Trends in Molecular Medicine

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Review

Untangling Galectin-Driven Regulatory Circuits in Autoimmune Inflammation

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Although progress has been made in understanding the mechanisms implicated in the pathogenesis of autoimmune inflammation, studies aimed at identifying the mediators of these pathways will be necessary to develop more selective therapies. Galectins, a family of glycan-binding proteins, play central roles in immune cell homeostasis. Whereas some members of this family trigger regulatory programs that promote resolution of inflammation, others contribute to perpetuate autoimmune processes. We discuss the roles of endogenous galectins and their specific glycosylated ligands in shaping autoimmune responses by fueling, extinguishing, or rewiring immune circuits. Understanding the relevance of galectin–glycan interactions in autoimmune inflammation could help to uncover novel pathways of tolerance breakdown, define molecular signatures for patient stratification and therapy responses, and open new avenues for immune intervention.

Highlights

Autoimmune diseases are heterogeneous dynamic conditions involving not only activation of autoreactive immune cells but also interruption of tolerogenic circuits.

Biological agents have considerably improved therapeutic options for patients; however, current standard treatments solely reduce clinical symptoms and present severe side effects and variable response rates.

Understanding the relevance of galectin-driven regulatory circuits and their alterations in autoimmune pathologies could pave the way for more rational tailored treatments.

Galectins are endogenous lectins displaying specific, partially overlapping, and/or opposing functions in the regulation of immune cell programs; whereas some members of the galectin family (such as Gal1) mostly contribute to resolution of inflammatory responses, others (e.g., Gal3) can trigger proinflammatory signals or may even contribute to tissue repair.

Galectins Shape the Immune Landscape in Autoimmune Inflammation

Autoimmune diseases include a broad range of chronic disabling conditions such as **multiple sclerosis** (MS; see Glossary) and **rheumatoid arthritis** (RA) with an estimated prevalence of 5% and a dramatic increase in their incidence over the past decade [1]. The occurrence of these of pathologies is associated with genetic predisposition and environmental factors, including persistent infections leading to breakdown of **immune tolerance** [2]. These inflammatory disorders involve a plethora of pathogenic mechanisms and clinical manifestations targeting almost any organ and tissue, precluding the design of single treatment modalities. Current therapies improve life quality by reducing the severity of symptoms but do not provide a definitive cure and have substantial side effects including increased susceptibility to infections and malignancies. Furthermore, owing to the natural heterogeneity of these diseases, the incidence of patients not responding to immunosuppressive therapies is elevated [1–3].

Galectins, a family of soluble glycan-binding proteins initially identified by their roles in embryogenesis and development, are currently emerging as powerful modulators of innate and adaptive immune responses (Box 1) (extensively reviewed in [4]). Through specific interactions with glycoconjugates on the cell surface (Box 2), galectins can control the proliferation, differentiation, migration, and survival of lymphoid and myeloid cells [4]. Although the majority of galectin functions rely on glycan recognition, glycan-independent roles for these proteins have also been described [5].

Because current treatments for autoimmune diseases do not achieve a definitive cure but are limited to relief of clinical symptoms, exploring alternative molecular pathways of immune

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Box 1. Biochemical Properties and Biodistribution of Galectins

Galectins are ubiquitously expressed and highly conserved in eukaryotic taxa throughout evolution [82]. In mammals, 15 members of this family have been identified that share a common structural fold and contain at least one carbohydrate recognition domain (CRD). Traditionally galectins have been classified according to their biochemical structure into three groups: (i) prototype galectins that have one CRD and can act as non-covalent dimers or monomers (Gal1, 2, 5, 7, 10, 11, 13, 14, and 15), (ii) tandem-repeat galectins that contain two CRDs connected by a short linker peptide (Gal4, 8, 9, and 12), and (iii) the chimera-type Gal3 that contains a CRD connected to a non-lectin terminal region that is responsible for its oligomerization [4,83]. Interestingly, Gal1, 3, 8, and 9 are ubiquitously expressed, whereas other members of the family such as Gal2 and 4 are preferentially distributed along the digestive and immune system, while Gal7 is mainly expressed in stratified squamous epithelium. Although originally defined as β-galactoside-binding proteins, galectins display different preferences for glycan structures mainly based on lactosamine residues: Gal1 recognizes only terminal *N*-acetyllactosamine (LacNAc) in the absence of α(2,6) sialic acid, Gal3 can bind to internal LacNAc repeats in poly-LacNAc structures and is not affected by α(2,6) terminal sialylation, while Gal8 preferentially recognizes α(2,3)-sialylated LacNAc [84]. These biochemical differences may contribute to their differential action in diverse cells and tissues, depending on the global regulation of the cellular glycome and the particular glycosylation state of particular receptors [83,84]. Nevertheless, despite their conserved glycan-binding activity, some galectins can also exert biological functions via protein–protein interactions [85].

Galectins exhibit both intracellular and extracellular functions; their biosynthesis takes place in the cytoplasm and, despite the fact that they do not contain a classical secretory signal, many members of this family are secreted through an unconventional endoplasmic reticulum (ER)/Golgi-independent route. Galectins may exert diverse extracellular functions through crosslinking LacNAc-containing glycoconjugates and possibly by forming galectin–glycan structures (often termed lattices) on the cell surface [84]. Interestingly, Gal1, 3, 8, and 9 are ubiquitously expressed, whereas other members of the family such as Gal2, 4, and 6 are preferentially distributed along the digestive system, bone marrow, and immune system, while Gal7 is mainly expressed in stratified squamous epithelium [86]. This different expression profile also occurs in immune cells: while Gal1 is present in a wide variety of cells (including activated T and B lymphocytes, DCs, $Foxp3^+$ Tregs, and macrophages), Gal10 is predominantly expressed in eosinophils and basophils [86].

regulation could shed light on the mechanisms underlying autoimmune diseases and open new roads to more rational and effective therapies. In this review we discuss emerging functions of galectins in autoimmune disorders with the major goal of dissecting redundant, opposite, and overlapping activities of distinct members of the family. In particular, we highlight the impact of individual galectins, as reported in experimental models and patients samples, in the most prevalent autoimmune diseases including MS, RA, type 1 diabetes (T1D), inflammatory bowel disease (IBD), and systemic lupus erythematosus (SLE).

Galectins in MS and Experimental Models of Neuroinflammation

MS is an autoimmune inflammatory and degenerative disease of the brain and spinal cord that is characterized by focal lymphocytic infiltration leading to demyelination and neurological disability [6]. A comprehensive study aimed at delineating the galectin signature in the white matter from healthy individuals revealed variable expression of galectins Gal1, 3, 8, and 9 in **astrocytes**, microglia, and endothelial cells. However, a significant upregulation of these lectins was consistently observed in active lesions (demyelinated areas with reactive immune cells) of MS patients [7].

Although they do not fully recapitulate human disease, experimental models of MS, such as **experimental autoimmune encephalomyelitis** (EAE), have proved useful to delineate the function of galectins and their roles in central nervous system (CNS) inflammation. In the EAE model induced by immunization of mice with the peptide myelin-oligodendrocyte glycoprotein $(MOG)_{35–55}$, Gal1 demonstrated immunomodulatory activity by limiting survival of pathogenic T cells and promoting the differentiation or expansion of regulatory immune cell populations [8,9] (Figure 1, Key Figure). *In vitro* experiments in different T cell subsets showed that Gal1 induces selective **apoptosis** of **T helper type 1 (Th1) cells** and **T helper type 17 (Th17) cells** through

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specific recognition of cell-surface glycans [8]. Accordingly, Gal1-deficient (*Lgals1*$^{-/-}$) mice showed more severe signs of EAE, characterized by a higher frequency of pathogenic Th1 and Th17 cells compared to wild-type (WT) mice [8]. Moreover, exposure to Gal1 during the differentiation or maturation of **dendritic cells** (DCs) induced a tolerogenic profile characterized by IL-27 production both *in vitro* and *in vivo.* Adoptive transfer of $MOG_{35–55}$-primed, Gal1-conditioned tolerogenic DCs during ongoing CNS inflammation attenuated the severity of the disease, limiting demyelination and immune cell infiltration of the spinal cord. These effects were mediated by DC-derived IL-27 which induced the differentiation of IL-10-secreting type 1 regulatory T cells (Tr1 cells) [9]. This tolerogenic circuit was further confirmed by experiments showing that Gal1 is central to tolerance induction induced by intravenous administration of $MOG_{35–55}$. Injection of *Lgals1*$^{-/-}$ mice with the encephalitogenic peptide resulted in reduced expression of tolerogenic cytokines by DCs and lower numbers of Tr1 and $CD4^+CD25^+Foxp3^+$ **regulatory T cells** (Tregs) compared to WT mice [10]. Notably, analysis by confocal microscopy showed that, within the CNS, Gal1 was mainly expressed by astrocytes during the resolution of CNS inflammation in the EAE model. Functionally, this lectin induced deactivation of microglia cells and their polarization towards an alternative **M2 phenotype**, preventing inflammation-induced neurodegeneration [11]. Thus, Gal1 synthesized by DCs, **macrophages**, or astrocytes activates tolerogenic circuits and favors the resolution of autoimmune neuroinflammation. Further investigations of these immunoregulatory properties will be necessary to develop Gal1-based therapeutic modalities for the treatment of T cell-mediated demyelinating diseases.

Gal9, a tandem-repeat member of the galectin family (Box 1), has also been implicated in suppressing neuroinflammatory processes. As evidenced by *in vitro* and *in vivo* studies, the immunoregulatory functions of Gal9 involved inhibition of proinflammatory responses through mechanisms that include induction of Th1 cell apoptosis via association with T cell immunoglobulin and mucin domain 3 (Tim3) and inhibition of Th17-derived cytokines [12,13]. Moreover, Gal9 also promoted differentiation and expansion of Tregs which dampen neuroinflammation [13,14]. In this context, administration of recombinant Gal9 to WT mice during ongoing inflammatory disease reduced disease severity, whereas Gal9-deficient (*Lgals9*$^{-/-}$) mice showed exacerbated inflammation and enhanced Th17-mediated responses compared to WT mice (Figure 1) [12,13]. Notably, proinflammatory cytokines such as IL-1β, IFN-γ, and TNF induced Gal9 expression in primary cultures of mouse and rat astrocytes [15]. This heightened expression reduced T cell viability and proliferation, as evidenced by coculture experiments of T cell lines and astrocytes from *Lgals9*$^{-/-}$ or WT mice [15]. Thus, Gal9 is upregulated in proinflammatory microenvironments and dampens pathogenic responses.

Studies performed on blood samples of MS patients revealed that Gal9-producing $CD4^+$ T cells are markedly reduced in **primary-progressive multiple sclerosis** (PP-MS), but not in **relapsing-remitting MS** (RR-MS) patients, relative to healthy individuals [16]. Moreover, antibody blocking experiments *in vitro* revealed that T cell-derived Gal9 induces apoptosis via specific interactions with Tim3. These results suggest that, in PP-MS, a defect in Gal9-mediated immunosuppression may contribute to sustained pathology [16]. Thus, the immunosuppressive role of Gal9 on activated T cells together with the elevated concentrations of Gal9 in the cerebrospinal fluid of patients suffering from **secondary-progressive MS** (SP-MS) and RR-MS (both compared to healthy volunteers) suggested that increased expression of this lectin may induce a compensatory mechanism to reduce CNS inflammation [17]. Altogether, evidence obtained from murine models and studies on MS patient specimens emphasize the crucial role of Gal9 as a potential regulator of CNS immunopathology.

Glossary

Adoptive T cell transfer model: model of mouse chronic experimental colitis induced by transfer of $CD4^+CD45RB^{high}$ T cells to mice of the same genetic background lacking T and B cells. Colitis and small bowel inflammation become evident 4–5 weeks after the induction of disease.

Anti-cyclic citrullinated peptide autoantibodies: antibodies present in the serum of most patients with rheumatoid arthritis (RA) that can be used in conjunction with other tests to reach a diagnosis.

Anti-double-stranded (ds)DNA circulating antibodies: antibodies directed to dsDNA. They are highly specific for systemic lupus erythematosus (SLE) patients and their detection is used for diagnosis of this pathology.

Apoptosis: regulated or programmed cell death.

Astrocytes: the most prevalent glial cells within the central nervous system (CNS). Functionally, they are involved in injury response but also contribute to neuronal development and plasticity.

Collagen type II-specific antibody-induced arthritis (CAIA): experimental acute model of rheumatoid arthritis induced by the administration of monoclonal antibodies directed to antigenic epitopes in type-II collagen, followed by endotoxin injection.

Collagen-induced arthritis (CIA): autoimmune chronic experimental model of rheumatoid arthritis induced by immunization with type II collagen and Freund's adjuvant, commonly used in mice and rats.

Dendritic cells (DCs): bone marrow-derived myeloid cells known for their ability to present antigens to naïve T cells and orchestrate adaptive immunity. However, DCs can also trigger inhibitory circuits that ensure immunological tolerance and tissue homeostasis.

Dermatomyositis: a systemic autoimmune disease with compromised microvasculature of the skin and muscles.

Dextran sulfate sodium (DSS)-induced colitis: model of experimental colitis induced by DSS dissolved in drinking water. This detergent decreases mucus barrier

In contrast to Gal1 and Gal9, Gal3 accentuates the demyelinating disease in two different mouse MS models: EAE and the viral model of **Theiler's murine encephalomyelitis virus** (TMEV). Consequently, Gal3-deficient (*Lgals3*$^{-/-}$) mice developed an attenuated form of neuroinflammatory disease (Figure 1) [18,19]. Moreover, addition of recombinant Gal3 to cultures of rat microglia cells favored the acquisition of an activated phenotype with enhanced phagocytic activity and elevated synthesis of IL-12, TNF, and IL-1β [20]. Gal3 was found to be upregulated in CNS lesions of MS patients compared to CNS samples from individuals with non-related diseases and to WT brain-resident immune cells exposed to proinflammatory mediators [19,20].

However, galectins have multifaceted roles beyond their immunomodulatory activities. Within the CNS, myelin production relies on oligodendrocytes, and dysfunction of these cells may lead to demyelinating disorders such as MS. In addition, the phagocytic removal of myelin debris is central for the remyelination process and tissue repair [21]. In this regard, experiments performed on *Lgals3*$^{-/-}$ mice challenged with cuprizone (to induce demyelination) revealed that Gal3 is expressed by activated microglia and favors a phagocytic phenotype on these cells, contributing to tissue repair through phagocytosis of myelin debris and differentiation of oligodendrocytes [22,23].

In summary, whereas some galectins such as Gal1 and Gal9 display a consistent immunosuppressive function and dampen CNS inflammatory responses, others such as Gal3 contribute to proinflammatory responses but at the same time facilitate wound-healing and tissue-repair processes.

Galectins in RA and Experimental Autoimmune Models of Osteoarticular Disease

RA is a chronic inflammatory disease that affects primarily the synovial membrane leading to bone and cartilage destruction. This multifactorial disease is characterized by the induction of autoantibody responses, infiltration of the joints with multiple leukocyte types, and activation of local fibroblast-like synovial cells [24]. Similarly to autoimmune CNS inflammation, early studies in the **collagen-induced arthritis** (CIA) model in mice demonstrated the ability of Gal1 to reduce disease severity: a single injection of synovial fibroblasts engineered to secrete this lectin or multiple injections of recombinant Gal1 suppressed clinical and histopathological manifestations of arthritis in the DBA/1 mouse strain [25]. This immunoregulatory effect involved a shift from a Th1-mediated pathogenic response towards a Th2-skewed profile, diminishing the inflammatory reaction [25]. *Lgals1*$^{-/-}$ exhibited increased susceptibility to CIA, with earlier disease onset and more severe clinical symptoms than WT mice [26]; these effects were accompanied by increased T cell proliferation and higher production of proinflammatory cytokines including IL-17 and IL-22 [26]. Based on the broad immunosuppressive activity of Gal1, different laboratories have evaluated innovative formulations and delivery vehicles, including those involving recombinant Gal1 conjugated to gold nanoparticles as well as lentiviral vectors designed for *Lgals1* gene delivery to inflamed joints in rat arthritis models. These formulations reduced disease severity in experimental models and provided evidence supporting the therapeutic potential of this lectin in clinical settings [27,28]. Further studies demonstrated a significant reduction of Gal1 levels and increased titers of anti-Gal1 autoantibodies in synovial fluid of RA patients compared to healthy individuals, suggesting that the immunosuppressive function of this lectin could be limited in arthritic joints, thus contributing to uncontrolled inflammatory responses [29]. Therefore, a potential therapeutic strategy for RA based on recombinant Gal1 should take into account the possible neutralizing ability of anti-Gal1

and injures the epithelium. In consequence, inflammation arises as a response to the influx of bacterial and food antigens. It can be either acute or chronic depending on the extent and frequency of DSS administration.

Dysbiosis: condition in which the microbial communities normally present in the intestinal system or skin are imbalanced. Dysbiosis has been related to many pathologies including inflammatory bowel diseases.

Experimental autoimmune encephalomyelitis (EAE): an experimental model of multiple sclerosis (MS) in which sensitization to myelin antigens is provoked by immunization with CNS homogenates or peptides of myelin proteins.

Fc receptors: receptors for the fragment crystallizable region (Fc) portion of immunoglobulins; often found on immune cells.

Glycome: repertoire of glycan structures present in a given cell, tissue, or organism.

Gut commensal microbiota: the set of microbes that normally inhabit the gastrointestinal tract in a mutualistic relationship with the host.

Immune tolerance: a state of unresponsiveness to self or external nonhazardous antigens that have the potential to induce an immune response. This state is achieved through central (in primary lymphoid organs including the thymic and bone marrow compartment) and peripheral mechanisms.

M2 phenotype: one possible activation phenotype of macrophages and microglia. M2 or alternatively activated macrophages favor wound healing, neuroprotection, angiogenesis, and tumor immune escape, and may be polarized in response to IL-4. They may be subclassified as M2a, M2b, M2c, and M2d according to their gene expression profile and function.

Macrophages: bone marrow-derived myeloid cells that are present in every tissue of the body and exert multiple functions including antigen presentation, phagocytosis, and immunoregulation. Their functional phenotype can change based on environmental signals and tissue localization.

autoantibodies; structural analysis of the epitopes recognized by these autoantibodies will be necessary to design Gal1 variants resistant to autoantibody-mediated blockade.

In contrast to the immunosuppressive properties of Gal1, Gal3 appears to play a proinflammatory role in RA, resembling its function in autoimmune demyelinating diseases. In a model of antigen-induced arthritis, *Lgals3*$^{-/-}$ mice showed mild disease associated with reduced concentrations of proinflammatory cytokines compared to WT mice. Furthermore, administration of recombinant Gal3 reestablished the histopathological signs of synovitis in Gal3-deficient arthritic mice (Figure 1) [30]. Lentivirus-mediated Gal3 silencing using short hairpin RNA (shRNA) strategies resulted in reduced disease scores, lower T cell infiltration, and lower microvessel density in joints of arthritic mice [27]. However, and in addition to its proinflammatory roles, Gal3 showed a protective effect preventing the excess of bone destruction by hindering pathological osteoclastogenesis. *In vitro*, this lectin inhibited the differentiation of the mouse macrophage cell line Raw-D to osteoclasts in a glycan-independent manner [5]. *In vivo,* administration of recombinant Gal3 during rat adjuvant-induced arthritis reduced the extent of bone destruction and osteoclast recruitment [5]. Moreover, this endogenous lectin also appeared to activate a proinflammatory circuit in the synovia of RA patients because synovial fibroblasts exposed to recombinant Gal3 showed enhanced proinflammatory cytokine and chemokine secretion *in vitro* [31]. These results suggest that galectins may exert different functions depending on the target cell type. In this particular case, Gal3 might simultaneously function as an activator of innate and adaptive immune mechanisms, an inhibitor of osteoclast differentiation, and a regulator of synovial fibroblasts function. Several studies using patient specimens partially recapitulated the findings described in animal models. Patients with juvenile idiopathic arthritis (JIA) and newly diagnosed RA patients showed increased Gal3 expression in serum and synovial fluid in comparison to healthy individuals [32,33]. The increased plasma levels of Gal3 positively correlated with the clinical severity score and disease markers such as the **anti-cyclic citrullinated peptide autoantibodies** [32,33]. Follow-up studies in patients with **undifferentiated arthritis** showed that expression of Gal3 is increased in those patients who progressed to RA after 12–13 months, underscoring the possible prognostic value of this lectin in autoimmune osteoarticular conditions [34]. Though these results may appear promising, additional studies with an increased number of patients and uniform criteria for patient classification would be fundamental to elucidate the potential role of Gal3 as a prognostic biomarker in RA.

Other members of the galectin family may also modulate the biology of immune cells in the context of RA. *In vitro* analysis showed that Gal8 is secreted by synovial fluid cells from RA patients, and interacts with CD44vRA variant and fibrinogen forming a trimeric complex that sequestered this lectin, blocking its pro-apoptotic activity (Figure 1) [35]. These data suggest that decreased availability of Gal8 may contribute to the proinflammatory arthritogenic response. In addition, analysis of single-nucleotide polymorphisms in Caucasian populations indicated that F19Y substitution in Gal8 is associated with RA development [36]. By contrast, Gal9 was highly expressed in synovial tissues and fluids of RA patients and promoted *in vitro* apoptosis of fibroblast-like synoviocytes (key cells that can perpetuate disease and increase joint destruction) in a carbohydrate-dependent manner [37]. However, peripheral blood CD4$^+$ T cells from RA patients showed decreased sensitivity to Gal9-induced apoptosis (Figure 1), an effect that was associated with decreased expression of Tim-3 compared to CD4$^+$ T cells from healthy donors [38]. Thus, different mechanisms may operate to enhance or limit the pro-apoptotic activity of this lectin depending on the target cell type. Moreover, further studies in the mouse CIA model showed that genetic deletion of the *Lgals9* gene led to increased disease severity, whereas administration of recombinant Gal9 reduced clinical signs of the disease, as

Multiple sclerosis (MS): an autoimmune chronic and degenerative disease characterized by immune-mediated damage to myelinated axons in the CNS.
Plasma cells: late stage of B cell differentiation characterized by the ability to produce large amounts of antibodies and exert immune regulatory effects.
Primary progressive multiple sclerosis (PP-MS): form of MS disease characterized by a sustained worsening neurologic function from the onset of symptoms, without early relapses or remissions.
Regulatory T cells (Tregs): subset of T cells that promote immune tolerance by downregulating the activation and proliferation of effector T cells. Several populations have been described including CD4$^+$CD25$^+$Foxp3$^+$ cells (Tregs) and IL-10-producing Foxp3$^-$ (type 1 regulatory T cells, Tr1) cells. These cells play key roles in preserving tissue homeostasis and protecting against the detrimental effects of autoimmune inflammation.
Relapsing-remitting MS (RR-MS): the most common course for MS characterized by relapses with new or increasing neurologic symptoms followed by periods of partial or complete recovery (remissions).
Rheumatoid arthritis (RA): a chronic autoimmune disease characterized by injury to bone and cartilage mediated by autoantibody responses and synovial membrane inflammation.
Secondary progressive MS (SP-MS): a type of MS that follows an initial RR-MS. Most patients will eventually transit this secondary course in which a progressive worsening of neurologic function over time is observed.
Sjögren syndrome: a systemic autoimmune disease affecting exocrine glands and other organs.
T follicular helper (Tfh) cells: subset of CD4$^+$ T cells that produce IL-21 and provide B cell help in the germinal centers for antibody affinity maturation and the development of memory B cells and long-lived plasma cells.
T helper type 1 (Th1) cells: subset of CD4$^+$ T cells that typically produce IFN-γ. Th1 cells are involved in controlling intravesicular pathogens

measured by paw-swelling, through mechanisms involving Treg cell expansion and reduced Th17 differentiation (Figure 1) [39]. These effects were not limited to the CIA model because treatment of recombinant Gal9 also reduced clinical signs of arthritis in the **collagen type II-specific antibody-induced arthritis** (CAIA) model by negatively regulating the activation of peritoneal macrophages. Mechanistically, Gal9 altered the balance of inhibitory and stimulatory **Fc receptors** on these cells and impaired the secretion of proinflammatory cytokines [40].

Thus, in immune-mediated osteoarticular disorders, a complex network of galectins is involved in the regulation of differentiation and fate of immune and synovial cells. Some members including Gal1, Gal8, and Gal9 contribute to resolution of the arthritogenic process, while others such as Gal3 augment the inflammatory response but prevent bone erosion and tissue damage.

Pro- and Anti-Inflammatory Roles of Galectins in T1D

T1D results from destruction of insulin-producing β cells by immune-mediated reactions involving the activation and infiltration of $CD8^+$ and $CD4^+$ T cells, B cells, and macrophages [41]. Current research efforts are focused on halting autoimmune destruction and restoring immune tolerance to β cell-derived antigens [42]. Similarly to the above-mentioned effects, Gal1 displayed anti-inflammatory activities in an experimental model of T1D mainly by down-regulating pathogenic T cell responses [43]. In the spontaneous model of non-obese diabetic (NOD) mice, treatment with recombinant Gal1 not only prevented the onset of hyperglycemia, and reverted 60% of overtly diabetic NOD mice to normoglycemia (Figure 1), but also diminished inflammatory infiltrates in pancreatic islets, tilting the balance towards a Th2-dominant anti-inflammatory response [43]. *In vitro* experiments with effector T cells from prediabetic NOD mice demonstrated that these cells were less sensitive to Treg suppression mediated by Gal1 compared to cells isolated from BALB/c and C57BL/6 mice [44]. Thus, mechanisms underlying the anti-inflammatory effects of exogenous Gal1 might differ from those involving communication between Tregs and effector T cells. Of note, peripheral blood mononuclear cells from T1D patients, particularly monocytes, secreted lower amounts of Gal1 compared to healthy

and contribute to autoimmune disease pathogenesis.

T helper type 17 (Th17) cells: subset of $CD4^+$ T cells that typically produce IL-17, IL-21, and IL-22 and are involved in mucosal immunity and chronic inflammation.

T helper type 2 (Th2) cells: subpopulation of $CD4^+$ T cells characterized by the secretion of IL-4, IL-5, and IL-13 that play crucial roles in allergic reactions, parasite immunity, and reducing T cell-mediated autoimmune diseases.

Theiler's murine encephalomyelitis virus (TMEV): an encephalitogenic virus that causes a biphasic CNS disease commonly used as an experimental model for MS. The disease starts with an acute phase characterized by poliomyelitis followed by a chronic phase involving inflammatory demyelination.

Undifferentiated arthritis: a classification used to categorize patients with arthritis symptoms that cannot be diagnosed according to current criteria.

Box 2. It Takes Two To Tango: Galectins and Glycans

The functional consequences of galectin binding are exquisitely regulated by the availability of specific permissive glycan structures. Because galectins recognize glycans based on the LacNAc disaccharide, mouse strains devoid of genes necessary for the biosynthesis of galectin ligands have been instrumental in unraveling the complex network of biological processes triggered by these lectins in autoimmune pathologies. Deficiency in two different glycosyltransferases involved in the biosynthesis of complex *N*-glycans produces altered immunological phenotypes that resemble human autoimmune diseases: α-mannoside β1,6-*N*-acetylglucosaminyltransferase 5 (*Mgat5*$^{-/-}$) null mice, which are unable to synthesize β1,6-branched complex *N*-glycans [87], and mannosyl-oligosaccharide 1,3-1,6-α-mannosidase II (*Man2a1*)-deficient mice, which display no complex *N*-glycans on cells of the erythroid lineage and an altered *N*-glycome relative to other cell types [88] owing to incomplete conversion of high-mannose to complex *N*-glycans and a truncated *N*-glycan maturation pathway (Figure I). While *Mgat5*$^{-/-}$ mice exhibit altered cytokine signaling and increased T cell activation, *Man2a1*$^{-/-}$ null mice develop an age-related autoimmune disease resembling SLE. In the first case, and despite of the fact that Mgat5-deficient mice exhibit an increase in Tregs compared to WT mice, the development of spontaneous demyelinating disease indicates both hyperactive effector cell responses and ineffective Treg cell functions. In the case of *Man2a1*$^{-/-}$ mice, the hybrid *N*-glycan structures exposed on extracellular glycoproteins are recognized by specific lectin receptors on innate immune cells that initiate immune responses in the kidney glomeruli, leading to chronic inflammation and the consequent development of autoimmune disease. Finally, mice lacking the *B3gnt2* gene (encoding a poly-LacNAc synthase that is capable of extending complex *N*-glycan antenna, Figure I) presented hypersensitivity to immune stimulation and macrophage hyper-responsiveness to lipopolysaccharides (LPS), indicating that poly-LacNAc extensions are key factors determining immune activation, probably through galectin binding [89].

With respect to mucin-type *O*-glycosylation, the core 1 β1,3-galactosyltransferase-specific molecular chaperone (Cosmc), an X-linked chaperone important for core 1/core 2 *O*-glycan biosynthesis and glycocalyx formation, was identified by genome-wide association studies as an IBD risk gene on the X chromosome. *Cosmc* deletion in mouse intestinal epithelial cells led to reduced mucosal diversity, and a **dysbiotic** mucosal but relatively preserved luminal microbiota as seen in human IBD patients. Considering that core 2 *O*-glycans are synthesized based on the core 1 structure, and many members of the galectin family can recognize both core 1 and extended core 2 *O*-glycans (Figure I), a potential involvement of galectins in the development of experimental colitis by loss of epithelial Cosmc should be considered [90].

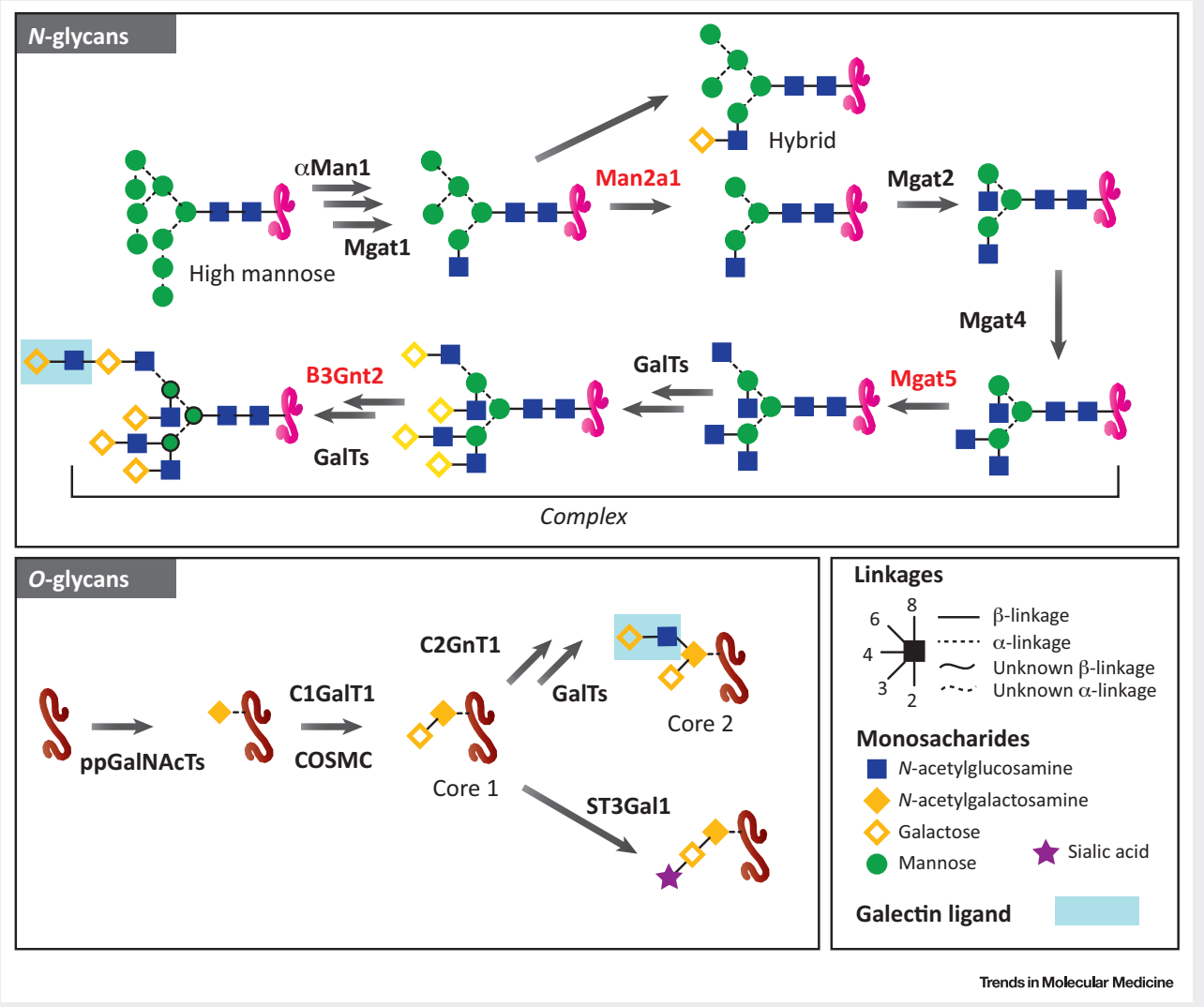

Figure I. Schematic Representation of *N*- and Mucin-Type *O*-Glycan Biosynthesis. This scheme is simplified showing only glycan-modifying enzymes relevant to this review. Briefly, complex *N*-glycans are obtained by trimming of high-mannose structures by α-mannosidases (i.e., αMan1, Man2a1) and their different antennas are initiated by the action of *N*-acetylglucosaminyltransferases (Mgat1, 2, 4, 5). These GlcNAc residues are extended to LacNAc structures by galactosyltransferases (GalTs) and can be further elongated by poly-LacNAc synthase B3Gnt2. In the case of *O*-glycans, biosynthesis starts by the addition of a GalNAc residue to the protein, a process mediated by polypeptide *N*-acetylgalactosamine (GalNAc) transferases (ppGalNAcTs). Biosynthesis of core 1 is achieved by the action of β1,3-galactosyltransferases (C1GalT1) with the assistance of core 1 β1,3-galactosyltransferase-specific molecular chaperone (Cosmc). The resulting disaccharide can be sialylated by an α2,3-sialyltransferase (ST3Gal1), or branched to core 2 *O*-glycans by β1,6-GlcNAc transferases such as C2GnT1. Finally, core 2 *O*-glycans can be further decorated by GalTs with the consequent synthesis of LacNAc.

individuals, suggesting that Gal1-driven immunoregulatory circuits might be impaired by a lower expression of this lectin during the course the disease [45].

Gal9 has also shown a protective role in T1D through mechanisms involving downregulation of pathogenic Th1 responses. NOD mice overexpressing Gal9 were protected from the development of diabetes and displayed reduced insulitis compared to control NOD mice [46]. In addition, overexpression of this lectin in pancreatic islets prolonged islet graft survival in

Key Figure

Impact of Galectins on Different Immune Cell Functions and Their Implications in Autoimmune Inflammation

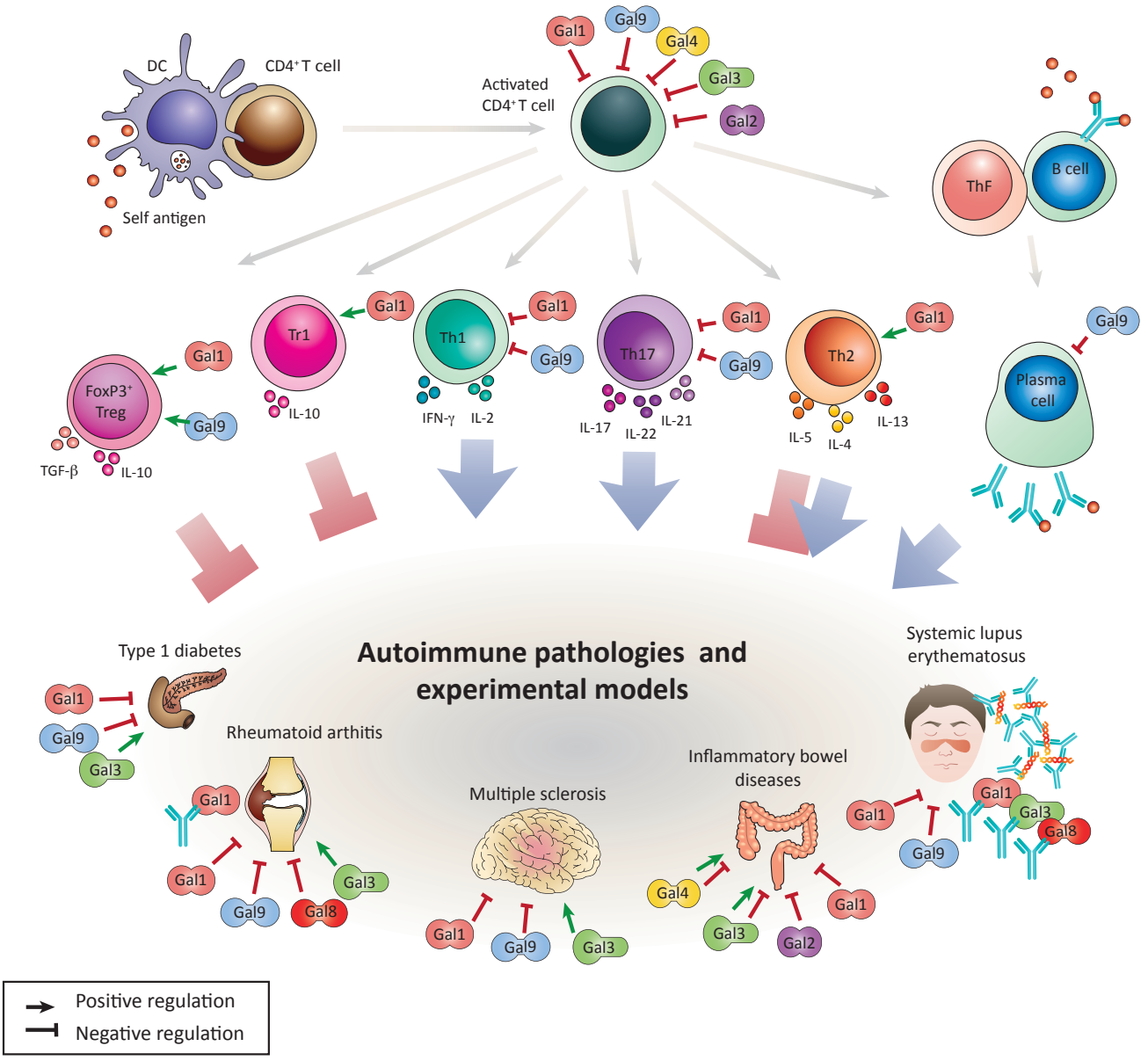

Figure 1. Galectins control a wide range of T cell processes, including T cell activation, survival, cytokine secretion, and differentiation. By modulating immune cell programs, galectins may influence the development and course of autoimmune diseases. Studies performed in mice lacking individual members of the galectin family, or following gene or protein delivery in wild-type mice, have revealed immunoregulatory activities of these lectins in T cell- and antibody-mediated autoimmune conditions. Specifically, galectins may selectively alter the survival and/or cytokine production by activated T cell subsets (Th1, Th2, and Th17 effector cells). In addition, some members of the galectin family may induce the differentiation and/or expansion of Foxp3$^+$ Tregs, IL-10-producing Foxp3$^-$ Tr1 cells, and tolerogenic DCs, thus

(See figure legend on the bottom of the next page.)

streptozotocin-induced diabetic NOD/severe combined immunodeficiency (SCID) mice compared to control islets after challenge with diabetogenic splenocytes [47]. Furthermore, preventive long-term treatment with recombinant Gal9 given once per week until 50 weeks of age delayed the onset of the disease in NOD mice (Figure 1) [48].

Finally, Gal3 is extensively expressed by both rat and human isolated islets in response to IL-1β *in vitro* and *in vivo* during spontaneous development of T1D in the Bio-Breeding diabetes-prone rat strain [49]. Interestingly, *Lgals3*$^{-/-}$ mice develop attenuated diabetes in response to multiple low-doses of streptozotocin, as demonstrated by reduced glycemia and lower insulitis [50]. The elicited immune response was characterized by low expression of IFN-γ and complete absence of TNF and IL-17 in pancreatic lymph nodes, implicating a reduced inflammatory reaction against β cells [50]. These results suggest that Gal3 may have a pathogenic proinflammatory role in streptozotocin-induced diabetes. However, contradictory findings regarding the pro-survival function of this lectin on β cells have been reported, precluding definitive conclusions to be reached [49,51]. While one study described that overexpression of Gal3 protects pancreatic β cell lines against IL-1β-induced toxicity [49], another study indicated that *Lgals3*$^{-/-}$ islet cells display enhanced survival and function in response to TNF, IFN-γ, and IL-1β [51]. These apparent conflicting observations might be associated with differences in the experimental approaches used, involving cell lines or primary cell cultures from different animal species. Comparative analysis of basal expression of galectins in each cellular model, the levels of Gal3 reached by overexpression, and possible compensatory pathways generated by other members of the family in Gal3-deficient mice could offer a mechanistic basis for these discrepancies. Thus, the potential role of Gal3 in regulating survival of pancreatic β cells and immune-mediated pathology remains controversial and further investigation will be necessary to understand the underlying mechanisms.

Dual Roles of Galectins in IBD

IBD refers to chronic immune-mediated disorders of the bowel, including Crohn's disease (CD) and ulcerative colitis (UC). These are generally believed to arise from an aberrant immune response to **gut microbiota** and pro-inflammatory CD4$^+$ T cells have been shown to play key roles in these pathologies [52,53]. Studies analyzing galectin expression and distribution in the gastrointestinal tract of healthy individuals as well as CD and UC patients indicated that several members of the family, including Gal1, 2, 3, 4, 8, and 9, are extensively expressed in the gut and their expression patterns change in pathological conditions [54–56]. In fact, based on a linear discriminant analysis of mRNA galectin expression in biopsies of CD, UC and non-IBD donors, a galectin signature has been proposed as a biomarker to discriminate IBD samples from other intestinal inflammatory conditions [56]. Studies aimed at dissecting the role of galectins in gut homeostasis and IBD were performed both in patient samples and in experimental models with variable results. In the **dextran sodium sulfate (DSS)-induced acute colitis** model, *Lgals3*$^{-/-}$ mice showed more severe pathological signs, while administration of recombinant Gal3 prevented colitis manifestations through mechanisms that required the presence of Foxp3$^+$ Treg cells and suppression of colonic IL-6 production [57,58]. Conversely, in another study, *Lgals3*$^{-/-}$ mice displayed attenuated acute DSS-induced colitis that was associated

modulating the balance between effector and regulatory immune cell populations in autoimmune settings. In addition, galectins can regulate the differentiation and survival of antibody producing-plasma cells. Notably, their bioavailability may be influenced by the presence of circulating anti-galectin autoantibodies that are detected in rheumatoid arthritis, uveitis, and systemic lupus erythematosus patients. Abbreviations: DC, dendritic cell; Foxp3$^+$ Treg cell, CD4$^+$CD25$^+$Foxp3$^+$ Treg cell; Th1, T helper type 1 cell; Th2, **T helper type 2 cell**; Th17, T helper type 17 cell; ThF, **T follicular helper cell**; Tr1, IL-10-producing Foxp3$^-$ Treg cell; Treg, regulatory T cell.

with a reduced number of infiltrating macrophages, inflammatory DCs, and polymorphonuclear neutrophils, with lower production of proinflammatory mediators [59]. These apparently contrasting findings may reflect the variability of housing conditions, and particularly the intestinal microbiota composition that could impact on the development of the disease. In clinical settings, several groups have described Gal3 downregulation in inflamed colonic tissue via TNF-dependent mechanisms [56,60–62]. However, a recent study reported that Gal3 expression was augmented in the inflamed colon of UC patients compared to non-UC controls [59]. The diverse factors affecting development of intestinal inflammation, the different criteria used for patient stratification, and finally the inherent difficulties in studying small size samples could explain these disparities.

The potential role of Gal4 in experimental colitis is also controversial. Some authors have reported that this galectin aggravates colitis and delays the recovery of mice treated with DSS through stimulation of $CD4^+$ T cells [63]. Moreover, another study reported that downregulation of the core-2 synthase (C2GnT1, Box 2) generated an inducible colitis-associated **glycome** which increased Gal4 binding to local memory $CD4^+$ T cells and promoted their survival, sustaining the inflammatory process [64]. This particular T cell glycome as well as C2GnT1 downregulation were observed in experimental mouse models including the **adoptive T cell transfer model**, spontaneous colitis in TCRα-deficient mice, and DSS-induced acute colitis, and was confirmed in UC patients. By contrast, administration of recombinant Gal4 was found to reduce mucosal inflammation in mouse DSS-induced acute colitis via induction of mucosal T cell apoptosis and reduction of proinflammatory cytokines [65]. These conflicting data recapitulate the contradiction previously described for Gal3, and might reflect the complex nature of the immunobiology of mucosal tissue and its dynamic interaction with the microbiome, highlighting the possibility that immune and epithelial cell glycosylation could be differentially regulated in distinct experimental models and might vary according to the microbiota typically present in each animal strain and facility. Although our understanding of the role of Gal4 in diverse physiological and pathological conditions is rapidly increasing, further studies will be necessary to completely define the role of endogenous Gal3 and Gal4 in IBD and other gastrointestinal pathologies by using more selective targeting technologies including tissue-specific disruption of individual members of the galectin family or their glycosylated ligands in epithelial, immune, or endothelial compartments.

Gal2, a prototype galectin (Box 1) associated with gut epithelial cells, was downregulated in DSS-induced colitis, but could be restored to normal levels after treatment with the immunosuppressant drug tacrolimus [66]. Moreover, administration of recombinant Gal2 induced apoptosis of mucosal T cells and decreased histopathological and immunological signs of inflammation both in acute and chronic DSS-induced colitis mouse models (Figure 1) [66].

Similarly to its effects in other autoimmune diseases, recombinant Gal1 demonstrated a protective role in the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced mouse model of colitis through selective elimination of mucosal activated T cells [67]. In this model, Gal1-treated mice showed a higher frequency of apoptotic T cells in the lamina propria and decreased production of proinflammatory Th1 cytokines in colon compared to non-treated mice (Figure 1) [67]. Furthermore, studies in intestinal epithelial cells isolated from UC and CD patients or mice with TNBS-induced colitis revealed differential binding of Gal1 to epithelial cells that was dependent on the prevalent inflammatory conditions [68]. This glycan-dependent interaction selectively controlled epithelial cell survival and promoted the secretion of epithelial growth factors including thymic stromal lymphopoietin (TSLP), epidermal growth factor (EGF), and transforming growth factor (TGF)-β1 [68]. These findings support a dual role for Gal1 in

regulating inflammatory responses and modulating homeostasis of epithelial intestinal tissues. Thus, galectins and their glycosylated ligands may play diverse and non-overlapping roles in the pathogenesis and/or resolution of gut inflammation.

Immunomodulatory Roles of Galectins in SLE

SLE is an antibody-mediated autoimmune systemic disease characterized by high titers of **anti-double-stranded (ds)DNA circulating antibodies** with variable clinical presentations [69]. In SLE mouse models such as (NZB × NZW) F1 and MRL/lpr mice, administration of recombinant Gal1 or Gal9 attenuated the clinical manifestations of the disease and reduced the titers of anti-dsDNA circulating antibodies [70,71]. Mechanistically, Gal1 promoted expansion of Tregs, and Gal9 induced **plasma cell** apoptosis [70,71]. Hence, treatment of SLE with Gal1, Gal9, or both merits further preclinical examination.

Exploratory studies on the expression of Gal1 and Gal3 in SLE patients and healthy individuals showed that activated T cells from SLE patients had lower expression of Gal1 compared to healthy T cells, and were less sensitive to Gal1-induced apoptosis [72], whereas Gal3 serum levels were upregulated in SLE patients [73]. Notably, the frequency of anti-galectin autoantibodies was increased in sera from SLE patients compared to healthy individuals [74–76]. In particular, anti-Gal3 and anti-Gal8 antibodies were found to be tightly associated with clinical signs of lupus, cutaneous vasculitis, and lymphopenia [75,76]. In this regard, antibodies isolated from SLE patients inhibited Gal8-induced T cell apoptosis and modulated T cell adhesion *in vitro* [77]. Hence, dysregulation of galectin expression as well as the presence of anti-galectins autoantibodies in the sera of SLE patients warrant further studies to analyze their contribution to SLE immunopathology. Finally, although less well documented, the role of galectins has also been explored in other autoimmune pathologies (Box 3).

Concluding Remarks and Future Perspectives

Autoimmune diseases arise from genetic and environmental interactions that ultimately lead to disruption of self-tolerance and autoinflammatory manifestations, but only a fraction of these disorders are associated with germline genetic variations. Homeostatic control of these pathologies involves a complex network of regulatory cells and multiple immune checkpoint programs that represent attractive targets for therapeutic intervention. By influencing both immunological and non-immunological compartments, galectin-driven regulatory circuits have emerged as novel homeostatic pathways, shaping the landscape of autoimmune diseases.

The information discussed in this review provides an opportunity to delineate emerging patterns in the functional divergence of galectins. Like many cytokines and growth factors, individual members of the family may display predominant pro- or anti-inflammatory effects, and these biological activities appear to be context- and target cell-dependent. In fact, whereas Gal1 and Gal9 display mostly immunosuppressive activities, Gal3 has general proinflammatory functions that exacerbate autoimmune processes. However, these functions are not always detrimental because phagocyte activation and removal of damaged cells may be productive processes intrinsically associated with wound healing and tissue repair [21]. Moreover, galectins might exert double-edged effects depending on inherent biochemical factors, including their dimerization or oligomerization status, stability, oxidation, and concentration in cells and tissues, as well as extrinsic factors such as the glycosylation status of specific target cells and receptors [78–80]. Thus, galectins may function as soluble regulatory checkpoints that safeguard tissue homeostasis either by dampening deleterious responses or by promoting tissue repair.

Box 3. Galectins in Other Autoimmune Diseases

Autoimmune uveitis (AU) is a T cell-mediated intraocular inflammatory disease that compromises the functionality of the eye [91]. Treatment with recombinant Gal1 or Gal8 in a mouse model of AU (induced by immunization with the interphotoreceptor retinoid binding protein, IRBP) was sufficient to suppress ocular pathology through mechanisms comprising inhibition of leukocyte infiltration and promotion of non-pathogenic Th2 and Treg cell responses [92–95]. Thus, galectins can actively participate in regulatory networks that preserve the eye as an immune-privileged organ.

Autoimmune hepatitis (AIH) is a chronic immune-mediated liver disease that may lead to liver cirrhosis, organ failure, and death [96]. Similarly to the effect of recombinant galectins in other models, administration of recombinant Gal1 or Gal9 prevented liver damage by inducing apoptosis of activated T cells, reducing the production of proinflammatory cytokines, and increasing the number of Tregs in a mouse model of AIH [97,98]. In addition, Gal9 expression was upregulated in the liver of animals with AIH, an effect that was accompanied by an increased frequency of hepatic Tregs [99]. However, studies performed in AIH patients indicated a reduced percentage of circulating $Gal9^{+}$ T cells compared to healthy individuals [100]. This evidence reflects the intrinsic differences between the animal models and the human pathology as well as the experimental approaches used in each case. Furthermore, $Lgals3^{-/-}$ mice presented reduced susceptibility to experimental AIH and reduced tissue damage associated with lower production of proinflammatory mediators as well as augmented synthesis of IL-10 by T cells, DCs, and natural killer T (NKT) cells, suggesting a proinflammatory role of Gal3 in this pathologic setting [101,102].

Autoimmune orchitis (AO) is an organ-specific autoimmune disease characterized by testicular inflammation and the presence of anti-sperm antibodies that leads to male infertility [103]. Experimental induction of autoimmune orchitis was significantly less severe in $Lgals1^{-/-}$ mice than in WT mice. Paradoxically, however, exogenous administration of Gal1 in WT mice attenuated the severity of the disease [104]. These opposing results may reflect differential roles of endogenous versus exogenous Gal1 in regulating germ cell survival and immune responses [104].

Autoimmune thyroid diseases (AITD) are paradigmatic organ-specific autoimmune maladies which are most frequently represented by Graves' disease and Hashimoto's thyroiditis [105]. Studies performed in patients revealed that Gal9 expression is reduced in peripheral blood DCs from Graves' disease patients but not from Hashimoto's thyroiditis patients or healthy controls [106].

Finally, analysis of galectins expression in blood serum revealed that Gal9 is increased in **dermatomyositis** patients, and Gal3 is augmented in **Sjögren syndrome** patients as compared to healthy individuals [107,108].

A major limitation hindering research progress in autoimmune diseases is the lack of experimental models capable of recapitulating clinical features of human pathologies. However, mouse models provide insightful details on potential etiological and immunological factors. Notably, the current literature documenting the role of galectins in autoimmune pathologies exhibits a degree of discrepancy probably due to (i) diversity in cause and nature of autoimmune responses (e.g., spontaneous autoimmunity, autoimmune responses in genetically-modified mouse strains, active/passive immunizations, or chemically induced inflammatory diseases), (ii) variations in the intrinsic expression of galectins in each mouse strain, (iii) study of endogenous galectins using knockout mouse strains versus administration of exogenous recombinant galectins, (iv) the dose and route of administration of recombinant galectins, and (v) the environmental context including the dynamics of the microbiome. More importantly, limiting factors (i), (ii), and (v) may also be relevant in the evaluation of clinical findings in human disease, considering their multifactorial and heterogeneous nature as well as the discrepancies in patient stratification.

The cell-surface glycome emerges as an important factor that could be altered during disease evolution, influencing the activation, differentiation, migration, and survival of immune cells, as demonstrated by experiments in glycosyltransferase-deficient mice (Box 2). Glycosylation of cell-surface receptors adds an additional layer of complexity to immune regulation and delineates a future scenario in which an integrated analysis of glycan remodeling and the expression of glycan-binding proteins (including not only galectins but also C-type lectins and siglecs) might provide novel opportunities for differential diagnosis and therapeutic intervention (see Outstanding Questions and Box 4).

Box 4. Clinician's Corner

Different carbohydrate-binding proteins including C-type lectins, siglecs, and galectins control immune cell homeostasis by conveying glycan-containing information into immune signaling programs, thereby regulating immune cell activation, differentiation, trafficking, and survival.

By either amplifying, suppressing, or rewiring immune cell circuits, galectins, a family of β-galactoside-binding lectins, can shape the immune landscape in autoimmune inflammatory processes.

Understanding the relevance of galectin–glycan interactions in normal or inflamed tissues could help to uncover new circuits of immune tolerance breakdown, define molecular signatures for patient stratification and therapy responses, and offer new therapeutic modalities for treating autoimmune diseases.

Whereas several galectin-tailored agents (either agonists or antagonists) have been designed, progress thus far might represent only the starting point of a therapeutic potential that awaits future discovery.

Outstanding Questions

What is the degree of functional redundancy among different members of the galectin family?

Do galectin regulatory circuits interact with other immune checkpoint pathways to control immune tolerance and homeostasis?

Can regulatory galectins provide a selective treatment for autoimmune diseases that avoids the side effects of general immunosuppressant drugs?

Would combinatorial strategies including galectins and other immunomodulatory agents be beneficial for treatment of autoimmunity patients?

The original concept of autoimmunity as a result of aberrant activation of autoreactive immune cells has been currently replaced by the notion that these pathologies are dynamic conditions arising from the interruption of tolerogenic or immunoregulatory circuits [81]. Current therapies for autoimmune diseases are mainly based on broad immunosuppressive drugs; the recent incorporation of biological agents (such as anti-TNF, anti-CD20, and anti-$\alpha_{4\beta7}$-integrin antibodies) has significantly expanded the therapeutic options for patients, but the frequency of non-responding patients is still an issue. The long-term use of immunosuppressive drugs has diverse systemic side effects and may undermine the immune response of the patient against infection and cancer [81]. Hence, research in autoimmune diseases is in need of more specific and rational therapeutic strategies aimed at reestablishing immune tolerance and silencing harmful immune cells [81]. In this regard, the findings discussed in this review provide evidence for a promising area of research in which galectins are homeostatic mediators capable of fueling, extinguishing, or reprogramming immune responses through glycosylation-dependent or -independent mechanisms. Understanding the relevance of galectin–glycan interactions in immunity will help to uncover new molecular pathways leading to autoimmune disorders and identify possible biomarkers to either predict or monitor treatment responses. Future studies at the interface of immunology, glycobiology, and molecular medicine are anticipated, paving the way for the design of galectin-based tailored therapies aimed at restoring immune cell homeostasis and rewiring tolerogenic circuits in a wide range of autoimmune conditions.

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