-isolated lymphocytes such as T cell lines or T cell clones are administered intravenously into naïve recipients [16]. Interestingly, recent studies revealed that MS is not only T-cell dependent disease as previously demonstrated, but it is also mediated by innate immune cells and B cells [17].

In contrast to MS, which occurs in a spontaneous and unpredictable manner, the classic EAE model needs to be induced in genetically-predisposed animals. Commonly used rodent models often involve immunization with peptides that are too short to induce a pathogenic B-cell response. In contrast, immunization with MOG in Dark Agouti rats, marmosets, or C57BL/6 mice is critically dependent on B cells and elicits both autoreactive T-cell and B-cell responses [18]. To study disease aspects without confounding factors present in conventional EAE, such as adjuvants, EAE models in which disease occurs spontaneously have also been developed. In these transgenic models mice are introduced with transgenes encoding myelin-specific human T cell receptors and human major histocompatibility complex antigens [19,20].

Although the information learned from EAE models has led to a huge advance in understanding the mechanisms and pathogenesis of MS, extrapolation of results obtained in rodents to humans has sparked-off significant debate. Potential therapeutic molecules showing promising effects in rodent EAE showed no beneficial properties in patients, or sometimes presented unexpected adverse effects in clinical trials. These drawbacks can be avoided using animal models more closely related to humans, such as non-human primates. In marmosets, EAE can be induced by immunization with recombinant human MOG_{1-125} in complete Freund's adjuvant [21]. Marmosets are of outbreed nature, which mirrors the genetic diversity of the patient population. Another beneficial aspect of marmoset EAE is that it can be induced at an adult age, where the immune system and the CNS are fully developed. Thus, possible medical side effects may be detected prior to clinical trials. Nevertheless, high costs and ethical issues limit studies on non-human primates to a minimum.

Thus, although EAE recapitulates many features of the pathogenesis of MS, discrepancies between MS and EAE models still remain an important issue. When using EAE rodent models, extrapolations should be made with caution if the goals are to study the pathogenesis of the disease, the search for useful biomarkers and/or the validation of novel therapeutic approaches [22]. One critical limitation is that most EAE studies are based on injection with antigenic material to induce disease onset. This clearly contrasts with the spontaneously arising nature of MS. Transgenic murine models offer a possibility to overcome this limitation. However, these mice are highly manipulated and the precise antigen(s) involved in triggering inflammation and demyelination are still uncertain. Adding complexity to this picture, another potential limitation is that investigation in EAE models is mostly conducted on highly inbred animals. Finally, the degree of clinical disease in the EAE model is typically reflected by the lesion load within the spinal cord [23]. Although some animal strain/species may particularly develop cerebellar lesions, in many EAE models the brain is relatively unaffected [24]. Problematic aspects of animal models are in no way confined to MS alone, and limitations in models should be acknowledged when placing research findings into perspective. Clearly, studies in the EAE model need to be carefully tailored to the pathogenesis or therapy question. Furthermore, results showing a high degree of consistency between various models, experimental conditions and clinical outcomes are more likely to lead to translation into therapeutic success.

1.3. From EAE to MS: immunopathogenic mechanisms

Plaques of inflammatory demyelination within the central nervous system (CNS) constitute the pathologic hallmarks of MS.

Typical features of the acute plaque include ill-defined margins of myelin loss, infiltration by immune cells, and parenchymal edema [25]. The constituents of immune cell influx around vessels include T cells, B cells, dendritic cells (DCs), monocytes and macrophages. The presence of lymphocytes within plaques and bordering areas suggests that inflammatory destruction observed in MS is driven by antigen-specific cells targeting myelin and other CNS components. Access of immune cells to the CNS is restricted, at least in part, by the blood brain barrier (BBB), and only through activation of a hierarchical sequence of cellular events, autoreactive lymphocytes can actually enter the CNS compartment. Initially, leukocyte engages in rolling, activation, and arrest along the BBB endothelium. This initial step is greatly facilitated by up-regulation of endothelial cell adhesion molecules, including ICAM-1 and VCAM-1 [26]. Changes in the vascular endothelium could result from pro-inflammatory mediators circulating within the vasculature, including TNF, and IFN-γ. The repertoire of molecules that leukocytes rely on to entry into the CNS, involves a number of integrins. Among them, very late antigen-4 (VLA-4 or $\alpha_4\beta_1$) has been identified as the most critical molecule involved in transmigration of encephalitogenic T cells toward the CNS [27]. Indeed, natalizumab, a humanized monoclonal antibody that is directed against the α4β1 integrin, has been shown to substantially reduce disease activity in clinical trials involving MS patients [28,29]. In addition, leukocyte migration to the CNS is facilitated through the concerted action of chemokines and their specific receptors [30]. In this regard, a proof-of-concept of the relevance of immune cell traffic in the pathogenesis of MS is provided by the emergence of a new drug, FTY720 (fingolimod), a sphingosine 1-phosphate (S1P) receptor modulator that inhibits egress of lymphocytes from the lymph nodes, preventing entry into the blood and infiltration into the CNS. Importantly, this is the first oral disease-modifying therapy to be approved for the treatment of RR-MS [31].

It has been demonstrated that EAE is induced by abnormally activated $CD4^+$ T cells recognizing CNS antigens; yet the mechanisms underlying activation of these cells remain unclear [32]. Moreover, dysregulation of the balance between Th1 and Th2 cytokines has long been implicated in MS immunopathogenesis: Th1 cells produce pro-inflammatory cytokines such as IFN-γ which activates macrophages and $CD8^+$ cytotoxic T cells, while Th2 cells secrete IL-4, IL-5 and IL-13 and suppress Th1 responses. Induction of Th17 cells, a distinct lineage of effector T cells capable of synthesizing pro-inflammatory cytokines such as IL-17A and IL-17F, is promoted by TGF-β and IL-6 and amplified by IL-23 produced by DCs and macrophages [33]. Interestingly, Th17 to Th1 ratio appears to be a critical determinant of CNS inflammation where high Th17 to Th1 ratios have been linked to T cell infiltration and CNS inflammation [34]. Natural occurring or inducible $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells (Treg) contribute significantly to dictate the evolution and severity of MS. Although Treg cell numbers in peripheral blood and cerebrospinal fluid (CSF) appear to be similar in MS patients as compared to healthy controls, several studies highlighted defects in the capacity of Treg cells from MS patients to suppress myelin-specific T cell activation in the periphery [35]. Interestingly, in addition to $CD4^+CD25^+FoxP3^+$ Treg cells, *in vitro* antigen activation leads to Foxp3 expression in $CD8^+$ T cells. Studies indicated that $CD8^+$ T cells present in MS lesions may contribute to tissue damage by attacking oligodendrocytes and transecting axons [36,37], and Foxp3 expression in $CD8^+$ T cells enables them to acquire additional suppressive activity [38].

Although autoreactive T cell-mediated responses have been considered critical for MS pathogenesis, increasing evidence indicates that B cells also play an important role in the development and resolution of the disease. B cells, plasma cells, immunoglobulins and complement deposition, along with immunoglobulin-myelin complex within macrophages have been found in autopsy tissues from MS patients [39]. Synthesis of intrathecal IgG and the occurrence of B-cell lymphoid follicles in the meninges of MS patients with progressive disease further support this concept [40,41]. In addition, B cells can promote neuroinflammation in MS *via* secretion of pro-inflammatory cytokines such as TNF, and lymphotoxin in the presence of the T cell-derived cytokine IFN-γ [42]. Conversely, B cells are also likely to have immunosuppressive activity. For example, IL-10 secretion by B cells can serve to limit pro-inflammatory T-cell responses [43]. Noteworthy, the effectiveness and safety of the anti-CD20 monoclonal antibody rituximab in relapsing and progressive forms of MS emphasize the essential role of B cells in the pathogenesis of the disease [44].

Antigen-presenting cells (APCs), including DCs and macrophages, play an important role in the initiation, progression and resolution of MS. DCs are present in perivascular spaces, the choroid plexus and the meninges of healthy brains [45]. In MS, DCs among other cell types are recruited to the CNS, representing the major APCs during cognate interactions with $CD4^+$ T cells within the CNS [46]. In addition to DCs, microglia are resident APCs localized in active plaques that play an important role in antigen presentation, immunoregulation and neuromodulation. Microglia are resident myeloid cells of the CNS that play essential roles both in normal and inflamed CNS. They are distinguished from peripheral macrophages by the lower expression of CD45. Recently, Butovsky and colleagues identified 239 genes and 8 microRNAs that are uniquely or highly expressed in microglia *versus* macrophages, myeloid cells and other immune cells. Of these genes, the authors identified a TGF-β-dependent signature that is required for the generation of adult microglial cells [47]. Importantly, under resting conditions, microglia constantly surveys the CNS microenvironment, suggesting that these cells are critical for maintaining CNS homeostasis [48]. Quiescent microglia express undetectable levels of MHC I and MHC II, CD80, CD86, and CD40. However, in response to inflammatory stimuli, microglia become activated and upregulate the expression of CD45, MHC II and costimulatory molecules, promoting pro-inflammatory responses and secreting high amounts of nitric oxide (NO) and reactive oxygen species (ROS) [49,50]. Functionally, this pro-inflammatory microglia, termed 'M1-type' or 'classically-activated microglia' can be generated *in vitro* using IFN-γ or Toll-like receptor (TLR) agonists including lipopolysaccharides (LPS). Conversely, 'M2-type' or 'alternatively-activated microglia' can be generated with IL-4, secrete low amounts of NO and high amounts of arginase 1 and prevents inflammation-induced neurodegeneration [51]. Probably due to their lower MHC II expression compared to DCs or macrophages, microglia cells serve as poor activators of naïve T cells. Rather, these cells, depending on whether they are differentiated into M1 or M2 phenotypes, may play a role in the reactivation or de-activation of T cells infiltrating the CNS during MS. In addition, due their ubiquitous localization within the CNS, microglia cells are a potent source of inflammatory cytokines and chemokines that are essential for the initiation and propagation of a CNS-localized immune response [50,51].

Astrocytes are the most abundant cell type in the brain and serve a variety of functions both related and unrelated to immunomodulation. Briefly, they are involved in maintaining the BBB, glutamate metabolism, stabilizing the extracellular concentrations of potassium and producing trophic survival factors for neurons and other glial cells [52]. Although the function of astrocytes as APCs is controversial, they can provide an optimal environment for T-cell activation or de-activation during inflammation. In fact, these cells are producers of a variety of cytokines including IL-1, IL-6, TNF, IL-10 and TGF-β [53], which can positively or negatively regulate innate and adaptive immune

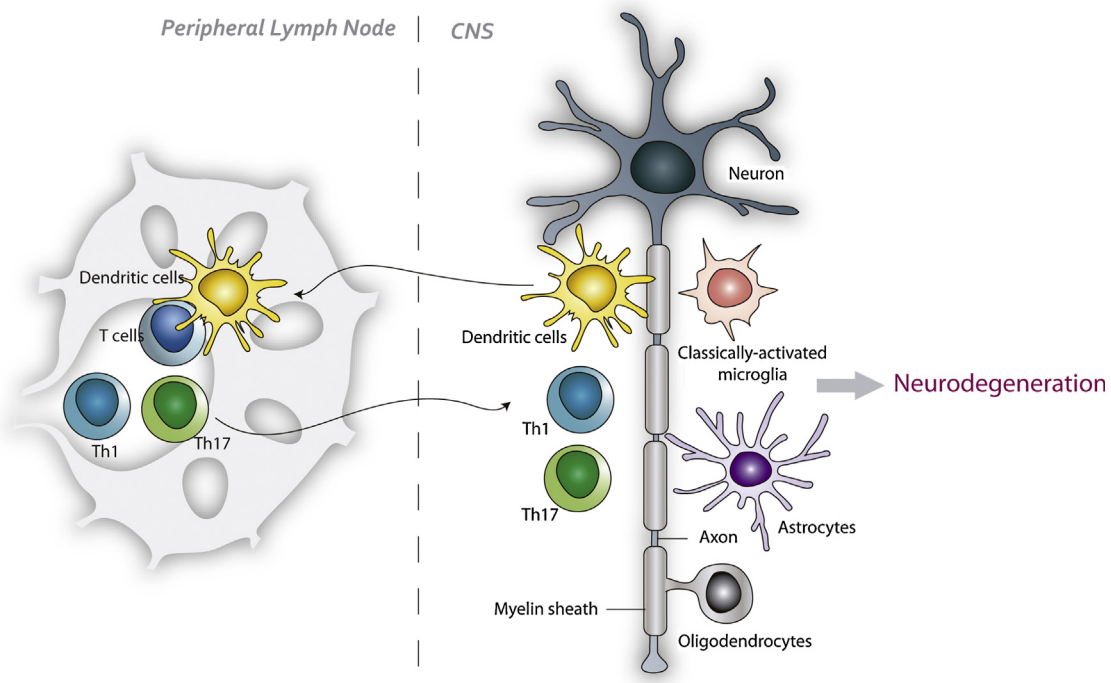

Fig. 1. Contribution of distinct cell populations to the pathogenesis of EAE and MS. Different immune cells contribute to the pathogenesis and severity of EAE and MS promoting inflammation and neurodegeneration. These include Th1 and Th17 pathogenic cells, DCs and microglia, which are reciprocally regulated by CNS resident cells including neurons, astrocytes and oligodendrocytes. Glycan-binding proteins including siglecs, C-type lectins and galectins modulate the function, survival and signaling of these cell types and modulate the course of the disease.

responses. Additionally, they can secrete a variety of chemokines including RANTES, MCP-1, IL-8, and IP-10 [53].

Although MS was originally described as a disease characterized by gradual loss of myelin, axonal loss has also been observed early during the pathology, suggesting that neural defects may in some cases precede immunopathology. Mechanisms for axonal damage are manifold and include but are not restricted to specific immunological attack to axons [37,54]; the presence of soluble mediators such as proteases and free radicals released as part of the inflammatory microenvironment present in the CNS of MS patients [55]. In addition, axonal loss may be due to lack of neurotrophic factors provided by oligodendrocytes as a result of demyelination [56]. As a consequence of immune-mediated injury to myelin, higher energy demands on demyelinated axons and glutamate-mediated excitotoxicity may impart further unsustainable damage [57,58]. A summary of the mechanisms involved in MS pathogenesis, including Th1 and Th17 cells, dendritic cells and CNS microglia, astrocytes and oligodendrocytes is illustrated in Fig. 1.

In spite of considerable evidence highlighting the contribution of genomics, proteomics and lipidomics to demyelinating disease [59,60], the relevance of glycomics to the pathogenesis of MS is just unfolding. In the next section we illustrate selected examples of lectin–glycan recognition systems that play essential roles in the pathogenesis, severity and resolution of MS and its animal model EAE.

2. Glycobiology of MS

Data on the role of glycans in autoimmune CNS inflammation has blossomed with the generation of mice deficient in glycosyltransferases and lectins and the availability of analytical tools for the study of the cellular glycome [61]. Dennis and Demetriou [62] were pioneers in demonstrating a role of *N*-glycans in T-cell activation, autoimmunity and MS. They demonstrated that mice deficient in β1,6 N-acetylglucosaminyltransferase 5 (MGAT5 or GnTV), a limiting enzyme in the *N*-glycosylation pathway, had increased susceptibility to EAE [62]. Remarkably, lack of β1,6 *N*-glycan branching in Mgat5-deficient mice lowers the threshold for T-cell activation by enabling T cell receptor (TCR) clustering and signaling characterized by sustained TCR-dependent tyrosine phosphorylation and robust proliferation. This effect results in enhanced delayed-type hypersensitivity reactions and increased susceptibility to autoimmune neuroinflammation [62]. Further dissection of the underlying mechanisms revealed that, in the absence of cognate ligand, cross-linking of *N*-glycans prevented filamentous actin-dependent targeting of the TCR, CD4, and Lck tyrosine kinase to GM1-enriched membrane microdomains. This effect prevented spontaneous TCR activation by favoring Lck inactivation and specifically retaining the CD45 phosphatase at these membrane domains [63]. In this regard, β1,6-GlcNAc branching appears to be sensitive to the availability of metabolites of the hexosamine pathway because higher concentrations of the cellular donor UDP-GlcNAc increases the capacity of MGAT5 to catalyze *N*-glycan branch formation. Supporting this notion, oral administration of GlcNAc enhanced *N*-glycan branching *in vivo*, dampened TCR signaling, blunted Th1 and Th17 cell responses, and attenuated autoimmune neuroinflammation [64]. Thus, MGAT5-modified complex *N*-glycans presented on cell surface glycoprotein receptors can control T-cell activation threshold and tailor adaptive immunity and autoimmune diseases. Supporting these findings, studies in MS patients revealed that dysregulated Golgi *N*-glycosylation is a final common pathway in which disease associated environmental factors and multiple genetic variants (IL-7RA, IL-2RA, and CTLA-4) converge [65].

On the other hand, recent work from Kanekiyo and colleagues [66] revealed that selective loss of N-acetylglucosaminyltransferase-IX (GnT-IX or GnT-Vb), a brain-specific glycosyltransferase that catalyzes the branched formation of *O*-mannosyl glycan structures in astrocytes, leads to accelerated remyelination in the cuprizone inducible model [66]. Whether *N*- and *O*-glycans play a

role in the transition from normal to inflamed tissue during the course of the disease remains to be determined. In the next sections we will dissect the contribution of glycan-binding proteins including siglecs, C-type lectins (CLRs) and galectins to autoimmune neuroinflammation and neurodegeneration *in vivo*.

2.1. Siglecs

Siglecs are a family of type I membrane proteins, which can be divided into CD33-related siglecs, sialoadhesin, MAG and CD22. These lectins are well known for their ability to selectively recognize sialic acid-containing glycans as well as for their capacity to discriminate among specific linkages (α2,3; α2,6 or α2,8). For CD33-related siglecs and CD22, specific cellular functions are determined by the presence of immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tails [67]. The carbohydrate-binding capacity of these lectins resides in one N-terminal variable (V)-set immunoglobulin-like domain followed by a variable number of C2-set immunoglobulin domains in the extracellular region.

Siglec-1 (sialoadhesin; Sn or CD169) and Siglec-7 are expressed on monocytes from MS patients and have been proposed to play an important role in the pathogenesis of the disease. While Siglec-1 is up-regulated in inflammatory monocytes of patients with PP-MS, and to a lesser extent in patients with SP-MS, Siglec-7 is up-regulated only during clinical relapses in RR-MS patients [68]. Siglec-1 is specifically induced by type-1 IFN and TLR agonists. Siglec-1-positive antigen-presenting cells have been demonstrated the ability to present antigens to naïve T cells and to polarize T cells toward a Th2 response [69]. Interestingly, Siglec-1 expressed on resident and activated tissue-infiltrating macrophages directly binds to T regulatory (Treg) cells and regulates their expansion in EAE, providing direct evidence of the pro-inflammatory role of this lectin [70].

Siglec-7, on the other hand, is known to be a target of the suppressor of cytokine signaling 3 (SOCS3), a negative regulator of the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway that is up-regulated during inflammation. The predominant expression of Siglec-7 in monocytes of relapsing MS patients, suggests its possible role in the regulation of the inflammatory response. Malhotra et al. [68], investigated whether differences in the expression of Siglec-1 and Siglec-7 among healthy volunteers, PP-MS, RR-MS and SP-MS patients were secondary to differences in the activation status of blood monocytes. The conclusions indicated important roles for Siglec-1 in the chronic progressive phases of MS and for Siglec-7 in acute disease activity [68].

Recently, Claude et al. [71], showed that Siglec-E, a mouse orthologue of human Siglec-7, is expressed on microglia cells, inhibits phagocytosis of neural debris and prevents the production of superoxide radicals induced by challenge with neural debris. Co-culture of mouse microglia and neurons demonstrated a neuroprotective effect of microglial Siglec-E that was dependent on neuronal sialic acid residues [71]. On the other hand, murine Siglec-H functions as an endocytic receptor that internalizes antigens for T-cell presentation. Siglec-H-mediated delivery of a T cell epitope derived from MOG to plasmacytoid DCs effectively delayed the onset of EAE and suppressed disease severity. Mechanistically, this effect involved interference with the priming phase of the response, but did not imply differentiation or expansion of MOG-specific Foxp3$^+$ Treg cells [72,73].

Finally, Siglec-4 (myelin-associated glycoprotein, MAG) is preferentially expressed on the myelin sheath, where it delivers signals that affect the cytoarchitecture, structure and long-term stability of the axon. Its main ligand is represented by glycans bearing the structure NeuAcα1,3Galβ1,3GalNAc, which can be found on the terminals of the main gangliosides of the brain: GD1a and GT1b. As this lectin serves as an inhibitor of nerve regeneration, it has been proposed to be involved in the pathogenesis of MS [74]. Thus, siglecs can deliver inhibitory or stimulatory signals that modulate different stages and types of MS and CNS inflammatory processes.

2.2. C-type lectins

C-type lectin receptors (CLRs) are a heterogeneous family of calcium-dependent carbohydrate-binding proteins that can be classified based on glycan recognition specificity. Most CLRs contain one or more carbohydrate-recognition domains (CRDs) that are present on the surface of numerous cell types including macrophages, microglia and DCs.

Previous studies suggested the involvement of CLRs in neurological diseases such as MS. Tetranectin, a homotrimeric CLR has been suggested to play a role in tissue remodeling due to its ability to stimulate plasminogen activation. Tetranectin binds to complex sulphated polysaccharides such as apolipoprotein A, plasminogen and fibrin in a calcium-dependent fashion. The affinity of tetranectin for plasminogen suggests its involvement in central events leading to the proteolysis of matrix proteins [75]. Tetranectin is present in most nerve cells and in myelinated fibers of the white matter, brain and cerebellum, and has been proposed to modulate scarring of MS lesions [76]. However, it is still not clear whether tetranectin is present only in the CSF from MS patients or also in healthy individuals and patients with other neurological disorders. Although discrepancies exist among different reports, a decreased tetranectin index has been proposed to serve as a pre-diagnostic tool in neurological disorders aiming to differentiate early stages of MS and other CNS diseases [77,78].

Interestingly, a CLR called C-type lectin-like domain family 16A (CLEC16A) has been shown to exhibit single nucleotide polymorphisms (SNPs) associated with MS along with other autoimmune diseases [79]. In rat brain, CLEC16A is found in astrocytes and neurons, but not in microglia, and its expression is considerably up-regulated in rat astrocytes upon intraspinal LPS injection [80]. Finally, targeting a mannosylated encephalitogenic peptide inhibits the onset of EAE probably by targeting the mannose receptor, a CLR on immature DCs [81].

2.3. Galectins

Galectins are a family of soluble lectins with a common structural fold and a conserved CRD, which interacts with high avidity with N-acetyl-lactosamine-(Galβ1,4-GlcNAc; LacNAc) residues present both in *N*- and *O*-glycans [7,82,83]. They are present in the intracellular space (cytoplasm, nucleus) and are secreted to the extracellular milieu where they cross-link poly-LacNAc-containing glycoconjugates on the cell surface or extracellular matrix [7]. Galectins play multiple roles in a variety of biological processes including inflammation, angiogenesis, embryogenesis and neuromodulation. Stancic and colleagues evaluated the expression of different members of the galectin family in MS lesions and found that galectin-1, -3, -8 and -9 are present at detectable levels in the control white matter and increase substantially in MS lesions [84].

Galectin-1 is a 'proto-type' member of the galectin family that occurs in a monomer–dimer equilibrium. Expression of this lectin is prominent at sites of inflammation and immune privilege (including the CNS) and is up-regulated during the peak of EAE [85]. Recombinant galectin-1 or its genetic delivery to inflammatory sites has demonstrated immunoregulatory activity in several experimental models of autoimmune disease including collagen-induced arthritis, experimental autoimmune uveitis, 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis and EAE [4,86,87].

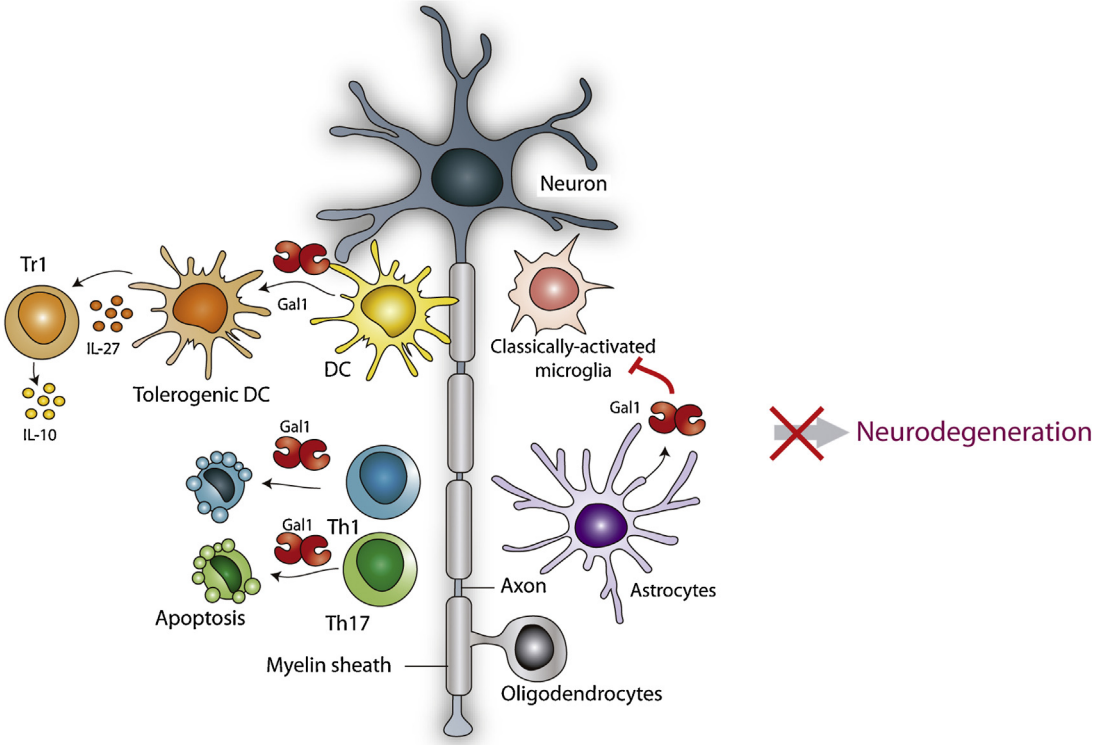

Fig. 2. Role of Gal-1 in autoimmune CNS inflammation. Galectin-1 (Gal-1) contributes to prevent inflammation-induced neurodegeneration through different mechanisms: (a) within the peripheral compartment, Gal-1 selectively eliminates Th1 and Th17 pathogenic lymphocytes; these cells express the complete repertoire of cell surface glycans critical for Gal-1 binding; (b) Gal-1 contributes to create a tolerogenic circuit mediated by IL-27, triggering the differentiation of IL-10-producing T-regulatory type 1 (Tr1) cells; (c) within the CNS, Gal-1 produced by TGF-β-stimulated astrocytes functions by deactivating classically-activated (M1-type) microglia and preventing inflammation-induced neurodegeneration.

Offner and colleagues [86] were pioneers in demonstrating a disease-modifying role for galectin-1 in EAE in Lewis rats [86]. Further studies showed that galectin-1 acts mechanistically by selectively eliminating Th1 and Th17 cells [87] (Fig. 2). Investigation of the mechanism underlying this effect revealed that Th1 and Th17 cell subsets share the repertoire of glycans required for galectin-1 binding and cell death, including the up-regulated expression of core-2-*O*-glycan branched structures and lower exposure of α2,6-linked sialic acid as compared to Th2-polarized cells [87]. Consistent with these findings, mice lacking the galectin-1 gene (*Lgals1*) showed increased Th1 and Th17 responses and more severe EAE following immunization with MOG_{35-55} [87]. This effect was recapitulated in mice lacking *N*-glycan branching (important galectin ligands) which developed a spontaneous disease that resembles progressive MS [88]. The immunoregulatory activity of galectin-1 is controlled, at least in part, by the regulated expression of glycosyltransferases at sites of inflammation which act in concert to create poly-LacNAc ligands on complex *N*-glycans or core 2-*O*-glycan structures. However, it is also regulated by intrinsic biochemical factors, including its monomer–dimer equilibrium, its avidity for multivalent glycans and the redox status of the inflammatory microenvironment [7,12]. Interestingly, Wang et al. showed that galectin-1 expressed by Treg cells binds the ganglioside GM1 on effector T cells and controls TRPC5 channel activation and immunoregulation during the course of EAE [89]. Interestingly, galectin-9, a 'tandem-repeat' member of the galectin family, also blunts Th1 responses during the induction of EAE [90]. Supporting these findings, Steelman et al. recently showed that galectin-9 is induced in astrocytes *via* the JNK/c-Jun pathway and functions as a T-cell regulatory protein in response to ongoing CNS inflammation [91].

In addition to its pro-apoptotic activity, galectin-1 also contributes to the resolution of EAE by inducing the differentiation of IL-27-producing tolerogenic DCs which in turn promote the expansion of IL-10-producing Tr1 cells during the course of EAE [85]. Phenotypically galectin-1-differentiated DCs express low amounts of CD11c and CD86 and high amounts of CD45RB and phospho-STAT3 (Fig. 2). When adoptively transferred into EAE recipient mice at the day of the disease onset, galectin-1-conditioned tolerogenic DCs blunted Th1 and Th17 responses and halted autoimmune neuroinflammation. These effects were abrogated when galectin-1-differentiated DCs were transferred into mice lacking IL-27Rα (*Il27ra*$^{-/-}$) or IL-10 (*Il10*$^{-/-}$) [85]. On the other hand, galectin-3, a 'chimera-type' galectin composed of a non-lectin N-terminal domain and a C-terminal CRD, often displays pro-inflammatory activities. Accordingly, Jiang and colleagues demonstrated that this lectin has a disease-exacerbating and pro-inflammatory role in EAE through prevention of immune cell apoptosis, increased IL-17 and IFN-γ synthesis and decreased IL-10 production [92].

Despite considerable evidence indicating a role for galectin-1 within peripheral immune compartments, the function of this lectin in CNS immunity has just emerged. Recent studies in the EAE model revealed that astrocytes can de-activate 'classically-activated' (M1-type) microglia *via* secretion of galectin-1 which prevents inflammation-induced neurodegeneration [93] (Fig. 2). Galectin-1 is dramatically up-regulated in TGF-β-stimulated astrocytes and a subset of microglia and CNS-resident Foxp3^{+} Treg cells. Mice devoid of galectin-1 showed increased microglia activation, astrogliosis, demyelination and axonal regeneration [93]. In contrast, galectin-9 promotes microglia and macrophage activation through binding to the T-cell immunoglobulin and mucin domain-3 (Tim-3) [94], suggesting that galectin-9-Tim-3

interactions can deliver either pro-inflammatory or anti-inflammatory signals depending on whether they function within the T-cell or the microglia/macrophage compartments. Interestingly, galectin-3, but not galectin-1, promoted oligodendrocyte differentiation and contributed to maintain myelin integrity and function [95]. Moreover, this chimera-type lectin limited cuprizone-induced demyelination by influencing microglia activity [96]. Furthermore, galectin-4, a 'tandem-repeat' galectin plays a key role in oligodendrocyte differentiation through interaction with sulfatide-enriched membranes [97], suggesting complementary and synergistic roles of galectins in the modulation of the myelination process.

A possible mechanism governing the regulatory functions of galectins at the cellular level involves the retention of glycoprotein receptors at the cell surface, an effect which prevents their endocytosis, prolongs intracellular signaling and increases their responsiveness to extracellular inputs [4]. In this regard, galectin-1 fine-tunes the threshold of microglia activation through binding to core 2-*O*-glycans on CD45, promoting its cell surface retention and augmenting its phosphatase activity [93]. Moreover, interaction between galectin-3 and Mgat5-modified *N*-glycans on transforming growth factor-β receptor (TGF-βR) prolongs Smad-dependent signaling and increase macrophage responsiveness to TGF-β_1 [98]. Likewise, complexes formed between galectins and complex *N*-glycans on cytotoxic T lymphocyte antigen-4 (CTLA-4) prevent endocytosis of this immunoinhibitory receptor and enhances its immunoregulatory signal. In contrast to growth-promoting receptors, which display a high number of *N*-glycosylation sites per peptide (multiplicity), arrest-promoting receptors like TGF-βR and CTLA-4 have few *N*-glycosylation sites and display ultrasensitive responses to metabolic flux of UDP-GlcNAc to attain the branching required for lectin binding, surface retention, and growth arrest [99]. Prolonged TCR signaling facilitates GlcNAc branching and formation of galectin-*N*-glycan complexes, which enables CTLA-4 surface retention and delivery of TCR suppressive signals [5]. More recently, intronic variants of the Mgat5 glycosyltransferase have been identified that are associated with reduced *N*-glycan branching, CTLA-4 surface expression and MS [100]. Collectively, these mechanisms contribute to amplify tolerogenic circuits that restore homeostasis and prevent inflammation-induced neurodegeneration.

3. Conclusions

In the past decades we have witnessed a revolution in our understanding of genomics and proteomics and their contribution to chronic inflammation, autoimmunity and neurodegeneration. However, the role of glycans and glycan-binding proteins in modulating the onset, severity and resolution of these processes is just emerging. Glycans have long been undervalued in the context of immunity and considered as mere decorative structures that are present on the cell surface or extracellular space. However, there is currently no doubt that glycans can store important biological information which can be decoded by endogenous glycan-binding proteins or lectins. The complexity of glycan structures is challenging and stimulating, and their heterogeneity is inherent to their biosynthesis and critical for their multifunctional activities. Glycan remodeling through the concerted action of glycosyltransferases and glycosidases governs a diversity of biological functions including those operating in immune, neural and glial cells. In this review we summarized the contribution of glycans and glycan-binding proteins including siglecs, C-type lectins and galectins to the development, severity and resolution of MS and its animal model EAE. These glycan-binding proteins can act either alone or in concert with canonical ligands to modulate immune cell fate, activation, cytokine production, differentiation, apoptosis and signaling. In this regard, these divergent families of lectins have evolved to serve as exquisite translators, capable of interpreting the different profiles of glycan structures into diverse cellular responses. Experiments using knockdown strategies or knockout mice revealed the hierarchical roles of lectins and glycans in the control of T-cell fate, DC function and glial cell responses. Understanding the complexity of lectin–glycan recognition systems during the course of autoimmune CNS inflammation will contribute to delineate novel therapeutic strategies for chronic inflammatory diseases including MS.

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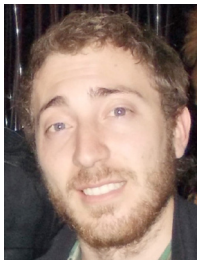

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Mauricio Farez is an associate researcher in Dr. Jorge Correale's laboratory at the Institute for Neurological Research Dr. Raúl Carrea (FLENI) in Buenos Aires, Argentina. Mauricio received his M.D. degree with honors in 2006 and continued his training as a research fellow in Dr. Howard Weiner's laboratory at Harvard Medical School followed by a Master of Public Health at Harvard School of Public Health. He has received several awards including the Bruce S. Schoenberg International Award in Neuroepidemiology of the American Academy of Neurology, the Investigator Award by the Allende Foundation and the Young Investigation award of the Argentinean Multiple Sclerosis Society. He is coauthor of 27 papers in international peer reviewed journals and has contributed to several book chapters.

Karina Mariño, PhD, is an adjunct researcher at the National Research Council (CONICET, Argentina) and her main area of interest is Glycobiology of inflammatory chronic diseases. In the laboratory of Functional and Molecular Glycomics (IBYME-CONICET) and through the establishment of a glycoanalytical platform, Dr. Mariño is studying the influence of diverse inflammatory factors in the structural alteration of *N*- and *O*-glycans in serum and tissue glycoproteins.
She has a multidisciplinary profile with cross-discipline training across the Chemistry/Biology interface, with 26 publications in international peer reviewed journals. Her initial training as an Organic Chemist in the University of Buenos Aires, and her postdoctoral positions at the University of Dundee (United Kingdom) and the National Institute for Bioprocessing, Research and Training (Dublin, Ireland) prompted her into the analysis of glycoproteins and the Glyco-biomarker field.

Jorge Correale M.D. is the head of Neuroimmunology and Demyelinating Diseases at the Institute for Neurological Research Dr. Raúl Carrea. Research in his group is focused into understanding the role of environmental factors and immunological mechanisms in the development and progression of multiple sclerosis. Likewise, Dr. Correale's group is investigating the immunological factors induced by pregnancy, and assisted reproductive techniques, as well as the role that sexual hormones play under these conditions. He graduated with honors from Buenos Aires University School of Medicine in 1981. Between 1983 and 1988 he completed his Neurology Residency training at the José María Ramos Mejía Hospital in Buenos Aires and was appointed Chief Resident. During 1989 he was visiting physician at the Department of Neuropathology at the same center. Dr. Correale continued his training as fellow in Neuroimmunology at the Karolinska Institute in Stockholm, Sweden between 1989 and 1990. In 1990 he moved to the University of Southern California, receiving a post-graduate fellowship in Neuroimmunology from the National MS Society of New York. In 1993 he was appointed Assistant Professor of Neurology at this University, and in 1995 Assistant Professor of Microbiology and Molecular Immunology. In 1997 Dr. Correale returned to Buenos Aires as Head of Neuroimmunology and Demyelinataing Diseases at the Institute for Neurological Research Dr. Raúl Carrea, position he holds to this day. Between 1999 and 2001 Dr. Correale was Head of the Department of Neurology at the same Institution, and between 1998 and 2010 he was Associate Professor at the Austral University in Buenos Aires. He is member of the Argentine Medical Advisory Board for Multiple Sclerosis and member of the Medical and Scientific Board of the International Federation of Multiple Sclerosis Societies (IFMSS). He is a member of the Editorial Board of several neurological and immunological journals. Likewise, Dr. Correale has served as an *ad hoc* reviewer for different agencies and Multiple Sclerosis societies. At present he published 120 original and reviews papers in pre-review journals, and is Editor of 4 books related to Neurology, Neuroimmunology, and Multiple Sclerosis.

Gabriel A. Rabinovich, PhD, is a principal investigator of the Argentinean National Council Research (CONICET), professor in Immunology of the University of Buenos Aires, vice-director of the Institute of Biology and Experimental Medicine (IBYME) and member of the Argentinean National Academy of Sciences. He heads the Laboratory of Immunopathology at IBYME and the Laboratory of Functional Glycomics at the Faculty of Exact and Natural Sciences of the University of Buenos Aires. Dr. Rabinovich's lab aims to identify different therapeutic targets, based on protein–glycan interactions, to differentially modulate immune-mediated disorders including cancer, autoimmunity and chronic inflammation. Dr. Rabinovich has authored over 170 articles and 30 book chapters, serves as Editor of several journals and received a number of awards including those from the John Simon Guggenheim Foundation (New York), Third World Academy of Science Award in Medical Sciences (TWAS), Baron Foundation (Argentina), Bernardo Houssay (Argentina), Bunge & Born Foundation (Argentina) and Platinum Konex Award (Argentina).