Role of galectins in inflammation and remyelination in multiple sclerosis

Alex Coolen

Multiple sclerosis is a chronic, heterogeneous disease caused by attacks on the myelin sheaths in brain white matter by autoimmune T-lymphocytes. No cure or effective treatment exists because it is not fully known what triggers the onset of the disease and why remyelination is often impaired. It is likely the result of a dysregulation of one or (more likely) multiple processes involved in the autoimmune inflammation-mediated demyelination and remyelination. Galectins are a group of beta-galactoside sugar-binding proteins known for their involvement in various extracellular and intracellular mechanisms, for example cell-matrix, matrix-matrix interactions, cell cycle regulation and pre-mRNA splicing. They are also known to modulate the immunesystem in various ways. This review analyzes the role galectins play in the various processes that are instrumental to inflammation mediated demyelination and remyelination in multiple sclerosis.

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Supervised by Wia Baron

Department of Cell Biology, Section of Membrane Cell Biology, University Medical Center Groningen, University of Groningen

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INTRODUCTION

"…the chief curse of the illness...I must ask constant services of people I love most closely...it is an illness accompanied by frustration...it is an illness that inflicts awareness of loss...sporadically it is, in its manifestations, a disgusting disease"

Brigid Brophy, 1929-95 (Brophy 1987)

Multiple sclerosis (MS) is a disease that is characterized by an autoimmune attack against the myelin sheaths of the central nervous system (CNS). MS patients may suffer from a variety of clinical symptoms, including sensory deficits, visual problems, muscle weakness and difficulties with coordination and speech (Frohman, Racke and Raine 2006). The disease usually has its onset in young adulthood and has a chronic, long disease duration (Flachenecker and Stuke 2008).

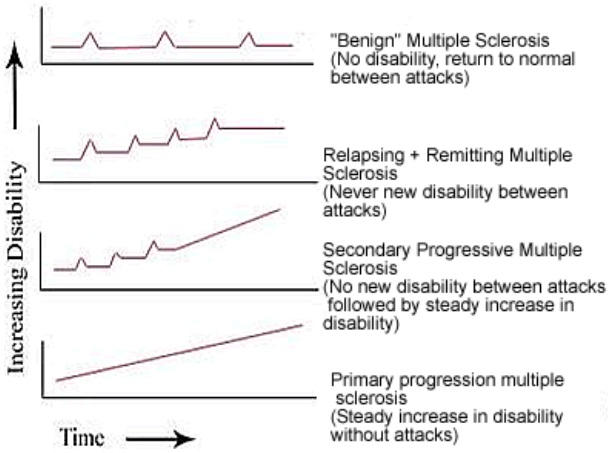

Figure 1. Graphical display of the four forms of MS. (UK Department for Work and Pensions 2008)

Four types of MS can be distinguished by their form of progression: benign, relapse+remitting, secondary progressive and primary progressive MS (see figure 1). There are four major hallmarks that characterize MS: (i) The primary inflammation of the CNS and (ii) subsequent demyelination. These two account for the relapses, visualized as disability peaks in figure 1. When demyelination is followed by remyelination (i.e. replacement of damaged myelin sheaths) disability symptoms diminish (remittance of the disease). The final two hallmarks account for the progressive phase of MS: (iii) failure of remyelination and (iv) axonal degeneration. The major problem and mystery of MS is that remyelination does *not* always seem to happen in the later stages of the disease. In this stage of a lesion the immune response is usually inactive but remyelination is impossible. This leads to irreversible axonal degeneration. Conduction can't be restored in these axons leading to permanent loss of function. This is the linear increase of disability seen in figure 1, the progressive phase of MS. There are various types of lesions in MS: chronic active, acute active, chronic inactive and remyelinated lesion (for more information see box 1).

Recently galectins, a family of β-galactoside binding lectins, have come under attention in the field of MS research. The presence of galectin-1 (Gal-1) autoantibodies in the cerebrospinal fluid (CSF) of MS patients, but not in the CSF of controls, was hypothesized to be associated with the impairment of the regulation of the immune system in MS (Lutomski, et al. 1997). At first galectins were mostly studied for their properties in modulating the immune system, which generally consists of shifting the response from a T-helper 1 (Th1) to a Th2 response (Motran, et al. 2008) (Jiang, et al. 2009). Th1 cells contribute to the chronicity and the effector phases, while Th2 cells plays a role in the humoral response and the recruitment of macrophages (Ozdemir, Akdis and Akdis 2009). Shifting the overall balance to a Th2 profile is beneficial for MS recovery.

Later, galectins were also found to have various effects in modulating the extracellular matrix (ECM). Galectins bind glycoconjugates that contain suitable galactose-containing oligosaccharides. They are able to crosslink cell surface glycoconjugates, thereby triggering transmembrane signaling events. They're able to enhance or block ECM-cell or cell-cell signaling, mostly through integrins.

Intracellularly, galectins are involved in various basic functions such as pre-mRNA splicing, regulation of cell growth and cell-cycle progression.

Gal-1 and -8 have been found in the brain. Gal-1 in relation to (neural) stem cells in the brainstem (Sakaguchi, Imaizumi and Okano 2007). Gal-1, -3 and -9 have been found in relation to T cells. All of them perform various physiological functions making them interesting to study in relation to MS. The purpose of this review is to analyze what properties galectins have in autoimmune demyelination in the CNS and cellular mechanisms that are relevant to remyelination such as cell migration, proliferation, differentiation and intracellular trafficking. This includes research done on experimental autoimmune encephalomyelitis (EAE, the animal model for MS), which can be linked to MS directly. It also includes research in neighboring fields that study mechanisms similar to those in inflammation mediated demyelination and remyelination, which can thus be linked to MS indirectly.

Box 1: Types of lesions in MS

Different types of MS lesions can be distinguished. An area of white matter of a brain afflicted by MS can be characterized as one of five things: normal appearing white matter (NAWM), chronic active, acute active, chronic inactive and remyelinated. Each type is very different from another when considering myelination of the axons, inflammation activity and the status of the ECM.

Active lesions are characterized by an active inflammatory response, involving activated T cells, macrophages and a compromised BBB. Active lesions can be distinguished into an acute and chronic type. Chronic lesions are the result of repeated and prolonged inflammatory responses. Chronic inactive lesions are areas of demyelinated white matter that no longer show any inflammatory activity, but cannot be remyelinated (more on this in the sub-section Remyelination: a delicate procedure). When a demyelinated lesion could be remyelinated saltatory conduction is restored and the axons function as normal. More on this in the sub-section (Re)myelination in the healthy brain.

Gene expression comparison between chronic inactive and chronic active human lesion material showed the genes related to inflammation, e.g. cytokines TNF and IL6 were present in active lesions, whereas they were underrepresented in inactive lesions. In contrast, the genes underrepresented in chronic active lesions are genes associated with apoptosis and stress-induced response, e.g. heat-shock-proteins (Mycko, et al. 2004).

(RE)MYELINATION IN THE HEALTHY BRAIN

"We propose that myelination is initiated by the establishment of cell polarity by extrinsic cues provided by the axon. These localized cues trigger a multi-step process of myelin assembly. Assembly is carried out in spatially distinct cell regions and starts by the preassembly of myelin proteins and lipids during their transport through the biosynthetic pathway."

Mikael Simons and Jacqueline Trotter - (Simons and Trotter 2007)

Primary myelination occurs during early fetal stages and is carried out by oligodendrocyte progenitor cells (OPCs). The progenitor cells arise from multiple foci distributed along the neural tube from where they migrate into the future white matter (Colognato and ffrench-Constant 2004). There they differentiate into oligodendrocytes (OLGs), undergoing dramatic changes in their morphology with the formation of a large network of branching processes. A single OLG can form as many as 40 separate myelin segments, in contrast to Schwann cells in the peripheral nervous system, which only

form one myelin sheath each. The formation of processes requires the downregulation of RhoA GTPase linked to the oligodendroglial cytoskeleton. This appears to be initiated by ECM components and requires the β1-integrin receptor and depends on soluble factors released by neurons (Simons and Trotter 2007). Galectins are likely candidates to modulate this mechanism since some have been shown to interact with the ECM (Rabinovich, Liu, et al. 2007). The best candidate for an axonal surface molecule required for the induction of myelination is laminin-2 (Maier, Hoekstra and Baron 2008). Following the formation of an excess of processes only the ones attached to an axon survive and the rest are retracted. It is then required that myelin is assembled only at processes that have made contact with an axon, not anywhere else in the cell. This requires polarization of the cell body. Simons and Trotter speculate that the attachment of a process to an axon acts as a spatial cue for the recruitment of myelin-membrane components. Thus, the myelination of a CNS axon requires (i) proliferation and migration of OPCs, (ii) differentiation of the OPC to an OLG, (iii) formation of multiple processes, (iv) process contact with an axon, (v) production, directional transport and assembly of myelin sheath components and (vi) compaction of the myelin membranes wound around an axon.

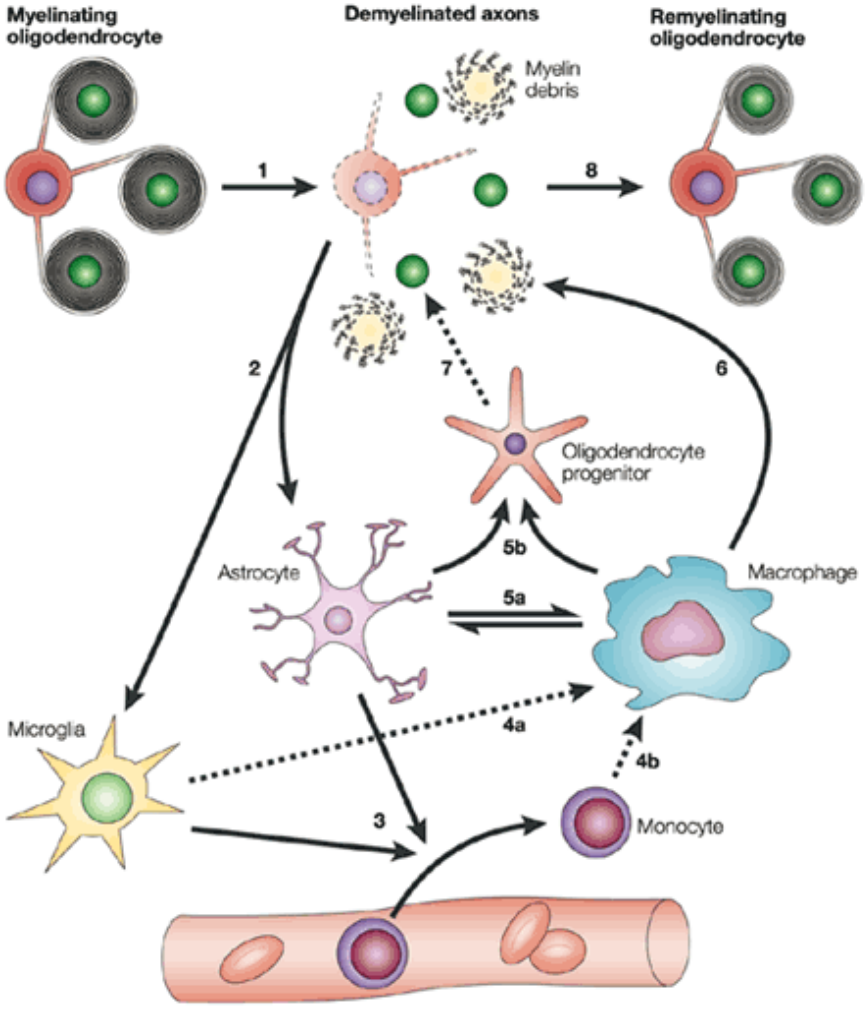

Figure 2: Remyelination involves a sequence of orchestrated steps, the dysregulation of which will result in remyelination impairment. In response to a demyelinating insult, the myelinated axons undergo demyelination (1), a process that generates myelin debris. Demyelination causes the activation of resident astrocytes and microglia (2). The activated astrocytes and microglia produce factors that contribute to the recruitment of monocytes from blood vessels (3). Microglia (4a) and recruited monocytes (4b) differentiate into macrophages. Activated astrocytes and macrophages produce factors that activate each other (5a). As a result of this activation, both produce growth factors that act on OLG progenitors (5b). Macrophages remove myelin debris (6), a function that may be beneficial to remyelination. Under the influence of factors that are produced by astrocytes and macrophages, recruited OLG progenitors engage demyelinated axons (7) and differentiate into remyelinating OLGs (8). From: (Franklin 2002)

Myelin sheaths and OLGs do occasionally get damaged under non-pathological conditions, thus a repair mechanism is required (see figure 2). Remyelination is the process in which new myelin sheaths are produced around demyelinated axons in order to restore their electric conduction. OLGs are unable to remyelinate naked axons after they are fully developed (Keirstead and Blakemore 1997), so another mechanism is required. The cells responsible for remyelination are the OPCs. Their task is two-folded: first they must migrate to the site of a lesion and secondly they must terminally differentiate to an OLG. The mechanism of remyelination is more or less similar to primary myelination. During adulthood OPCs continue to proliferate and are able to migrate to the site of a lesion and differentiate (Gensert and Goldman 1997). Remyelinated axons have more, shorter and thinner myelin sheaths, but their conduction is restored to normal (Smith, Blakemore and McDonald 1979) (Duncan, et al. 2009). As mentioned before in MS remyelination often fails, resulting in permanent scarring of the brain. Our understanding of why remyelination fails is far from complete. Understanding why remyelination fails in MS may prove to be the key to further alleviate the disease.

REMYELINATION IN MS: A DELICATE PROCEDURE

"there are no individual villains of the piece that are responsible for remyelination failure. Instead, the process fails because the complex and finely tuned mechanism by which it proceeds loses its precise coordination."
Robin J.M. Franklin - (Franklin 2002)

The above quote is what Franklin proposes as the 'dysregulation hypothesis': not one factor can cause failure of remyelination, but the delicate balance that is the process of remyelination is lost by a multitude of factors. It has been shown that a number of things can go wrong at either of these processes.

So what are these mechanisms that are malfunctioning in MS? There are many and some of them are still poorly understood. As mentioned in the previous section, even if OLGs survive the process of demyelination, they are unable to form new myelin sheaths (Targett, et al. 1996) (Keirstead and Blakemore 1997). Hence OPCs must differentiate to OLGs or, if there are not enough OPCs present at the site of the lesion, OPCs must migrate and proliferate until they reach the site of the lesion. Various paracrine signals stimulate migration and proliferation including: ECM molecules (Hu, et al. 2009), chemokines (Robinson, et al. 1998), cytokines (Benveniste and Merill 1986) and various growth factors (platelet derived growth factor (PDGF) and fibroblast growth factor (FGF)) (Redwine and Armstrong 1998) (Baron, Shattil and Ffrench-Constant 2002) (Hinks and Franklin 1999).

While the migration and proliferation of OPCs is fairly well understood, our understanding of differentiation and activation of OLGs is incomplete. A number of growth factors have been demonstrated to induce OPC differentiation: Insulin-like growth factor 1 (Ye, Carlson and D'Ercole 1995) and transforming growth factor β1 (McKinnon, et al. 1993). FGF and the axonally expressed neuregulin glial growth factor 2 (GGF2) inhibit differentiation of OPCs (Goddard, et al. 2001) (Canoll, et al. 1996). The effect of all these growth factors are rather complex, as for example GGF2 has also been shown to be necessary for terminal differentiation of OPCs (Park, et al. 2001). It is unlikely that the absence of a single of these factors provides a complete explanation (Franklin 2002).

The Notch-jagged signaling system also has a role in OPC differentiation. Adult neurons axonally express jagged, which acts at Notch-1 receptors on OPCs, blocking differentiation (Wang, et al. 1998). Blocking Notch signaling was shown to enhance tissue repair in EAE (Jurynczyk, et al. 2008). However contradictive results show that remyelination is not hindered even though Notch1 and Jagged1 are both expressed (Stidworthy, et al. 2004).

It is clear that neurons themselves have a great deal of influence in the (re)myelination process. For example electrical activity in the axon is of great importance for differentiation of OLGs (Demerens, et al. 1996). Charles and colleagues showed that demyelinated axons expressed the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), which acts as a negative regulator of myelination (Charles, Reynolds, et al. 2002). Remyelinated axons within shadow plagues do not express PSA-NCAM, which led to the conclusion that PSA-NCAM is likely to act as an inhibitor of remyelination.

The ECM can stimulate OPC differentiation through integrin signaling. At the time of myelination the ECM of the developing CNS is rich in laminin around axons, stimulating myelination and OLG survival (Colognato, et al. 2002). Additionally, laminin-2 deficiency causes myelination defects in mice (Colognato, ffrench-Constant and Feltri 2005). Fibronectin in the ECM is a likely candidate to inhibit OPC differentiation (Sisková, et al. 2006). It is proposed that fibronectin, leaking across the BBB or excreted by astrocytes, may impede myelination by interference with intracellular myelin sheet-directed membrane trafficking (Maier, Hoekstra and Baron 2008).

In conclusion, when attempting to understand why remyelination can fail, there are many factors to take into account. Cytokines, chemokines and growth factors influence OPC migration and the ECM also plays an important role in migration and differentiation of OPCs and OLGs. Because of their various interactions with the ECM galectins may alter these effects. Finally, axons are central to myelination and can secrete substances, e.g. growth factors, galectins, that can either hinder or enhance remyelination.

THE IMMUNE SYSTEM: FRIEND OR FOE?

"There are two components to the treatment of multiple sclerosis; the first is to prevent damage occurring, and the second is to repair the residual damage. While considerable progress has been made in the recent years with the former through the development of anti-inflammatory and immunomodulatory therapies, there are currently no effective repair therapies routinely used in MS patients."
Chao Zhao - (Zhao, et al. 2005)

Although a lot of research has been done on MS, a lot is still unknown about the cause of this disease, and an effective treatment or cure remains to be found. The immune system plays a central role in the development of MS, although it is not fully known what causes the disease to develop. As can be deducted from Zhao's quote above, the role of the immune system in MS it two-fold: (i) the primary inflammation in the CNS that is believed to be the cause of lesion formation (see figure 3), and (ii) the cleaning up of myelin-sheath debris and the immune system's role in remyelination.

First the role of the immune system in the onset of MS will be considered. It is generally assumed that an autoimmune response causes demyelination, although it is sometimes thought to be a response to demyelination rather than the cause (Zhao, et al. 2005) (Mason, et al. 2001). An impressive amount of circumstantial evidence points in the direction of activated autoimmune T-lymphocytes that target myelin structures (Wekerle 2008), but definite proof has yet to be produced. These activated auto-immune T-cells cross the BBB via a still unknown mechanism. Theories as to how this happens often include damage to the BBB, allowing T-cells, macrophages and other members of the immune system to cross into the brain (Kebir, et al. 2007) (Simka 2009). Increased BBB permeability is associated with the decreased expression of tight junctions (TJ). This was found predominantly in active lesions (42% of vessels affected), but also in inactive lesions (23%) and normal appearing white matter of MS patients (13%) (McQuaid, et al. 2009). They also indicate that

TJ abnormalities were associated with leakage of the serum protein fibronectin, which has been shown to be an activator of microglia (Milner and Campbell 2003) and is also shown to hinder the growth of OLG processes (Siskova, et al. 2009) and thereby remyelination.

The second role of the immune system in MS that has to be considered is the cleaning up of myelin debris and facilitating remyelination (see figure 2). Microglia are the innate immune cells of the CNS, similar to monocytes in the rest of the body. Like monocytes they can perform various functions. Microglia can release various potentially cytotoxic molecules such as proinflammatory cytokines, reactive oxygen intermediates, proteinases and complement factors. Upon activation, they can also secrete neurotrophic and neuron survival factors (Pivneva 2008).

Activated microglia have divergent effects on OPC's and OLGs, reducing OPC survival and increasing OLG survival (Miller, et al. 2007). Remyelination is most prominent in sites of active lesions (Raine and Wu 1993), suggesting that an active immune response *can* be beneficial to remyelination. Macrophage activity is necessary to clear myelin debris, which can impair remyelination either through receptor-mediated hindrance or simply by physical blockage (Copelman, et al. 2001). The necessity of an inflammatory response in remyelination is best demonstrated in studies using toxins rather than EAE to achieve demyelination (Zhao, et al. 2005). EAE is based on immunizing an animal with basic myelin proteins, hence the immune response is the cause of demyelination. By using toxins to demyelinate an area it was shown that an immune response was caused by demyelination (rather than the other way around), and said immune response was instrumental to the remyelination (Duncan, et al. 2009). Studies using knock-out mice show that in the absence of pro-inflammatory cytokines IL-1 and TNF-α remyelination is impaired (Mason, et al. 2001) (Arnett, et al. 2001).

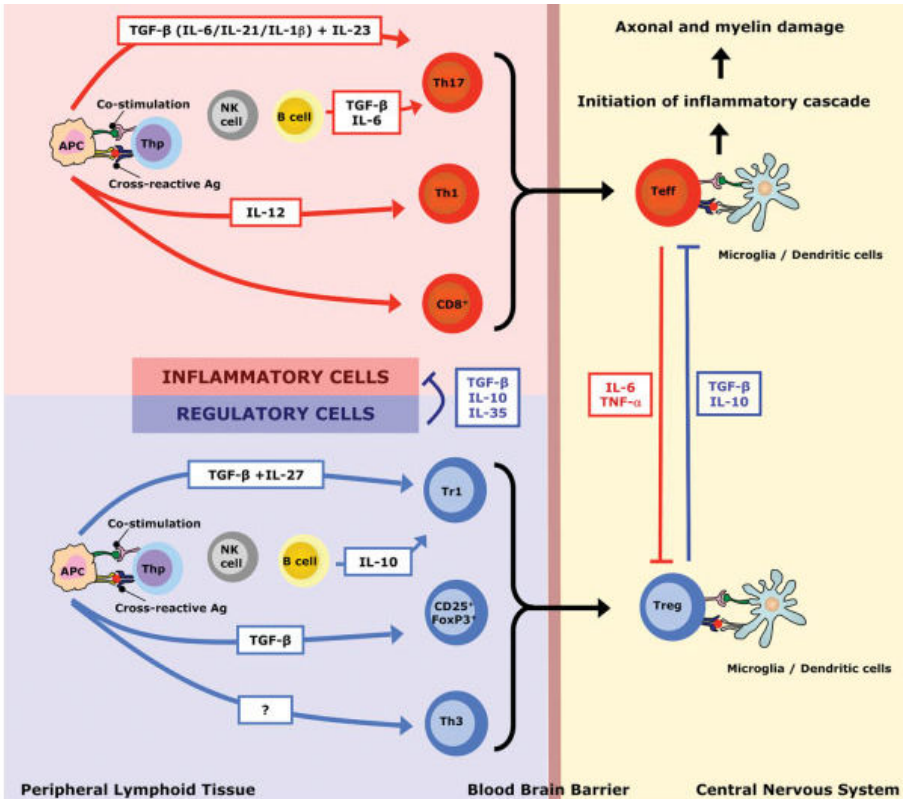

Figure 3. Immune pathways and adaptive immunity in the initiation of MS. MS is initiated by myelin reactive inflammatory T cells that cross the blood–brain barrier and initiate an inflammatory cascade in the CNS. These inflammatory T cells are modulated by regulatory T cells both inside and outside the CNS. B cells and NK cells may influence both inflammatory and regulatory T cells. APC = antigen-presenting cell; IL = interleukin; TGF = transforming growth factor; TNF = tumor necrosis factor; Th = T helper cell; Treg = regulatory T cell; NK = natural killer cell. (Howard and Weiner 2009)

Thus, the adaptive immune response is largely responsible for the onset of demyelination by means of activated T and B lymphocytes. The innate immune response while causing damage when activated and sensitized by the adaptive immune response, also has an important role when it comes to creating an environment suitable for remyelination. There is evidence that an inflammatory response is necessary to create an environment suitable for remyelination (Duncan, et al. 2009), but prolonged and repeated inflammation can cause a lot of damage in lesion areas (Zhao, et al. 2005) (Compston and Coles 2008).

GALECTINS: FORMS AND EXPRESSION

"God must love galectins; He made so many of them"
Modified from the comment attributed to Abraham Lincoln, "God must love common people, He made so many of them."
Douglas N.W. Cooper - (Cooper and Barondes 1999)

Galectins are a group of proteins that is part of the larger family of lectins. Lectins are proteins that bind to specific carbohydrate structures and can thereby recognize specific glycoconjugates. Galectins are defined by two properties that set them aside from other lectins: their shared characteristic amino acid sequence and affinity for β-galactoside sugars (Barondes, et al. 1994). Galectins appear both outside as well as inside the cell. Extracellularly, they bind to both the cell-surface as well as extracellular matrix glycans, thereby affecting a variety of cellular processes (Yang, Rabinovich and Liu 2008). Intracellularly they appear both in the cytosol as well as in the nucleus, where they influence signaling and trafficking pathways (Yang, Rabinovich and Liu 2008). At this time there are 15 known galectins in mammals. All galectins share a conserved sequence of amino acids called the carbohydrate-recognition domain (CRD) of about 130 amino acids that is responsible for carbohydrate binding. Three types of galectins can be distinguished based on their form (see figure 4. From: Yang, Rabinovich and Liu, 2008): (i) prototypical (1, 2, 5, 7, 10, 11, 13, 14, 15), contains one CRD and can form homological dimers. (ii) Chimaera-type (3), contains one CRD and an unusual N-terminal repeat allowing it to polymerize. (iii) Tandem-repeat type (4, 6, 8, 9, 12), contains two CRDs, connected by a linker.

Some galectins have a wide tissue distribution, while others are more tissue-specific. Galectin 1 can be found in most tissues (Chiariotti, et al. 2004); skeletal muscle, heart, placenta and within the immune system it can be found in lymphoid organs, T cells, activated macrophages and endothelial cells (Norling, Perretti and Cooper 2009). Within the CNS galectin 1 can be found in a wide array of neurons and glial cells (i.e. primary sensory neurons, motor neurons, OLG and astrocytes) (Sakaguchi, Imaizumi and Okano 2007) (Stillman, Mischel and Baum 2005). Galectin 2 is expressed throughout the gastro-intestinal tract (GI-tract) and was recently found in the nuclei of fibroblasts (Dvorankova, et al. 2008). Galectin 3 can be found in virtually all immune cell types (Norling, Perretti and Cooper 2009) and in the CNS it is found in fibroblasts, macrophages, dorsal root microganglia neurons, astrocytes and OLGs following injury (Stillman, Mischel and Baum 2005). Galectin 4 is, like galectin 2, distributed throughout the GI-tract and it also expressed in the brain (Le Mercier, et al. 2009). Galectin 8 has a wide tissue distribution and can be found in the liver, kidney, cardiac muscle, lungs, colon and in the brain in astrocytes (Chiariotti, et al. 2004) (Le Mercier, et al. 2009). And finally, galectin 9 is distributed in certain cells fundamental to the inflammatory response: endothelial cells, T cells and fibroblasts (Norling, Perretti and Cooper 2009), but it can also be found in the GI-tract and the kidneys (Chiariotti, et al. 2004), as well as in the brain (Le Mercier, et al. 2009).

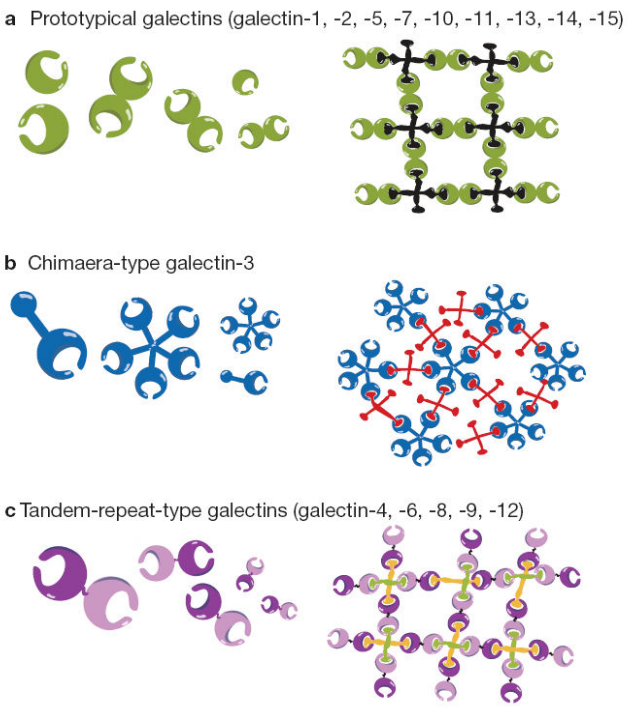

Figure 4. Galectin family members and formation of galectin–glycan lattices. Galectins can be subdivided into three groups: (a) prototypical galectins, containing one CRD; (b) galectin-3, a chimaera-type galectin consisting of unusual tandem repeats of proline- and glycine-rich short stretches fused onto the CRD; and (c) tandem-repeat-type galectins, which contain two distinct CRDs in tandem, connected by a linker. Many galectins are either bivalent or multivalent with regard to their carbohydrate-binding activities: one-CRD galectins often exist as dimers; galectin-3 forms petamers upon binding to multivalent carbohydrates; and two-CRD galectins have two different carbohydrate-binding sites. Thus, galectins can form lattices with multivalent glycoconjugates. (Yang, Rabinovich and Liu 2008)

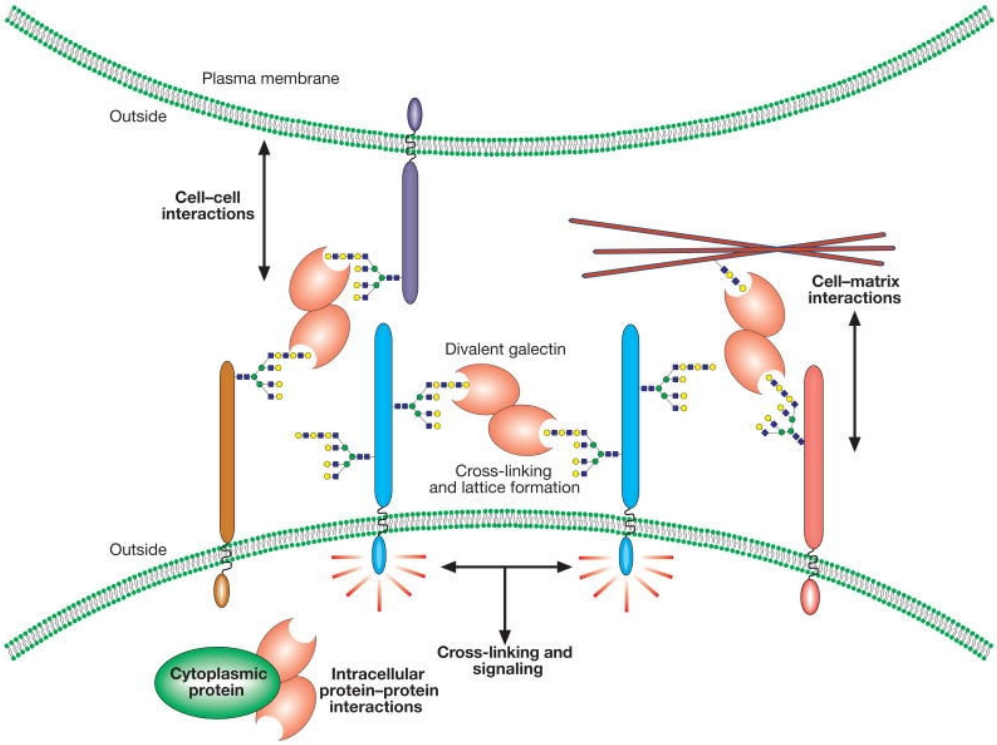

Figure 5. Functional interactions of galectins with cell-surface glycoconjugates and extracellular glycoconjugates can lead to cell adhesion and cell signaling. Interactions of galectins with intracellular ligands may also contribute to the regulation of intracellular pathways. From: (Cummings and Liu 2009)

"T-cell apoptosis is a sophisticated, naturally occurring mechanism that confers protection of vulnerable sites from tissue damage. At present, we do not know exactly how this is achieved in vivo, but we have now begun to uncover the mechanisms in order to take advantage of its therapeutic applications."

Ralf Gold - (Gold, Hartung and Lassmann 1997)

Activated autoimmune T-cells are the main concern for the development of MS. It has recently been shown that a number of galectins have modulatory effects on T-cell physiology (see figure 6 and 7) (Ilarregui, et al. 2005), while others influence the ECM in ways that is relevant to remyelination failure in MS. In the following section I will outline the properties of the galectins with properties in the processes mentioned above, based on recent research that has been done on them. The properties outlined here are by no means meant as a comprehensive summary of all properties of a described galectin. Rather, it's a summary of properties relevant to the context of MS.

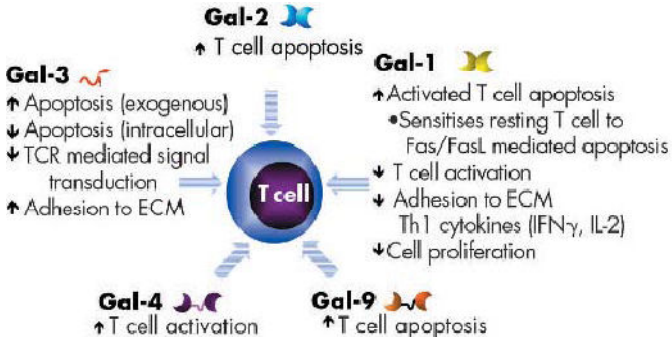

Figure 6. Influence of galectins in the regulation of T cell physiology. This scheme illustrates the influence of different members of the galectin family on different T cell functions including T cell apoptosis, activation, adhesion, and cytokine secretion. From: Ilarregui, et al. 2005

GALECTIN 1

Galectin 1 (Gal-1) is a prototypical galectin containing one CRD that is produced as a monomer but can form homological dimers. As early as 1990 this protein was already known to have a role in inflammation. It was shown then by Offner's group (Offner, et al. 1990) that injecting rats with recombinant human Gal-1 could prevent their EAE from becoming symptomatic. A lot of research has been done on how Gal-1 manipulates the immune system and it is now well established that Gal-1 induces apoptosis of activated T-lymphocytes. Motran *et al.* studied the effect of Gal-1 on the Th1/Th2 balance on murine cell lines (Motran, et al. 2008). Gal-1 did bind to both versions of T-helper cells, but the effect was radically different. On Th1 cells, responsible for activating macrophages, Gal-1 triggers apoptotic signals. On Th2 cells, responsible for sensitizing and activating B-lymphocytes, Gal-1 would bind to the same receptors, but the Th2 cell would be protected from apoptosis. This protection of Th2 cells was correlated with expression of anti-apoptotic intracellular galectin 3. Gal-1 works as a tuner for T-cell receptors (TCRs) of activated CD8+ T-cells and Gal-1-deficient CD8+ T-cells undergo greater cell division in response to TCR stimulation, with fewer cells going into apoptosis. Additionally, Gal-1 deficient mice are highly susceptible to EAE (Toscano, et al. 2007). By inducibly expressing Gal-1 on CD8+ T-cells it functioned as an autocrine negative regulator of peripheral CD8+ T-cell binding, signal transduction and burst size (Liu, et al. 2009). Since activated autoimmune T-cells are the main cause for MS lesion formation this targeted T-cell apoptosis by Gal-1 may prove promising for future research.

Adhesion and migration of immune cells across blood vessel epithelium and through the ECM is required for an inflammation to be maintained. The same applies for active MS lesions in the CNS: immune cells roll and adhere on the epithelium of the blood vessel and migrate through the compromised BBB. Rabinovich and colleagues (Rabinovich, Liu, et al. 2007) found that exposure to

Gal-1 inhibits T-cell adhesion to ECM glycoproteins like fibronectin and laminin. In addition, Gal-1 present on the surface of the ECM inhibited T-cell migration through the matrix. This showed that the immunosuppressive effect of Gal-1 was not solely based on apoptosis or suppression of pro-inflammatory cytokine secretion, but also includes the inhibition of migratory capacity of T-cells (Rabinovich, Liu, et al. 2007).

Besides its role in modifying T-cell physiology Gal-1 also plays a role in the neural stem cell maintenance. Sakaguchi and colleagues screened for factors that promoted proliferation of neural stem cells and identified Gal-1 as a candidate. Gal-1 is expressed in a subset of slowly dividing subventricular zone astrocytes, including the neural stem cells (Sakaguchi, et al. 2006). Kajitani and colleagues studied the hippocampus of adult wild type vs. Gal-1-knockout mice and found that Gal-1 promotes proliferation of neural progenitor cells (Kajitani, et al. 2008). Introducing anti-Gal-1 neutralizing antibody strongly inhibited neurogenesis and diminished neurological function. The reverse was found when Gal-1 was administered (Ishibashi, et al. 2007). Gal-1 induces astrocyte differentiaton and greatly enhances their production of brain-derived neurotrophic factor (BDNF), which is known to promote neuronal survival (Endo 2005). The effect of Gal-1 is astrocyte-specific and does not have any effect on neurons (Endo 2005). Nonetheless, this shows that Gal-1 is indirectly involved in neuron survival after lesion formation, as is the case in MS.

GALECTIN 2

Galectin 2 (Gal-2) is structurally and functionally related to Gal-1 and is also able to induce apoptosis in activated T-cells through β-integrin binding (Sturm, et al. 2004). Sturm and colleagues further showed that Gal-2 can modulate T-cell-derived cytokines and shift the balance towards Th2. Paclik and colleagues showed that Gal-2, expressed in intestinal epithelial cells, was downregulated in the inflamed colon, but this could be reversed by anti-inflammatory treatment (Paclik, Berndt, et al. 2008). They used an animal models for both chronic and acute experimental colitis which is, similar to EAE, characterized by a failure of the immune system allowing unrestricted expansion and an impaired apoptosis of T cells. They also demonstrated that administering Gal-2 to animals with experimental colitis abolished symptoms and, after histological and biochemical analysis, found a reduced inflammatory response. From the same lab as Sturm and Paclik, Loser and colleagues found similar results in an animal model for contact dermatitis (Loser, et al. 2009).

GALECTIN 3

Galectin 3 (Gal-3) is the only member of the galectin family with an extended N-terminal region composed of tandem repeats of short amino acid segments connected to a C-terminal CRD (Yang, Rabinovich and Liu 2008). This special N-terminal region allows this galectin to oligomerise in the presence of multivalent carbohydrate ligands (Yang, Rabinovich and Liu 2008).

Gal-3 can have opposite effects depending on its cellular location. Intracellular gal-3 can prevent the cell from going into apoptosis, while extracellular gal-3 induces apoptosis. The antiapoptotic activity of gal-3 has been shown in various types of cancer (Nakahara, Oka and Raz 2005). Extracellularly gal-3 can signal for apoptosis of human T-cells (Fukumori, et al. 2003). Demetriou and colleagues reported that extracellular Gal-3 may play a role in T-cell – APC (antigen-presenting cell) interaction by negatively regulating the immunological synapse of the TCR (Demetriou, et al. 2001). Gal-3 deficient mice were found to have a less severe EAE compared to WT mice. This was attributed to a decreased IL-17 and IFN-γ production and expanding populations of Th2 cells and Treg cells (Jiang, et al. 2009).

As discussed in the section 'The immunesystem: friend or foe?' ameliorating MS is not a simple case of shutting off the immune system, as it can also be beneficial. So far detrimental effects of Gal-3 have been discussed, but the presence of Gal-3 can also be considered beneficial as it is a marker for activated microglia (Roshenker 2003). As discussed earlier activated microglia and macrophages are critical for the removal of degenerated myelin by phagocytosis. Rotshenker suggests that inefficient myelin removal results from deficient microglia activation, reflected by the failure to up-regulate Gal-3. This is attributed to the absence of unknown native extracellular molecules to activate complement receptor-3 on microglia, instrumental to myelin phagocytosis.

Gal-3 also binds to ECM proteins in a carbohydrate dependent manner and can enhance cell adhesion to the matrix. It can also modulate cell adhesion through binding of integrins (Liu and Rabinovich 2005). Although not fully proven, Gal-3 may affect immune and inflammatory responses by modulating cell adhesion of immune cells (Hughes 2001) (Rabinovich, Liu, et al. 2007).

In vitro and *in vivo* studies suggest that Gal-3 may enhance inflammatory responses through its functions on cell activation, cell migration or inhibition of apoptosis. Data from studies using Lgals3 (the Gal-3 gene) knockout mice supports the role of Gal-3 in promoting inflammatory responses (Rabinovich, Liu, et al. 2007).

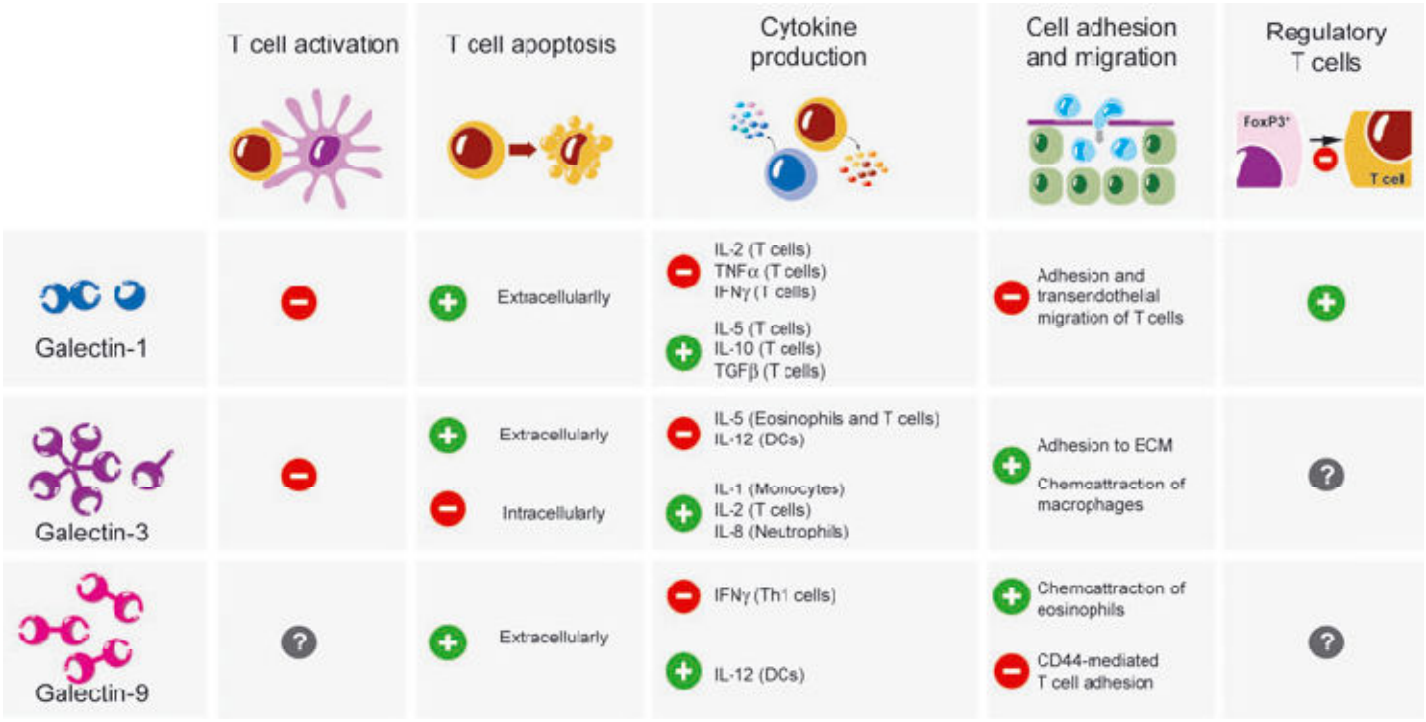

Figure 7. Role of galectin-1, -3 and -9 in the development and resolution of inflammatory responses. This scheme illustrates the influence of individual members of the galectin family in different immune processes, including immune cell activation, survival, cytokine production, cell adhesion and migration and the function of regulatory cells. Galectins are represented according to their biochemical structure: Galectin-1 is a prototype (one-CRD) galectin which can dimerize; galectin-9 belongs to the tandem-repeat family and has two distinct CRD in tandem, connected by a linker of up to 70 amino acids and galectin-3 consists of unusual tandem repeats of proline-and glycine-rich short stretches fused onto the CRD. From (Rabinovich, Liu, et al. 2007)

GALECTIN 4

Galectin 4 (Gal-4), a two-CRD galectin, is predominantly found in the gastro-intestinal tract. In a model of experimental colitis, galectin-4 ameliorated inflammation, induced apoptosis of T-cells and decreased the secretion of pro-inflammatory cytokines (Paclik, Danese, et al. 2008). They show that galectin-4 plays a unique role in the intestine and assign a novel role of this protein in controlling intestinal inflammation by a selective induction of T cell apoptosis and cell cycle restriction.

Returning to the brain, Wei and colleagues show that Gal-4 is involved in p27-mediated activation of the MBP promoter in OLGs (Wei, et al. 2007). In a yeast-2-hybrid essay Gal-4 was found to interact with p27. Furthermore Delacour and colleagues show that Gal-4 is involved in the trafficking of apical membrane components in polarized enterocyte-like cells. Sulfatides with long chain-hydroxylated fatty acids were identified as high-affinity targets for Gal-4. They concluded that interaction between Gal-4 and sulfatides plays a functional role in the clustering of lipid rafts for apical delivery in enterocytes (Delacour, et al. 2005). It would be interesting to study the effects of Gal-4 on OLGs and myelination in particular. These cells carry many sulfatides and the proper formation of lipid rafts is crucial for myelin sheath development (Baron, Colognato and ffrench-Constant 2005).

GALECTIN 8

Galectin 8 (Gal-8) is a tandem-repeat type galectin that is structurally and functionally similar to Gal-4. Gal-8 is different in that is has 6 isoforms, three with two CRDs and three with only one CRD (Yang, Rabinovich and Liu 2008). Gal-8 is secreted and acts as a physiological modulator of cell adhesion. When immobilized in the ECM it promotes cell adhesion by ligation and clustering of a selective subset of cell surface integrin receptors (Paclik, Danese, et al. 2008). However when gal-8 is present in excess as a soluble ligand, it (like fibronectin) forms a complex with integrins that negatively regulates cell adhesion and cell growth (Zick, et al. 2004).

Very recently Tribulatti *et al.* reported Gal-8 localization in the spleen where it was found to provide a costimulatory effect on antigen-specific T cell activation. Activating T cells at an antigen dose that would normally not evoke a response (Tribulatti, et al. 2009). They suggest that locally produced Gal-8 serves as an enhancer of otherwise borderline immune response and that Gal-8 might fuel the reactivity at inflammatory foci. This suggests that Gal-8 might enhance the Th1 immunity, an effect that is undesirable in the case of MS.

GALECTIN 9

The N-terminal CRD of galectin 9 (Gal-9) forms homodimers both in the crystal form and in solution (Yang, Rabinovich and Liu 2008). The lectin is produced and secreted by T-cells and serves as a chemoattractant for eosinophils (Matsumoto, et al. 1998).

Katoh and colleagues found that administering Gal-9 to guinea pigs with an experimental model of asthma reduced airway hyperresponsiveness (Katoh, et al. 2007). Although CD44 is expressed on most blood cells, few of them actively use it to bind hyaluronan (HA) under normal circumstances. However, the HA-binding ability of CD44 is inducible by activation of T cells. Expression of HA on microvascular endothelial cells is induced by proinflammatory stimuli, and CD44 is involved in the rolling and firm adhesion of leukocytes on endothelial cells (Katoh, et al. 2007). They show that administering Gal-9 prevents T-cell adhesion and migration, inhibiting Th2 cell-mediated airway inflammation. HA may also be expressed by astrocytes in chronic MS lesions (Back, et al. 2005). Back

and colleagues found that HA secreted by astrocytes accumulates in demyelinated lesions from individuals with MS, as well as from animals with EAE. The accumulated HA reversibly inhibits OPC differentiation and thus remyelination both *in vitro* and *in vivo* (Back, et al. 2005).

Gal-9 can also be expressed by astrocytes, this expression is stimulated by interleukin-1beta (IL-1β) (Yoshida, et al. 2001). IL-1β is secreted by activated microglia and macrophages, which can be further enhanced by contact with fibronectin in the ECM (Schmidt and Kao 2007). Gal-9 has also been shown to induce apoptosis is thymocytes in a dose dependant fashion (Wada, et al. 1997). Quite recently Gal-9 was found to be the ligand of the Tim-3 receptor on Th1 cells (Zhu, et al. 2005). It was already known that the Tim-3 receptor is involved in the regulation of Th1 immunity and peripheral tolerance, but the identity and mechanism by which this ligand operates was unknown. Administration of Gal-9 causes Tim-3 dependant Th1 apoptosis both *in vitro* and *in vivo* in the EAE model (Zhu, et al. 2005). Furthermore, knockout of Gal-9 using siRNA during the induction of EAE resulted in blunting of the disease (Zhu, et al. 2005). These data show that Gal-9 functions *in vivo* to eliminate Tim-3 Th1 cells and thereby eliminating Th1 immunity, shifting the balance to Th2, an effect beneficial for the recovery of MS lesions.

Taken together one could hypothesize inflammation in a MS lesion would activate microglia and macrophages, resulting in astrocyte activation through IL-1β (Schmidt and Kao 2007). They would, in turn, express Gal-9 (Yoshida, et al. 2001), inducing apoptosis in Th1 cells, shifting the balance to Th2 cells (Zhu, et al. 2005), further stimulating macrophages.

DISCUSSION

"The future challenge will be to tackle the technically challenging problem of distinguishing detrimental effects of inflammation from those that are beneficial to the repair process. In theory, this should allow the design of therapeutic strategies that both suppress myelin damage and promote its repair."

Chao Zhao - (Zhao, et al. 2005)

The purpose of this review is to analyze the link of a number of galectins in mechanisms that are relevant to inflammation-mediated demyelination and remyelination to MS, and if they could play a role in the disease pathology. To my view galectins are without doubt intimately involved with various processes that lead to demyelination and failure in remyelination in MS. Their involvement can be separated into various areas, one better studied than the other: (i) the adaptive immune response, mainly Th1 and Th2 regulatory cells, (ii) the innate immune system, most dominantly galectin involvement in macrophage biology, (iii) ECM modulation, (iv) involvement in OPC/OLG biology.

Galectins 1, 3 and 9 can be categorized as being the most potent immunomodulatory components, figure 7 provides a very nice summary of their properties. Generally Gal-1 is known to bestow a range of anti-inflammatory effects in various cell types, inhibiting cell trafficking, inducing apoptosis and modulating the release of mediators (Norling, Perretti and Cooper 2009). By contrast, Gal-3 is widely pro-inflammatory provoking leukocyte activation, wheres Gal-9 is most commonly known for its activity on eosinophils and as a negative regulator of Th1 cells (Norling, Perretti and Cooper 2009). Translating this to MS, the protective capacity of intracellular Gal-3 in for example Th2 cells is very important to shield them from Gal-1 induced apoptosis. The interaction of Gals-1, -3 and -9 with the ECM is something not to be overlooked. Gal-1 and -9 reduce adhesion and migration of T cells on the blood vessel epithelium and through the BBB. Both are important processes in the maintenance of

the inflammatory response in MS lesions, thus, these secreted galectins may reduce inflammation-induced demyelination.

On the case of macrophages a lot can be said about the involvement of galectins. First of all I must conclude that macrophages are vital to remyelination, based on all the latest research that has been done on the subject. Gal-3 is a marker for activated microglia and although its direct function in that situation is not specifically known, its purpose can be guessed. For example Gal-3 could facilitate the recruitment of other macrophages by enhancing ECM adhesion and chemoattraction (Rabinovich, Liu, et al. 2007). The involvement of Gal-9 in the interaction between macrophages, astrocytes and the Th1/Th2 balance described in the last paragraph of the 'Galectin 9' section is also very important, as demonstrated (Zhu, et al. 2005).

While the immunomodulatory properties of galectins are quite well studied, the properties of galectins in modulating the ECM, for example fibronectin and laminin, holds a lot of promise for progressing our understanding of MS. For this reason a lot more research would have to be done on gal-4 and -8, which have been shown to be potent modulators of ECM interactions. Gal-1 and -3 also have their various effects on the ECM, as is mentioned in the previous two paragraphs as well. Gal-1 and -3 can bind strongly to basement membrane laminin, cellular fibronectin and tenascin, whereas plasma fibronectin is poorly recognized (Hughes 2001). There is more and more evidence piling up that the ECM plays a crucial role in failure of remyelination, rather than just the immune system being the causing failure of remyelination.

OPC differentiation is a key process in remyelination, and it's a process that can be dysregulated by various events. Gal-4 is shown to be vital in the trafficking of lipid raft components in enterocytes (Delacour, et al. 2005). It would be interesting to find out if Gal-4 has any similar function in the trafficking of OLGs and if there is any impairment found in non-remyelinating lesions, although the polarization of OLGs is different from that of enterocytes (Maier, Hoekstra and Baron 2008).

As a final thought, there is a major problem when one attempts to modulate the immune response in MS. Various lesions can develop independent of each other in time and space, meaning that lesions may develop in various regions of the CNS and can be in various stages of development. A region undergoing demyelination due to inflammation benefits when the immune system is suppressed, reducing or possibly preventing lesion formation. However a demyelinated lesion needs at least some degree of inflammation to enable efficient remyelination in the case of MS, as recent research suggests. Current treatment of MS is based on suppressing the immune system, slowing lesion formation which generally slows or halts symptom progression. While this is effective in the way that the disease cannot further develop, it is by far not a cure. Curing MS would mean to both stop new lesions from forming while also remyelinating lesions that have already formed. Because a mild and controlled inflammation is needed to make a lesion area suitable for remyelination, merely suppressing the immune system prevents this, possibly being one of the causes why remyelination fails. Leaving MS untreated results in lesions where the inflammation is exacerbated, also impairing remyelination.

So far only indirect clues have been found linking galectins to MS. Research on EAE models suggests that the lack or overexpression of galectins at certain times and locations can be detrimental or beneficial for the course of MS. Except for the finding of Gal-1 autoantibodies in MS patients (Lutomski, et al. 1997) no data exists on the expression of galectins in MS (i.e. lesions, CSF). This article provides an overview of the role of galectins in virtually all factors involved in the development and sustaining of MS, providing a background to further investigate the prevalence and significance of galectins in MS pathology.

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