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Galectins: regulators of acute and chronic inflammation

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Galectins, β-galactoside-binding animal lectins, are differentially expressed by various immune cells as well as a wide range of other cell types. Extracellularly, galectins are able to exhibit bivalent or multivalent interactions with cell-surface glycans on various immune cells and exert various effects. These include cytokine and mediator production, cell adhesion, apoptosis, and chemoattraction. In addition, they can form lattices with cell-surface glycoprotein receptors, resulting in modulation of receptor functions, including clustering and endocytosis. Intracellularly, galectins can participate in signaling pathways and modulate biologic responses. These include apoptosis, cell differentiation, and cell migration. Thus, a large body of literature indicates that galectins play important roles in the immune and inflammatory responses through regulating the homeostasis and functions of immune cells. The use of mice deficient in individual galectins has provided additional evidence for the contributions of these proteins to these responses. Current research indicates that galectins play important roles in the development of acute inflammation as well as chronic inflammation associated with allergies, autoimmune diseases, atherosclerosis, infectious processes, and cancer. Thus, recombinant proteins or specific galectin inhibitors may be used as therapeutic agents for inflammatory diseases.

Keywords: galectin; immunity; inflammation; lectin; glycans

Introduction

Galectins are β-galactoside-binding animal lectins defined by shared consensus amino acid sequences in the carbohydrate-recognition domain (CRD) and affinity for β-galactosides. Presently, 15 members have been identified in mammals. The family is composed of one-CRD type (galectin-1, 2, 5, 7, 10, 11, 13, 14, and 15), which are monomers or homodimers of one CRD (∼15 kDa); two-CRD type (galectin-4, 6, 8, 9, and 12), which contains two distinct but homologous CRDs in a single polypeptide chain; and a chimeric type (galectin-3; ∼30 kDa), which contains a nonlectin part made of proline- and glycine-rich short tandem repeats connected to a CRD[1–3] (Fig. 1).

Some members are distributed widely in different cell and tissue types, while others are more selectively expressed. Although all galectins bind to galactose, they have specificity for different oligosaccharides. Most galectins studied are either bivalent or oligovalent with regard to their carbohydrate-binding activities. One-CRD galectins can exist as dimers; two-CRD galectins have two carbohydrate-binding sites; and galectin-3 forms pentamers upon binding to multivalent carbohydrates (Fig. 1). Thus, they can form ordered arrays of lectin–carbohydrate complexes, like lattices formed by antibodies binding to multivalent antigens.[1–3]

Galectin family members do not contain a classical signal sequence but they can be secreted through an as yet undefined secretory pathway and detected in the extracellular space. The proteins are known to be localized in the cytoplasm and can move into the nucleus or be associated with intracellular vesicles under certain conditions.[1]

The ability of recombinant galectins to engage glycans on the cell surfaces as well as extracellular matrix glycoproteins has been extensively and convincingly documented *in vitro*.[2] Some galectins have been shown to bind to a number of different cell-surface antigens or receptors through lectin–carbohydrate interactions. It appears that galectins do not have specific individual receptors,

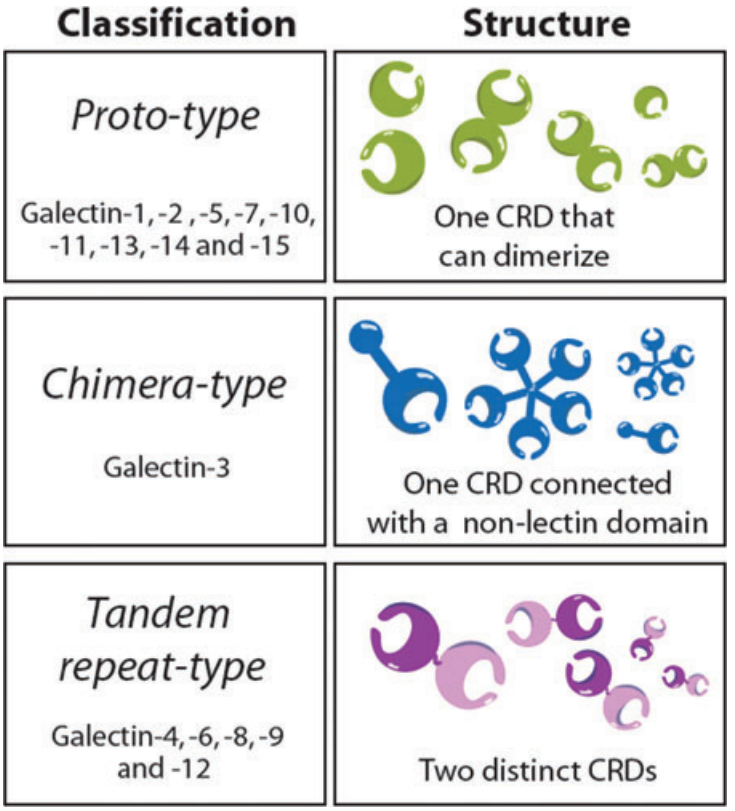

Figure 1. Galectin family members. The galectin family consists of those containing one carbohydrate-recognition domain (CRD); galectin-3, which consists of unusual tandem repeats of proline- and glycine-rich short stretches fused onto the CRD; and those containing two distinct CRDs in tandem, connected by a linker. One-CRD galectins can form dimers; galectin-3 forms pentamers upon binding to multivalent carbohydrates; and two-CRD galectins have two carbohydrate-binding sites. Thus, galectins can form lattices with multivalent glycoconjugates.

but each can bind to a set of cell-surface or extracellular matrix glycoproteins containing suitable oligosaccharides.[2] In addition, a model in which galectins form lattices with cell-surface glycans has been developed, as will be discussed below.

A number of studies have demonstrated that galectins can function inside the cell, and, interestingly, they may do so in a fashion that is independent of their carbohydrate-binding activities. Galectin-3, in particular, has been shown to bind to a number of intracellular proteins known to participate in intracellular signaling pathways (reviewed in Ref. 1). Intracellular functions of other galectins, including galectin-1, 9, and 10, have begun to emerge.

Since 1994 when the name *galectin* was first designated, significant progress has been made in our understanding of this family. Some members, especially galectin-1, 3, and 9, have been studied by researchers in a wide array of disciplines, and experimental results suggest that these lectins have diverse functions (reviewed in Refs. 2, 3).

Functions of galectins in immune cells demonstrated *in vitro*

A variety of functions of galectins have been demonstrated in various immune cells *in vitro*. A majority of the functions are shown with the responses measured after addition of recombinant proteins to cells. Others are revealed by using cells transfected with cDNA coding for a galectin or treated with small interfering (si)RNA or antisense RNA specific for a galectin, thus with overexpression or suppressed expression of the protein, respectively. Yet others are demonstrated by using cells from mice deficient-in one galectin. For some of these

functions, additional information supporting either an extracellular or intracellular action is provided.

Cells of innate immunity

Either in an extracellular or intracellular fashion, galectins can affect a variety of biological events of various cells participating in innate immunity. These include their adhesion and transmigration through endothelial cell surfaces; their ability to recognize, engulf, and kill intruders and damaged cells; and their capacity to produce pro- and anti-inflammatory cytokines and respond to chemotactic gradients.

Neutrophils

Galectin-1. In spite of significant advances in elucidating the role of galectin-1 within the T- and B-cell compartments (see below), the effects of this protein toward cells of innate immunity have not been studied in such detail. *In vitro*, neutrophils exposed to recombinant galectin-1 experience impaired chemotaxis and diminished capture, rolling, and adhesion on activated endothelial monolayers.[4,5] This inhibitory effect has also been demonstrated for the endogenous protein, as knocking down of endothelial galectin-1 using siRNA resulted in a significant increase in the number of extravasating neutrophils,[5] suggesting an essential role of galectin-1 in controlling neutrophil adhesion and trafficking.

Supporting the anti-inflammatory activity of galectin-1, it was shown that galectin-1–neutrophil interaction resulted in exposure of cell-surface phosphatidylserine, a ligand that targets these cells for phagocytic removal.[6–8] This phenomenon was reversible, required Ca^{2+} mobilization, and depended upon specific interactions with cell-surface complex-type N-glycans; however, it did not involve DNA fragmentation, changes in mitochondrial membrane potential, or caspase activation. This suggests an effect of galectin-1 in regulating leukocyte turnover independently of apoptosis.

Galectin-1 has also been shown to have a pro-inflammatory effect on neutrophils. It can activate the enzyme nicotinamide adenine dinucleotide phosphate oxidase and promote superoxide release from neutrophils.[9]

Galectin-3. Recombinant galectin-3 was shown to induce oxidative burst in neutrophils.[10–12] More recently it was found to induce L-selectin shedding and interleukin (IL)-8 production in naive and primed neutrophils. Interestingly, upon activating primed neutrophils, galectin-3 is degraded and inactivated by elastase released by the cells.[13]

Galectin-3 was previously found to promote neutrophil adhesion to extracellular protein laminin.[14] More recently it was found to promote adhesion of neutrophils to endothelial cells. This resulted from cross-linking of neutrophils to the endothelium and was dependent on galectin-3 oligomerization. The authors proposed that galectin-3 plays a role in neutrophil extravasation that occurs during alveolar infection with *Streptococcus pneumoniae*.[15]

Recombinant galectin-3 protects neutrophils from apoptosis.[13] While, as described below, galectin-3 induces both phosphatidylserine exposure and apoptosis in primary activated human T cells, it induces only phosphatidylserine exposure in neutrophils in the absence of cell death.[16] However, other studies suggest that galectin-3 can induce apoptosis in neutrophils as well their activation and granule release.[17]

Galectin-4. Similar to galectin-1, galectin-4 induces phosphatidylserine exposure in activated but not resting human neutrophils.[6]

Galectin-8. Recombinant galectin-8 binds to α_M integrin on neutrophils, enhances their adhesive properties, and induces them to produce superoxide, possibly through binding to this integrin.[18]

Monocytes/macrophages

Galectin-1. In keeping with its anti-inflammatory functions, galectin-1 inhibits arachidonic acid release,[19] blocks nitric oxide synthesis,[20] and increases arginase activity,[20] suggesting a role for this protein in triggering a state of alternative activation in cells of the monocyte/macrophage lineage. Accordingly, galectin-1 treatment inhibited interferon (IFN)-γ-induced FcγRI-dependent (type I IgG Fc receptor) phagocytosis and major histocompatibility complex (MHC) II expression in human monocytes and macrophages[21] and blocked IL-12 secretion from parasite-infected macrophages.[22] The results suggest that galectin-1-glycan lattices may have evolved to negatively regulate the antigen-presenting function and activation of monocytes/macrophages. More recently, studies highlighted a role of galectin-1 in facilitating HIV

infection of human macrophages through stabilization of viral adsorption.[23]

Galectin-3. There is a great deal of information on the function of galectin-3 in macrophages. Galectin-3 can trigger human peripheral blood monocytes to produce superoxide anion[24] and serve as a chemoattractant for these cells.[25] In addition, galectin-3 functions as an opsonin and enhances the macrophage clearance of apoptotic neutrophils.[26] It has also been shown that galectin-3 activates microglia cells, tissue macrophages of the central nervous system, to phagocytose degenerated myelin mediated by complement receptor-3 and scavenger receptor.[27]

Using macrophages from *Lgals3*$^{-/-}$ mice, galectin-3 has been shown to play a critical role in the phagocytic function of macrophages in ingesting opsonized sheep red blood cells and apoptotic thymocytes.[28] The former is mediated by FcγR, while the latter is mediated by receptors recognizing phosphatidylserine. However, other studies did not notice any difference between wild-type and *Lgals3*$^{-/-}$ macrophages with regards to phagocytosis of *Mycobacterium tuberculosis*[29] and *S. pneumonia*.[13] Nevertheless, the latter study, using *S. pneumonia*, did show *Lgals3*$^{-/-}$ cells to be defective in phagocytosis of apoptotic neutrophils. Thus, galectin-3 appears to be involved in the function of a subset of receptors engaged in phagocytosis. Moreover, galectin-3 was found to be translocated to the phagosomes.[28,29]

Recombinant galectin-3 is able to bind directly to a major xenoantigen, α-Gal [Galα(1,3)Galβ(1,4)GlcNAc], that is expressed on porcine endothelial cells.[30] Furthermore, neutralizing anti-galectin-3 antibody suppresses adhesion of human monocytes to porcine endothelial cells. The authors concluded that galectin-3 expressed in human monocytes is a receptor for this xenoantigen.[30]

Macrophages from *Lgals3*$^{-/-}$ mice exhibit reduced IL-4/IL-13-induced alternative macrophage activation *in vitro* compared to those from wild-type mice, but the two genotypes are comparable in IFN-γ/LPS-induced classical activation and IL-10-induced deactivation.[31] This defect was confirmed by using wild-type cells treated with galectin-3-specific siRNA to suppress galectin-3 expression and was noted also *in vivo* in *Lgals3*$^{-/-}$ mice. Furthermore, these authors demonstrated that recombinant galectin-3 was able to induce alternative activation and IL-4-induced alternative activation was blocked by a galectin-3 inhibitor, bis-(3-deoxy-3-(3-methoxybenzamido)-β-D-galactopyranosyl) sulfane.[31] The results suggest that extracellular galectin-3 mediates alternative macrophage activation.

Remarkably, LPS induced higher production of inflammatory cytokines, including IL-6, IL-12, and tumor necrosis factor (TNF)-α in *Lgals3*$^{-/-}$ macrophages compared to wild-type cells. This enhanced response was specifically inhibited by recombinant galectin-3, which binds to LPS.[32] The suppressive effect of endogenous galectin-3 is supported by the fact that a neutralizing antibody as well as the galectin-3 inhibitor lactose enhanced the response in wild-type macrophages. These studies suggest that galectin-3 represses LPS-induced inflammatory cytokine response in macrophages, possibly through directly binding to LPS. However, the authors recognized that intracellular galectin-3 may also contribute to this repressive effect.

Galectin-8. Galectin-8 induces phosphatidylserine exposure but not apoptosis in a human promyelocytic cell line, HL60.[33]

Galectin-9. Galectin-9 induces apoptosis in a monocytic cell line (THP-1) and HL-60.[34] Galectin-9 was shown to be able to activate two transcriptional factors, nuclear factor (NF)-IL6 (C/EBP β) and activator protein 1 (AP-1), and induce transcription of IL-1α, IL-1β, and IFN-γ. Because exogenously added galectin-9 did not induce production of these cytokines, these results suggested that endogenous galectin-9 functions intracellularly. The authors concluded that galectin-9 transactivates inflammatory cytokine genes in monocytes by functioning intracellularly, possibly through direct interaction with NF-IL6.[35] This is the first example of an intracellular function of galectin-9.

Dendritic cells

Galectin-1. While the role of endogenous galectin-1 within the dendritic cell compartment has yet to be established, exposure to recombinant galectin-1 resulted in the maturation of human monocyte-derived dendritic cells with an enhanced migratory profile.[36] Administration of recombinant galectin-1 *in vivo* favored the recruitment of a population of uterine dendritic cells with a regulatory cell-surface phenotype.[37]

Galectin-3. *Lgals3*$^{-/-}$ dendritic cells were found to produce significantly larger amounts of IL-12

than wild-type cells, suggesting a suppressive role of galectin-3 in the production of this cytokine.[38] Because IL-12 is important for promoting helper T (Th)1 response, these results suggest that galectin-3 may suppress Th1 responses through the generation of tolerogenic dendritic cells. This is further supported by the following results: When splenocytes from mice previously sensitized epicutaneously with antigen were re-stimulated with the same antigen *in vitro* (a model to be discussed further below), cells from *Lgals3*$^{-/-}$ mice produced higher levels of IL-12 than those from wild-type cells. When CD4^{+} T cells were exposed to antigen presented by wild-type or *Lgals3*$^{-/-}$ bone marrow-derived dendritic cells *in vitro* followed by CD3 and CD28 ligation, higher levels of IFN-γ, but lower levels of IL-4, were produced from *Lgals3*$^{-/-}$ cells relative to wild-type cells, indicating that *Lgals3*$^{-/-}$ dendritic cells drive a Th1 response.[39] Moreover, CD4^{+} T cells incubated with *Lgals3*$^{-/-}$ dendritic cells had higher rates of proliferation compared to those incubated with wild-type dendritic cells, suggesting that endogenous galectin-3 suppresses the antigen-presenting function of these cells.[40]

Galectin-3 also regulates the migratory pattern of dendritic cells as *Lgals3*$^{-/-}$ dendritic cells showed a lower migratory profile compared to their wild-type counterpart both *in vitro* and *in vivo*.[41] Galectin-3 functions intracellularly as migration is not affected by addition of lactose to the cell culture, which would inhibit the function of extracellular galectin-3. The study also showed the accumulation of galectin-3 on the lipid rafts and membrane ruffles in migrating cells.[41]

Recombinant galectin-3 added to mouse dendritic cells promoted their adhesion.[42] It has been shown that dendritic cell maturation results in pronounced changes in glycan expression, thus affecting recognition by galectins.[43] Therefore, one would expect that the influence of extracellular galectin-3 on dendritic cells is dependent on the maturation status of these cells.

Galectin-9. Galectin-9 was shown to stimulate the maturation of dendritic cells.[44] Galectin-9-maturated dendritic cells secreted IL-12 but not IL-10 and selectively elicited the production of Th1 cytokines by allogeneic CD4^{+} T cells. This effect may not be dependent on the lectin properties of this protein as a galectin-9 mutant lacking β-galactoside-binding activity retains its dendritic cell maturation-promoting activities. In addition, the effect of galectin-9 on dendritic cell maturation was only slightly inhibited by lactose.[44]

Mast cells

Galectin-1. Injection of galectin-1 in a model of phospholipase A2-induced inflammation resulted in reduced mast cell degranulation, although the underlying mechanisms involved in this effect are still uncertain.[19] This inhibitory effect does not seem to be related to an induction of cell death as recombinant galectin-1 is not able to trigger mast cell apoptosis, although galectin-3 is (see below).[45]

Galectin-3. Recombinant galectin-3 can induce mediator release from both IgE-sensitized and nonsensitized mast cells.[46,47] Interestingly, while galectin-3 was originally identified as a protein capable of binding to IgE (hence the designation IgE-binding protein), it also binds to IgE receptor (FcεRI).[46] It is thus possible that galectin-3 activates mast cells through cross-linking FcεRI-bound IgE, FcεRI, or both.

Prolonged treatment of mast cells with recombinant galectin-3 (18–44 h) resulted in apoptosis, and this effect involves caspase-3.[45] Galectin-3-induced apoptosis was abolished by agents that neutralize reactive oxygen species, such as dithiothreitol and superoxide dismutase, and galectin-3 induced the release of superoxide anion. Together, these results indicate that galectin-3-induced apoptosis involves oxidative stress and thiol oxidation. The authors also obtained evidence for the role of opening of the mitochondrial permeability transition pore in galectin-3 apoptotic effects. This effect involves the participation of RAGE (receptor for advanced glycosylation end products), as it was substantially inhibited by an anti-RAGE antibody. They thus concluded that galectin-3 exerts its pro-apoptotic activity through binding to RAGE.[45]

The function of endogenous galectin-3 in the mast cell response has been established by studying *Lgals3*$^{-/-}$ mice. *Lgals3*$^{-/-}$ mast cells exhibited lower degranulation and decreased cytokine production compared to wild-type cells when activated by cross-linkage of IgE receptor. The positive regulatory role of galectin-3 in mast cell response was supported by *in vivo* data: *Lgals3*$^{-/-}$ mice exhibited diminished IgE-mediated passive cutaneous anaphylactic reactions.[48]

Eosinophils

Galectin-3. Galectin-3 expressed on the surface of eosinophils has been shown to mediate IgE-dependent activation of this cell type.[49] Galectin-3 inhibits IL-5 production by human eosinophils both at the mRNA and protein levels.[50] Subsequent studies established a role for FcγRII in galectin-3-induced inhibition of IL-5 synthesis.[51]

Eosinophils from allergic donors were found to have higher levels of galectin-3 and significantly increased rolling and firm adhesion on immobilized vascular cell adhesion molecule 1 under conditions of flow. This effect was inhibited by specific monoclonal antibody and lactose and found to be mediated by engagement of α4 integrin on eosinophils.[52]

Galectin-9. Human galectin-9 was originally identified as a potent eosinophil chemoattractant.[53,54] Paradoxically, it was shown to significantly suppress apoptosis in eosinophils from eosinophilic patients but enhance apoptosis in those from healthy subjects.[55] Remarkably, eosinophils were found to adhere to the fibroblast cell line HFL-1 stimulated by IFN-γ , and the adhesion was inhibitable by both lactose and anti-galectin-9 antibody.[56]

Cells of adaptive immunity

Effector T cells

Galectin-1. Galectin-1 is present at sites of immunological synapse both in primary and secondary lymphoid organs and interferes with early T-cell signaling processes.[57–60] While in the thymus, expression of galectin-1 by thymic epithelial cells can shape the T-cell repertoire by differentially modulating positive or negative selection as it induces rapid and transient extracellular signal-regulated kinase (ERK) activation during negative selection but antagonizes ERK activity in thymocytes undergoing positive selection.[57] Once in secondary lymphoid organs, galectin-1 regulates the T-cell fate by modulating T-cell receptor (TCR)/co-stimulator-dependent clustering and signaling, thus defining appropriate T-cell activation thresholds.[58]

Undoubtedly, one of the most consistent observations in the literature is the ability of galectin-1 to blunt Th1- and Th17-mediated responses and skew the balance toward a Th2-polarized cytokine profile (see below). *In vitro* treatment of activated T cells with recombinant galectin-1 resulted in selective suppression of Th1-type cytokines, including IFN-γ, TNF, and IL-2, and enhanced secretion of Th2 cytokines, including IL-4, IL-5, IL-10, and IL-13.[16,61–66] In this regard, several reports demonstrated a strong induction of IL-10 in nonactivated and activated $CD4^+$ and $CD8^+$ T cells upon *in vitro* exposure to recombinant galectin-1[16,63] and following its administration *in vivo*.[37,67,68] Remarkably, recent findings indicate that Th1 cells may sustain TCR-induced Th2 cytokine production through secretion of galectin-1.[64]

In addition, galectin-1 interferes with T-cell trafficking by blocking T-cell adhesion to extracellular matrix[61] or by suppressing T-cell transendothelial migration through mechanisms involving CD43 clustering.[69] Using siRNA-mediated silencing strategies, it was found that galectin-1 limited T-cell capture, rolling, and adhesion to activated endothelial cells under flow.[70]

Through cross-linking specific glycoconjugates and forming ordered lattices on the T-cell surface, galectin-1 can regulate the viability of different T-cell subsets, particularly those imprinted with a "pro-inflammatory signature," which are endowed with a distinct glycosylation profile.[62,65,68,71–85]

Remarkably, T-cell susceptibility to galectin-1-induced cell death may be influenced by the expression of cell-surface glycoprotein receptors (such as CD45, CD43, CD2, and CD7[76,81,86,87]), the activity of a set of glycosyltransferases creating or masking specific cell-surface glycans,[84,86,88,89] and the activation of particular intracellular pathways (e.g., expression of Bcl-2 and the AP-1 transcription factor, activation of executor caspases and sphingomyelinase-mediated release of ceramide).[74,78,79,85]

Importantly, galectin-1-induced cell death requires the presence in the cell of intact activity of the core 2 β-1–6 *N*-acetylglucosaminyltransferase (GCNT1). This enzyme is responsible for creating the core 2 branch on *O*-glycans and allowing the exposure of poly-N-acetyllactosamine sequences through which galectin-1 binds and signals.[86,90] Moreover, the increased expression of the α2–6-sialyltransferase (ST6Gal1), an enzyme responsible for the addition of sialic acid in the α2–6 position of terminal galactose, may determine T-cell resistance to galectin-1 (Ref. 88).

Interestingly, the preferential expression of galectin-1 in effector but not resting T cells[91,92] suggests a potential autocrine regulatory mechanism by which this lectin may blunt T-cell responses after the

completion of an immune response. This was supported by a link among differential glycosylation of helper T cells, susceptibility to galectin-1-induced cell death, and termination of the inflammatory response.[84] While Th1- and Th17-differentiated cells express the repertoire of cell-surface glycans that are critical for galectin-1 binding and cell death, Th2 cells are protected from galectin-1 through differential α2–6 sialylation of cell-surface glycoproteins.[84] This selective pro-apoptotic effect may provide a rational explanation for the Th2 bias observed *in vitro* and *in vivo* following galectin-1 treatment. In line with this, it was shown that Th2 cells can promote Th1 cell apoptosis through secretion of galectin-1,[64] suggesting a lectin-dependent mechanism of cross-regulation between distinct helper T subsets. However, naive T cells, which are highly enriched in α2–6-linked sialic acid, are resistant to galectin-1-induced cell death; yet in this case galectin-1 supports T-cell survival without promoting proliferation.[59] Supporting the concept that galectin-1 is a negative autocrine regulator, recent findings showed that expression of this protein by $CD8^+$ T cells controls TCR binding, signal transduction, and burst size.[93]

In addition, it was reported that in the absence of a reducing agent, such as dithiothreitol, which is often used to keep the carbohydrate-binding activity of the protein, galectin-1 does not alter T-cell viability but can still regulate cytokine secretion.[16] This suggests that susceptibility to cell death may rely on extrinsic and intrinsic factors, including the nature of target T cells (e.g., resting, activated, or differentiated T lymphocytes) and the redox state of the microenvironment.

Galectin-3. In early studies, galectin-3 was shown to induce IL-2 production[94] and calcium influx[95] by Jurkat T cells. Subsequently, galectin-3 was found to induce apoptosis in activated T cells as well as T-cell lines.[81,96] In some cell lines, such as MOLT-4 cells, galectin-3 induces only phosphatidylserine exposure, an early event in apoptosis, but it is not followed by cell death.[16] Galectin-3 is more effective in inducing T-cell death than galectin-1 as comparable levels of apoptosis in T-cell lines are accomplishable with 1–3 μM galectin-3 and 20 μM galectin-1.[81] The sensitivity of T cells to lower concentrations of galectin-3 may reflect a greater efficiency of receptor ligation because of the ability of galectin-3 to form pentamers upon binding to multivalent glycans,[97] whereas galectin-1 exists as dimers.[98] Thus, the former may have higher avidity for cell-surface glycoproteins. Galectin-3 also induces apoptosis in mouse $CD4^-CD8^-$ thymocytes, with minor killing of $CD4^+CD8^+$ cells, while galectin-1 kills both populations of thymocytes.[81] Finally, galectin-3 induces apoptosis in both Th1 and Th2 cells, while, as mentioned above, galectin-1 acts selectively on Th1 cells.[84]

With regard to cell-surface receptors mediating the apoptosis-inducing function of galectin-3, one study identified CD7 and CD29,[96] but the other provided evidence for the involvement of CD45.[81] The basis for this difference remains to be clarified. The latter study also used affinity purification and proteomic analysis to identify over 15 cell-surface glycoproteins that are recognized by galectin-3.

In contrast to other galectins, endogenous galectin-3 has been shown to have anti-apoptotic activity. This was first shown in the human T-cell line Jurkat in which ectopic galectin-3 expression confers resistance to apoptosis induced by anti-Fas receptor antibody and staurosporine.[99] The current model is endogenous galectin-3 functions intracellularly and sustains cell survival by maintaining mitochondrial membrane potential[100] and delaying cytochrome *c* release.[101] Moreover, in primary T cells, endogenous galectin-3 was found to be necessary for IL-2-dependent cell growth as down-regulation of galectin-3 by treatment with a galectin-3-specific antisense oligonucleotide resulted in impaired cell growth.[102]

The glycosyltransferase β1–6 *N*-acetylglucosaminyltransferase V (GlcNAc-TV or Mgat5) catalyzes generation of branched glycans with N-acetyllactosamine groups, which are suitable ligands for galectin-3. Interestingly, T cells from Mgat5-deficient ($Mgat5^{-/-}$) mice were found to have significantly decreased thresholds for TCR activation induced by a number of stimuli compared to those from wild-type mice. The authors suggested that one mechanism by which this phenotype could arise is increased lateral motility of the TCR.[103] They further proposed that galectin-3 binds to N-acetyllactosamine groups and forms lattices with TCR, thus restricting the signals initiated from the latter. This is supported by the finding that wild-type T cells became hyper-responsive after treatment with lactose, which is capable of removing galectin-3 from the cell surfaces.[103]

A similar TCR restriction caused by galectin-3 has been demonstrated in $CD8^+$ tumor-infiltrating lymphocytes in which evidence was provided for direct interactions of galectin-3 with TCR. The authors demonstrated that galectin-3 caused separation of CD8 and TCR, thus making these cells anergic.[104]

Galectin-4. While some studies found that galectin-4 stimulates $CD4^+$ T cells to produce IL-6 and contributes to the development of inflammatory bowel disease,[105] other observations showed that galectin-4 induces apoptosis of mucosal T cells and promotes resolution of the inflammatory disease.[106] Which function prevails *in vivo* still remains to be explored in galectin-4-deficient mice.

Galectin-8. Galectin-8 was found to induce apoptosis in Jurkat T cells through activating the complex phospholipase-D/phosphatidic acid signaling pathway, which results in activation of ERK1/2 and type 4 phosphodiesterases.[107] ERK1/2 activation subsequently leads to expression of the death factor Fas ligand (FasL) that triggers apoptosis. Galectin-8 also induces apoptosis in human peripheral mononuclear cells activated by anti-CD3 and anti-CD28 (Ref. 107). Another research group showed that galectin-8 induced apoptosis in the $CD4^{high}CD8^{high}$ thymocytes through activation of caspases.[108] Galectin-8 also modulates the adhesive properties of T cells through specific binding to α4 integrins.[109]

Galectin-9. An early study found that recombinant galectin-9 induced apoptosis in thymocytes.[110] Subsequent studies demonstrated that it also induced apoptosis in Jurkat T cells and human peripheral blood $CD4^+$ and $CD8^+$ T cells, especially if these cells were activated.[34] Galectin-9 induces apoptosis via activation of caspase-1 but not caspase-8, 9, and 10 (Ref. 34). Other investigators demonstrated that galectin-9 induced apoptosis in Th1 cells but not Th2 cells and it does so through interaction with the Th1-specific cell-surface molecule T-cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3).[111]

Regulatory T cells

Galectin-1. In vitro exposure of T cells to galectin-1 resulted in considerable expansion of a population of $CD4^+CD25^{high}$ T regulatory (Treg) cells with high FOXP3 expression.[65] Interestingly, analysis of gene expression profiles of regulatory versus effector T cells revealed a substantial increase in *Lgals1*, the gene encoding galectin-1, in naturally-occurring Treg cells.[112,113] Importantly, antibody-mediated blockade of galectin-1 substantially reduced the suppressive effects of human and mouse $CD4^+CD25^+$ Treg cells, indicating that endogenous galectin-1 is required for maximal Treg cell function.[113] In addition, recent evidence revealed a critical role of the ganglioside GM1 as a primary target of galectin-1 expressed by Treg cells. Cross-linking of GM1 resulted in activation of the canonical transient receptor potential channel and suppression of autoimmune neuroinflammation.[114]

Galectin-10. By proteomic analysis, CD4+ CD25hi human Treg cells cells were found to express galectin-10. Additional results indicate that this protein primarily resides intracellularly and is essential for the suppressive function of Treg cells.[115]

B cells

Galectin-1. Research over the past few years has demonstrated key roles for galectin-1 during B-cell development, differentiation, and survival.[116–121] Within the bone marrow compartment, galectin-1 contributes to the formation of immune developmental synapse between human pre-B and stromal cells through binding to $\alpha_4\beta_1$, $\alpha_5\beta_1$, and $\alpha_4\beta_7$ integrins.[117,118] This effect was clearly verified in *Lgals1*$^{-/-}$ mice, which showed an arrest in B-cell development in pre-BII cell stage.[119] Once in the periphery, galectin-1 is upregulated by activation signals[116] and contributes to differentiation of activated B cells into antibody-secreting plasma cells.[120] Moreover, recent work demonstrated that enforced expression of galectin-1 can facilitate death of memory B cells,[121] thus confirming the role of this protein in favoring the plasma cell phenotype.

Galectin-3. When galectin-3 expression is suppressed by antisense oligonucleotides, B cells tend to differentiate into plasma cells rather than memory cells. Moreover, in these cells, IL-4 treatment did not result in a downregulation of the transcription factor Blimp-1 as it would normally in wild-type cells.[122] The results suggest that galectin-3 contributes to IL-4-induced downregulation of Blimp-1, which is essential for the development of memory cells. In addition, expression of galectin-3 results in less apoptosis in B-cell lymphoma cell lines,[123] but whether endogenous galectin-3 also inhibits apoptosis in primary B cells is not known.

Galectin-9. Recombinant galectin-9 also induces apoptosis in a human B-cell line.[124]

General comments

A plethora of functions have been demonstrated for various galectins in different cell types. It is probably safe to state that some responses will be induced when recombinant protein of any galectin is added to any of the immune cell types. The responses, which appear to be different for individual family members, range from apoptosis, cytokine production, reactive oxidative species production, and modulation of cell adhesion.

This type of function distinguishes galectins from cytokines. Each cytokine has its individual receptor, which in general has restrictive cellular distributions. Accordingly, cytokines usually act on selected cell types that express specific receptors. Galectins, in contrast, do not have specific individual receptors. They instead bind to an array of cell-surface glycoproteins and glycolipids that carry N-acetyllactosamine-containing oligosaccharides. Galectin-3, for example, has been shown to bind and to over 15 different known cell-surface glycoproteins on T cells[81] as well as a number of different glycoproteins on neutrophils[10] and mast cells.[46]

Because individual galectins have different carbohydrate specificity, it is conceivable that they bind to different sets of glycoproteins on any given cell. Indeed, glycoproteins recognized by galectin-1 and 3 on the surface of T cells[81] and neutrophils[9] are different. However, some proteins, such as CD45, are recognized by both galectins.

Because galectins bind to a large number of different glycoproteins, a challenge in the investigation of the functions of galectins is to determine which protein(s) mediates the functions exerted by a given galectin. Moreover, one galectin's binding to a glycoprotein does not mean that it functions through that glycoprotein; such relationship has to be demonstrated by using specific neutralizing antibodies against that glycoprotein or employing a cell line deficient in it.

Another feature that distinguishes galectins from cytokines (with the exception of IL-1) is the lack of a classical signal sequence in galectins. This, together with the fact that most of the functions demonstrated by using recombinant galectins require the use of micromolar concentrations of the proteins, makes it a challenge to determine whether the demonstrated functions are operative under physiological or pathological conditions. However, galectins are indeed found in cell culture supernatants and extracellular biologic fluid. In addition, it has been shown that a significantly lower amount of protein can be effective when presented by extracellular matrix.[125] Thus, various demonstrated functions may indeed be operative under appropriate conditions *in vivo*.

Some of the functions demonstrated *in vitro* have been validated by using cells from mice deficient in a given galectin or cells treated with specific siRNA or antisense oligonucleotides. However, in some of these studies it remains to be established whether the observed phenotypes are a result of the endogenous protein functioning intracellularly or extracellularly. Other studies used co-cultures of two different cell types and demonstrated the action of a galectin secreted from one cell type on another cell type. This approach, in conjunction with the use of neutralizing antibodies to support the action of the lectin being extracellular, is very convincing as these antibodies are not expected to penetrate inside the cells. Other studies similarly used small molecule inhibitors for the same purpose, yet it is not clear whether these inhibitors target the intracellular or extracellular galectins.

An intriguing development is the association of galectins with exosomes, which are small vesicles secreted by various cell types resulting from fusion of multivesicular bodies with the plasma membrane[126] and that contribute to various physiological processes. Galectin-3 has been found to be associated with exosomes derived from dendritic cells,[127] and galectin-9 has been found to be associated with exosomes produced by cancer cells.[128,129] Continued studies might establish that galectins carried by exosomes are the major functional species both *in vitro* and *in vivo*.

The data are growing to support the existence of intracellular functions of galectins. These are demonstrated mostly with the use of transfectants overexpressing a given galectin, cell lines with the expression of a given galectin suppressed by siRNA or antisense oligonucleotides, or cells from mice deficient in a specific galectin. In some studies the conclusion that galectin functions intracellularly is based on the demonstration of the intracellular

location of the protein and the fact that the demonstrated phenotypes are not inhibited by adding specific inhibitors to the extracellular space. A current challenge is the demonstration of the mechanism involved in these intracellular functions. In this regard it is promising that galectins have been shown to bind to a number of intracellular proteins known to play important regulatory roles in various cellular processes.

Interestingly, a majority of the intracellular functions shown are independent of carbohydrate binding. Glycoconjugates (including glycoproteins and glycolipids) are present inside the cells, mostly being en route to the external side of the cells, and many of these are recognizable by galectins. An intriguing possibility exists that they are in fact recognized by galectins inside the cells. However, these glycoconjugates are compartmentalized in vesicles, including endoplasmic reticulum, Golgi, and post-Golgi network vesicles, while galectins are located primarily in the cytosol. Thus, for galectins to recognize these glycoconjugates they need to gain access to these vesicles or these glycoconjugates need to be at least transiently present in the cytosol.

Nevertheless, the possibility that galectins may function by recognizing glycans intracellularly has begun to emerge. Galectin-3 was demonstrated to reside inside post-Golgi network vesicles that carry glycoproteins destined for export to the apical site of polarized epithelial cells. Blockade of galectin-3 results in mis-sorting of apical glycoproteins into the basolateral sides of epithelial cells.[130] Subsequently, the authors observed architectural defects in intestinal enterocytes from *Lgals3*$^{-/-}$ animals resulting from misdirection of membrane glycoproteins.[131] Thus, galectin-3, and possibly other galectins, may be critical for the intracellular transport of selected glycoproteins. How galectins exert this intracellular function and whether it involves their binding to glycoproteins remain to be addressed.

Functions of galectins in inflammation demonstrated *in vivo*

Acute inflammation

Galectin-1. The inhibitory effects of galectin-1 in neutrophil and macrophage functions have been also demonstrated *in vivo*. In a model of acute edema, injection of recombinant galectin-1 in Wistar rats suppressed bee venom phospholipase A_2-induced inflammation, which was correlated with diminished number of extravasating neutrophils and impaired mast cell degranulation.[19] Supporting these findings, galectin-1 administration resulted in reduced IL-1β-induced neutrophil recruitment into the mouse peritoneal cavity,[5] suggesting an essential role of galectin-1 in limiting acute inflammation by controlling neutrophil trafficking and extravasation.

In addition, peritoneal macrophages from *Lgals1*$^{-/-}$ mice displayed increased MHC II expression and allostimulatory capacity when recruited *in vivo* in response to inflammatory stimuli.[21] These broad anti-inflammatory activities of galectin-1 are apparently in contrast with its ability to trigger platelet activation,[132] although the effects of endogenous galectin-1 in platelet physiology still remain to be examined in the *in vivo* setting. Thus, the overall function of endogenous galectin-1 during the initiation and resolution of acute inflammation will result from the balance of different biological effects and remains to be investigated in null-mutant mice.

Galectin-3. Studies of *Lgals3*$^{-/-}$ mice have suggested a pro-inflammatory role of galectin-3 in acute inflammation. When treated with intraperitoneal injection of thioglycollate broth, *Lgals3*$^{-/-}$ mice exhibited a significantly reduced number of macrophages[133] and neutrophils[134] in the peritoneal cavity compared to wild-type mice. In addition, inflammatory cells from *Lgals3*$^{-/-}$ mice showed significantly reduced levels of NF-κB activation.[133] The role of galectins in acute inflammation and their impact on innate immune cells are illustrated in Figure 2.

Allergic inflammation

The role of galectins during allergic inflammation is illustrated in Figure 3.

Galectin-2. Recent findings demonstrated a role for galectin-2 in suppressing contact hypersensitivity induced by the hapten 2,4-dinitro-1-fluorobenzene.[135] Treatment with galectin-2 *in vivo* suppressed contact hypersensitivity by inducing apoptosis in activated $CD8^+$ T cells through binding to CD29 on the surface of these cells.[135]

Galectin-3. In a mouse model of asthma, in which mice are sensitized with ovalbumin systemically and then challenged with the same antigen through the airways, *Lgals3*$^{-/-}$ mice developed

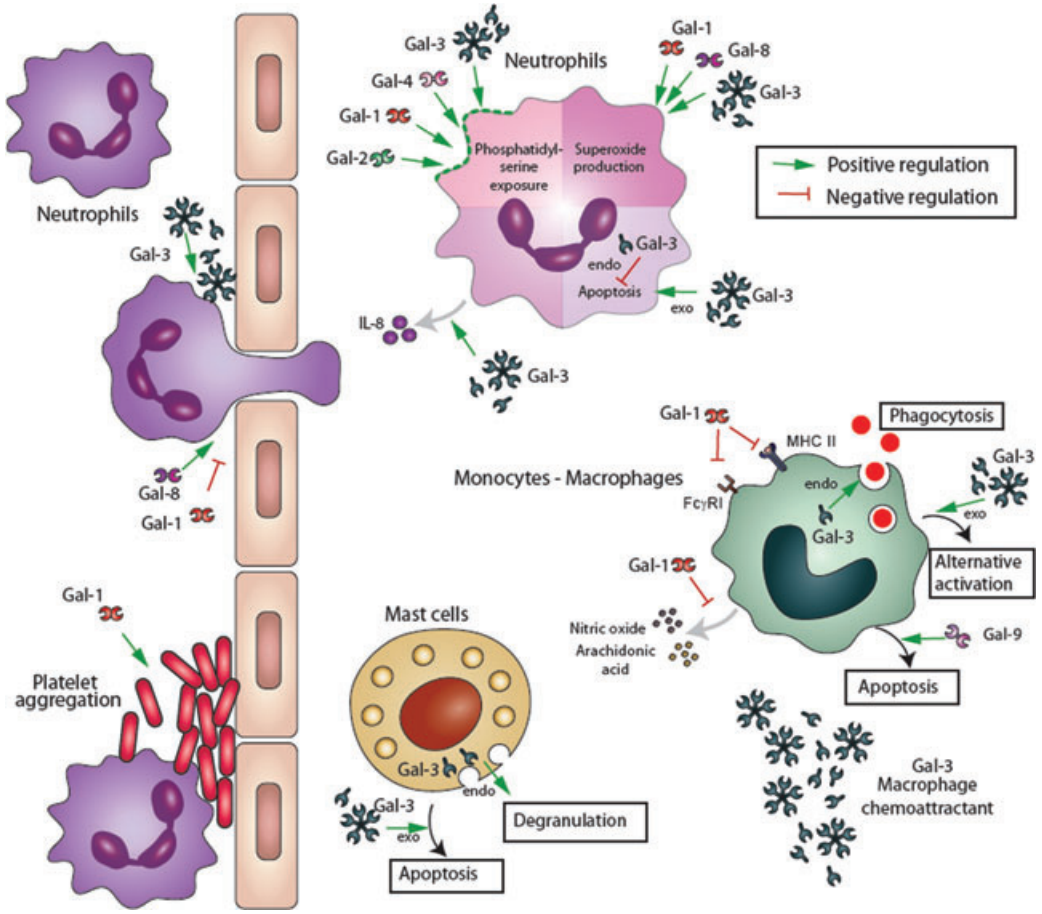

Figure 2. Galectins in acute inflammation and innate immunity. Galectins can affect a variety of biologic events of various cells participating in innate immunity and acute inflammation either in an extracellular or intracellular fashion. The functions illustrated here have been demonstrated by *in vitro* exposure to recombinant galectins, targeted delivery of galectins *in vivo*, or following challenge of galectin-deficient mice. While galectin-1 inhibits neutrophil adhesion, rolling, and extravasation, galectin-3 favors neutrophil adhesion to endothelia through its oligomerization processes. Galectins also affect the function of neutrophils by facilitating phosphatidylserine exposure without inducing apoptosis, promoting apoptosis, and regulating superoxide and IL-8 production. In addition, galectins can affect the functional fate of monocytes/macrophage by skewing the balance toward alternative versus classical activation, controlling nitric oxide production, and regulating inducible Fcγ receptor-mediated phagocytosis and major histocompatibility complex (MHC) II-dependent antigen presentation. Further, galectin-3 contributes to monocyte/macrophage chemotaxis and phagocytosis. Emerging evidence also indicates a role for galectins in the control of mast cell function. Endogenous and exogenous galectin-3 promotes mast cell activation and degranulation, although recent studies indicate that, under certain circumstances, galectin-3 but not galectin-1 favors mast cell apoptosis. However, in *vivo* gathered indirect evidence indicates that galectin-1 blocks mast cell degranulation through still unknown mechanisms. Thus, galectins modulate innate immune reactions through a plethora of mechanisms, including the control of adhesion and transmigration through endothelial cell surfaces; the ability to recognize, engulf, and kill intruders and damaged cells; and the capacity to produce pro- and anti-inflammatory cytokines and respond to chemotactic gradients. *Green arrows* indicate positive responses, and *red arrows* indicate inhibitory actions. "Endo" indicates an endogenous function of the protein demonstrated in galectin-deficient mice or following small interfering (si)RNA-mediated silencing or antibody-mediated blockade. "Exo" indicates an exogenous function demonstrated using the recombinant galectin. Abbreviations: Gal, galectin; FcγRI, type I receptor of Fc of immunoglobulin G.

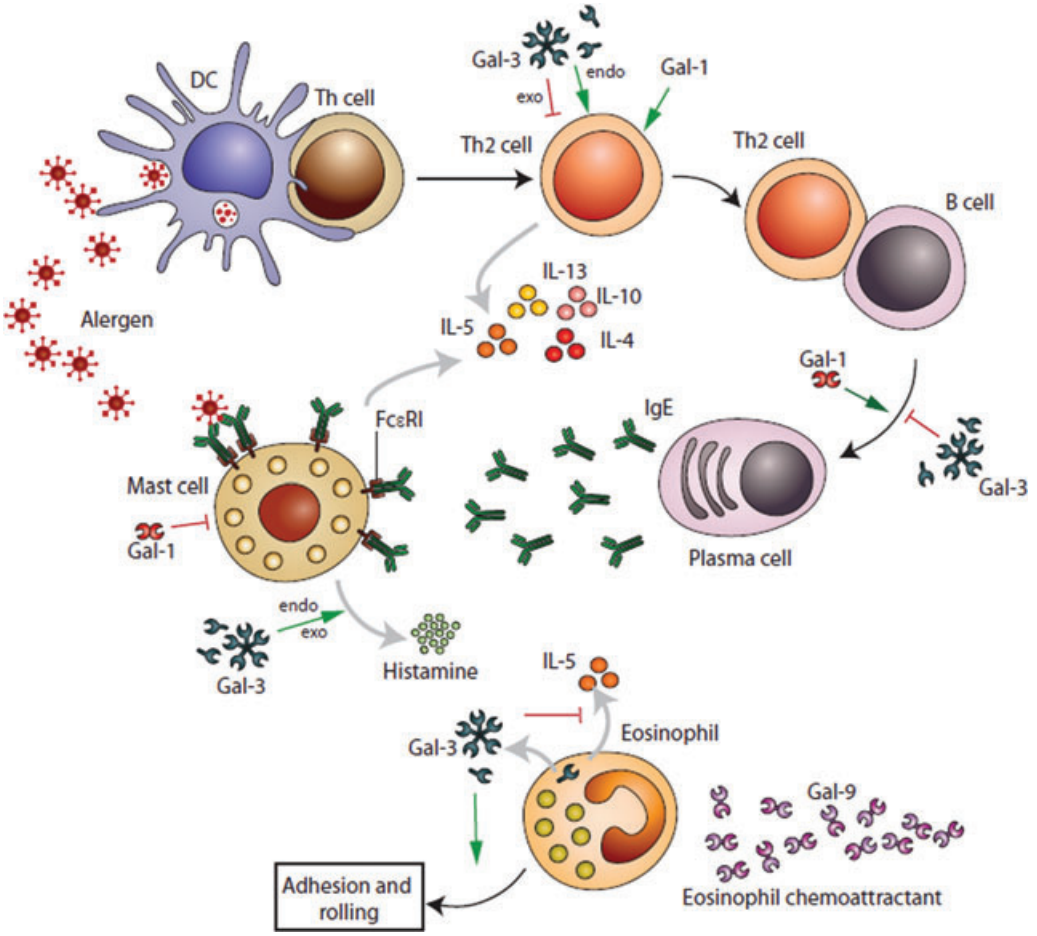

Figure 3. Galectins in allergic inflammation. Many key components of innate and adaptive immunity, including T helper (Th)2 cells, B cells, mast cells, and eosinophils, contribute to the development of allergic reactions. Galectins may affect the Th2 cell compartment through modulation of cytokine production. While endogenous galectin-3 promotes Th2 development, as shown by the Th1 skewing in *Lgals3*$^{-/-}$ mice subjected to allergenic challenge, exogenous galectin-3 silences Th2 responses by blocking IL-5 production, as demonstrated by delivery of a plasmid encoding galectin-3. On the other hand, Th2 cells are resistant to galectin-1-induced cell death, but this protein can enhance secretion of Th2-type cytokines, including IL-4, IL-5, IL-10, and IL-13. Galectins can also affect the B-cell compartment. While galectin-1 promotes the differentiation of B cells into a plasma cell phenotype, endogenous galectin-3 favors the differentiation into a memory B-cell phenotype. Of note, galectin-3 regulates mast cell function control of activation, degranulation, and survival. Also, galectin-3 promotes adhesion and rolling of eosinophils, while galectin-9 can act as an eosinophil chemoattractant. Abbreviations: DC, dendritic cell.

less severe allergic airway inflammation and airway hyper-responsiveness compared to wild-type mice.[136] *Lgals3*$^{-/-}$ mice also developed lower serum levels of IgE and lower levels of IgE and IL-4 but higher levels of IFN-γ in bronchioalveolar lavage fluid. These data suggest that galectin-3 promotes a Th2 response and its absence results in a Th1-polarized response and less severe disease.

In a model of atopic dermatitis in which mice are repeatedly sensitized with ovalbumin epicutaneously, *Lgals3*$^{-/-}$ mice developed reduced disease severity at antigen-sensitized sites; the epidermis was thinner and eosinophil infiltrations were lower, relative to wild type. Remarkably, this effect was associated with lower IL-4 but higher IFN-γ synthesis.[39] In addition, sera from *Lgals3*$^{-/-}$ mice

contained lower levels of IgE but higher ratios of IgG2a/IgG1 than those from wild-type mice. These results further suggest that endogenous galectin-3 is critical for promoting the Th2 response.

The role of galectin-3 within the T-cell compartment was studied by transferring to wild-type mice ovalbumin-sensitized $CD4^+$ T cells from wild-type or *Lgals3*$^{-/-}$ mice in which all T cells carry ovalbumin-specific TCR, and then epicutaneously treating the recipients with ovalbumin. Notably, mice receiving *Lgals3*$^{-/-}$ T cells exhibited lower allergic skin inflammation and a Th1-polarized response compared to those receiving wild-type cells.[39] These data imply a role for galectin-3 in driving allergic skin inflammation and Th2 responses, at least in part, through the control of T cells.

The above studies suggest that galectin-3 might be considered a therapeutic target for treatment of atopic asthma and atopic dermatitis. However, studies by other investigators showed reduction of eosinophil infiltration following airway antigen challenge in rats and mice treated by intranasal delivery of cDNA encoding galectin-3.[137,138] It thus appears that exogenously added galectin-3 may not exactly reproduce the function of endogenous galectin-3, probably because of the differences in the tissues and cells in which the protein is expressed and in the intracellular versus extracellular modes of action. Nevertheless, these studies demonstrate that recombinant galectin-3 and galectin-3 plasmid may also be used for the treatment of allergic inflammation.

Finally, in a mouse model of contact hypersensitivity in which mice are sensitized with the hapten oxazalone and then challenged with the same hapten at another skin site, *Lgals3*$^{-/-}$ mice developed a less severe response.[39] Thus, galectin-3 also promotes delayed type hypersensitivity, which is probably attributable to its positive regulation of dendritic cell migration, as mentioned above.

Galectin-9. Administration of recombinant galectin-9 in a mouse model of asthma was found to reduce Th2-associated airway inflammation and airway hyper-responsiveness.[139] This suggests a suppressive effect of galectin-9 in allergic airway inflammation and the therapeutic potential of the recombinant protein in treatment of human asthma. These authors further demonstrated that administration of galectin-9 inhibits the infiltration of Th2 cells into the airways through binding to the adhesion molecule CD44 on T cells and inhibiting its interaction with hyaluronan, which is necessary for T-cell trafficking in the inflammatory response.

In a mouse experimental allergic conjunctivitis, a Th2-mediated disease that is induced by active systemic immunization with ragweed (RW) followed by RW challenge as eye drops, treatment with anti-galectin-9 antibody did not significantly affect the severity of conjunctivitis. However, the role of galectin-9 was revealed in an adoptive transfer model in which RW-primed splenocytes were re-stimulated *in vitro* with RW and then transferred to recipient mice followed by challenge with RW in the eyes. The group that received cells re-stimulated in the presence of anti-galectin-9 antibody developed less severe conjunctivitis and produced significantly less IL-5 and IL-13 and more IFN-γ compared to those re-stimulated in the presence of control IgG.[140] Thus, galectin-9 may modulate the threshold of activation of splenocytes.

Autoimmunity and other chronic inflammatory disorders

The involvement of galectins in autoimmune inflammation is illustrated in Figure 4.

Galectin-1. Given its broad spectrum of anti-inflammatory effects, galectin-1 has been postulated as an attractive immunosuppressive agent to restore immune cell homeostasis in autoimmune and inflammatory settings.[3] In fact, gene or protein delivery of galectin-1 suppressed the clinical and histopathological signs of inflammation in several animal models, including experimental autoimmune myasthenia gravis,[141] experimental autoimmune encephalomyelitis (EAE),[84,142] collagen-induced arthritis,[143] concanavalin A-induced hepatitis,[144] inflammatory bowel disease,[145] graft versus host disease,[146] and experimental autoimmune uveitis[67] as well as experimental and spontaneous diabetes.[68,147]

Although the molecular mechanisms underlying these immunoregulatory effects were not explored in earlier studies, a careful examination of galectin-1-treated mice revealed a dramatic effect of this protein in blunting Th1 and Th17 pro-inflammatory responses and skewing the cytokine balance toward a Th2-polarized profile.[67,68,84,143–147]

In addition, treatment with recombinant galectin-1 late during the course of retinal inflammation ameliorated disease by promoting Treg

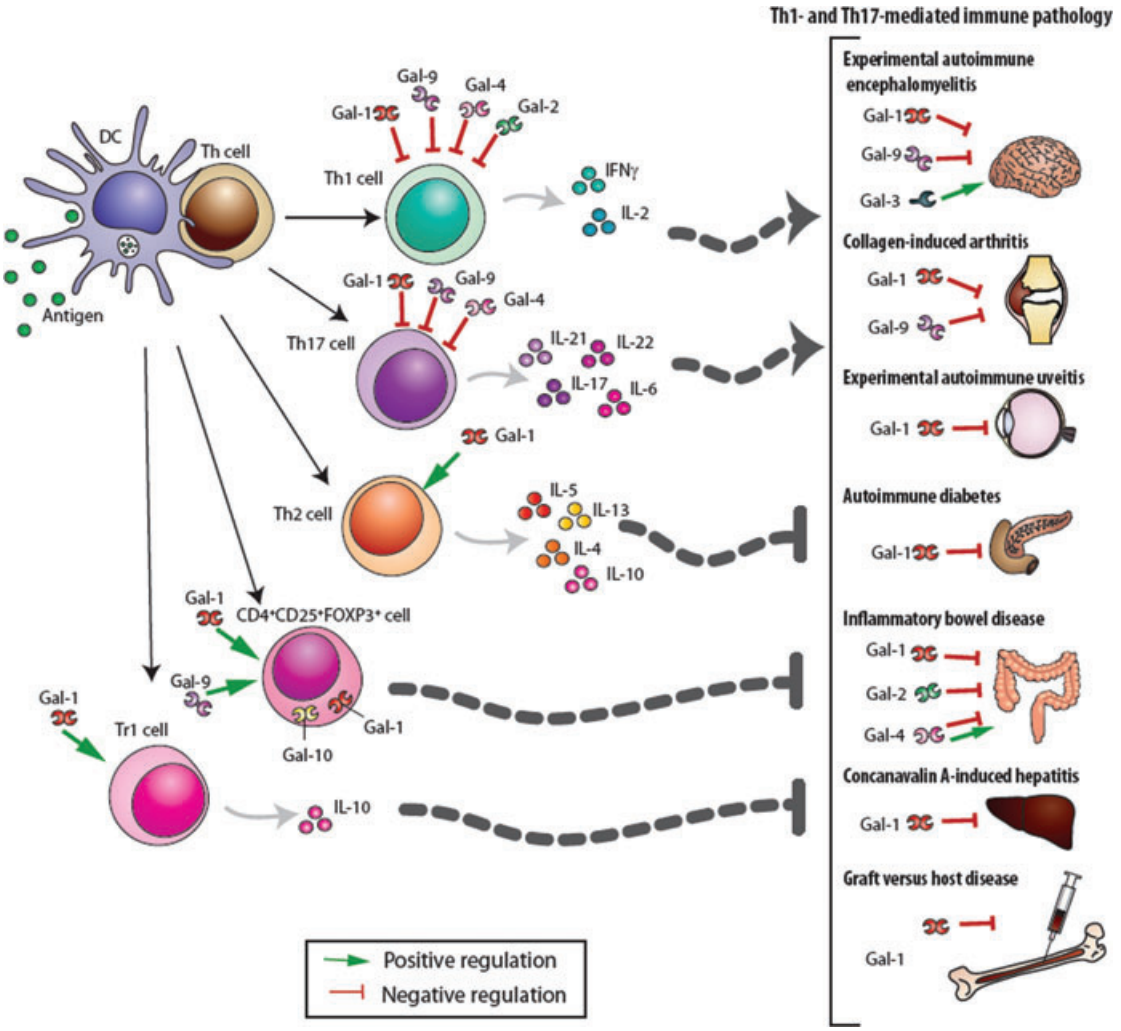

Figure 4. Galectins in autoimmune inflammation. Galectins regulate a broad range of T-cell processes, including T-cell signaling, activation, apoptosis, cytokine secretion, and T regulatory (Treg) cell expansion, which may act in concert to control the development of autoimmune disorders. In this context, targeted delivery of galectins, either by gene or protein therapy, control T-cell-mediated autoimmune and chronic inflammatory processes in several animal models, including encephalomyelitis, collagen-induced arthritis, uveitis, diabetes, inflammatory bowel disease, concanavalin A-induced hepatitis, and graft versus host disease. In addition to those functions illustrated in the figure, galectin-3 exerts either an inhibitory or stimulatory effect on diabetes induced by streptozotocin. Whether the protein participates in the process through inflammation remains to be clarified. Galectins can alter the survival and cytokine production by Th1 and Th17 effector cells and the differentiation and/or expansion of $CD4^+CD25^+FOXP3^+$ regulatory T cells (Treg cells) or IL-10-producing $FOXP3^-$ regulatory T cells (Tr1 cells), thus modulating the balance between effector and regulatory T cells in autoimmune settings. In addition, galectin-1 and galectin-10 contribute to the immunosuppressive capacity of $CD4^+CD25^+FOXP3^+$ Treg cells.

cell-mediated anti-inflammatory responses.[67] Adoptive transfer of IL-10-producing $CD4^+$ T cells obtained from galectin-1-treated mice prevented the development of uveitogenic responses in syngeneic recipients.[67] Interestingly, transferred cells did not show substantial expression of the FOXP3 transcription factor, suggesting that galectin-1 treatment *in vivo* promotes the expansion of

type 1 Treg cells (these cells, termed *Tr1 cells*, produce IL-10 but do not express FOXP3). In more recent studies, injection of soluble galectin-1 could prevent the onset of hyperglycemia, revert β-cell-specific autoimmunity, and dampen Th1 and Th17 responses in non-obese diabetic (NOD) mice,[68] indicating the ability of this protein to suppress autoimmune inflammation not only in experimentally-induced but also in spontaneous models of autoimmune pathology.

In spite of considerable progress, the function of endogenous galectin-1 was lacking until recently when novel findings emerged with the careful analysis of null-mutant mice.[37,84] Consistent with a galectin-1-permissive glycophenotype of Th1 and Th17 cells, *Lgals1*$^{-/-}$ mice showed greater antigen-specific Th1 and Th17 responses and exhibited more severe autoimmune inflammation than their wild-type counterpart, suggesting a critical role of endogenous galectin-1 in controlling T-cell homeostasis. In addition, the effects of galectin-1 in suppressing transendothelial migration have been confirmed in knockout mice where trafficking of T cells to mesenteric lymphoid organs and inflamed tissues was substantially augmented compared to wild-type littermates.[70]

Galectin-3. In an animal model of EAE induced by immunization with a myelin oligodendrocyte glycoprotein peptide, *Lgals3*$^{-/-}$ mice were found to develop significantly less severe disease compared to wild-type mice, including lower infiltrations of macrophages and dendritic cells in neural tissue relative to wild-type mice. Moreover, *Lgals3*$^{-/-}$ mice produced lower levels of pro-inflammatory cytokines in both isolated T cells and neural tissue compared to wild-type littermates. Significantly, higher frequencies of FOXP3^{+} Treg cells were found in the spleen and central nervous system of *Lgals3*$^{-/-}$ mice.[148] The authors concluded that immunosuppressive effects of Th2 cytokines and Treg cells are both responsible for milder disease in *Lgals3*$^{-/-}$ mice.

There are a large number of studies documenting the role of galectin-3 in development of diabetes. When rendered diabetic with streptozotocin, *Lgals3*$^{-/-}$ mice developed accelerated glomerulopathy compared to wild-type mice. This effect was associated with a pronounced accumulation of advanced glycation end products (AGE) in the kidneys.[149] Because galectin-3 is known to bind to AGE and is considered a receptor for these substances, these authors suggested that galectin-3 serves as an AGE receptor and protects the host from AGE-induced tissue injury. This conclusion was supported by these authors' subsequent studies of *Lgals3*$^{-/-}$ mice treated with Nε-(carboxymethyl)lysine-modified or unmodified mouse serum albumin as AGEs.[150] These studies were followed by other findings indicating more pronounced age-dependent changes in *Lgals3*$^{-/-}$ mice compared to age-matched wild-type mice.[151]

Another group studied ischemia and neovascularization in retina in a mouse model of oxygen-induced proliferative retinopathy after perfusion of preformed AGEs. While wild-type mice showed a significant increase in inner retinal ischemia and a reduction in neovascularization compared with untreated controls, such response was not seen in *Lgals3*$^{-/-}$ mice.[152] However, *Lgals3*$^{-/-}$ mice were more resistant to diabetes induced by multiple low doses of streptozotocin.[153] Furthermore, these mice showed significantly reduced expression levels of IFN-γ, TNF-α, and IL-17 in the draining lymph nodes. The authors attributed this, in part, to the function of galectin-3 in macrophages, which are known to play an important role in this model and infiltrate the islets. In fact, macrophages from *Lgals3*$^{-/-}$ mice produced lower amounts of TNF-α and nitric oxide compared to wild-type mice.[153]

Galectin-4. As mentioned above, it was shown that galectin-4 stimulated CD4^{+} T cells to produce IL-6, an effect that was associated with the development of colitis. Administration of a galectin-4 antibody into mice that have developed intestinal inflammation suppressed the severity of the disease.[105] Another group showed that galectin-4 induced apoptosis in activated peripheral and mucosal lamina propria T cells. Furthermore, galectin-4 reduced the secretion of pro-inflammatory cytokines, including IL-17. In a model of experimental colitis, administration of recombinant galectin-4 resulted in lower secretion of pro-inflammatory cytokines, increased apoptosis of mucosal T cells, and lower mucosal inflammation.[106]

Galectin-9. As mentioned above, galectin-9 is capable of inducing selective apoptosis of Th1 cells. The authors confirmed the role of this lectin in controlling the Th1 response *in vivo* by demonstrating that treatment of immunized mice with galectin-9 during the induction phase of EAE resulted

in a decrease in myelin-specific IFN-γ-producing T cells.[111] Furthermore, suppression of galectin-9 expression *in vivo* by using siRNA strategies resulted in lower disease scores during the induction of the disease.[111]

Interestingly, a recombinant galectin-9 variant, which does not contain the linker peptide and is resistant to proteolysis, significantly induced apoptosis in fibroblast-like synoviocytes from rheumatoid arthritis patients and in rheumatoid synovial tissue implanted into severe combined immune deficient (SCID) mice.[154] In addition, recombinant galectin-9 successfully reduced the severity and incidence of the disease in a collagen-induced arthritis model, which was associated with an *in vivo* expansion of Th17 cells and increased frequency of Treg cells.[155] These results suggest the potential use of recombinant galectin-9 in the treatment of rheumatoid arthritis.

Studies in another autoimmune disease model also supported the suppressive role of galectin-9 through its apoptosis-inducing activity. This is nephrotoxic serum nephritis induced in Wistar Kyoto rats by injecting rabbit antiglomerular basement membrane serum, which is characterized by the influx of $CD8^+$ cells into glomerular capillaries in the kidneys. Here administration of recombinant galectin-9 was noted to induce apoptosis in $CD8^+$ cells.[156]

Autoantibodies against a number of galectins, including galectin-1, 3, 8, and 9, have been detected in autoimmune diseases, but their pathological roles are largely unknown.[157] It is possible that these galectins are capable of suppressing autoimmunity by inducing apoptosis in autoreactive T cells and their respective autoantibodies contribute to autoimmunity by neutralizing these effects. In this regard, anti-galectin-8 autoantibodies were detected in the sera from patients with systemic lupus erythematosus, and these antibodies were found to block the apoptotic effect of galectin-8 on T cells.[107,158]

Inflammation related to atherosclerosis and myocardial infarction

Galectin-2. Macrophages of human atherosclerotic lesions express galectin-2, which binds to lymphotoxin-α and amplifies the inflammatory cascade.[159] Interestingly, a single-nucleotide polymorphism in the *Lgals2* gene, which affects its transcription, is associated with increased susceptibility to myocardial infarction, at least in the Japanese population.[159]

Galectin-3. Galectin-3 was found to be expressed in foam cells and macrophages in atherosclerotic lesions.[160] These authors subsequently demonstrated a role of this protein in the development of atherosclerosis by comparing apolipoprotein (Apo)E-deficient mice and ApoE/galectin-3 double-knockout mice. Compared to the former, the latter developed a significantly lower number of atherosclerotic lesions and atheromatous plaques. This was associated with a lower number of perivascular inflammatory infiltrates in the latter.[161] Thus, galectin-3 contributes to atherosclerosis by promoting inflammation.

Immune privilege, tolerance, and inflammation

The ability of galectin-1 to control multiple regulatory checkpoints[162] prompted the investigation of its role in the establishment of immune privilege at the fetomaternal interface. In a mouse model of stress-induced inflammation leading to pregnancy failure, injection of recombinant galectin-1 prevented fetal loss and restored tolerance *in vivo*.[37] Consistently, *Lgals1*$^{-/-}$ female mice showed higher rates of fetal loss compared to their wild-type counterpart in allogeneic but not syngeneic matings.[37] Investigation of the mechanisms underlying these regulatory effects revealed the ability of progesterone-regulated galectin-1 to restore the Th1/Th2 cytokine balance, promote the expansion of IL-10-producing Treg cells, and favor the recruitment of uterine dendritic cells with an immature functional phenotype.[37] In keeping with this is the finding that human decidual natural killer (NK) cells secrete considerable amounts of galectin-1, which induces apoptosis of decidual but not peripheral T cells.[75]

Cancer-associated inflammation

Galectin-1. Studies have found that galectin-1 contributes to the immunosuppressive activity of tumor cells, suggesting a role for this protein in tumor-immune escape.[163] Antisense-mediated blockade of galectin-1 in melanoma cells stimulated the generation of a tumor-specific Th1-type response in tumor-draining lymph nodes, which rendered mice resistant to tumor challenge.[163] Likewise, Reed Sternberg cells, the typical cells in classical Hodgkin lymphoma, selectively overexpress galectin-1 through activation of an AP-1-dependent

enhancer, which favored the secretion of Th2-type cytokines, induced Treg cell expansion, and suppressed Epstein–Barr virus-specific T-cell immunity.[65,66,164]

Additionally, prostate cancer cells, which have low expression of core-2-*O*-glycans, were resistant to killing by galectin-1 but were capable of inducing apoptosis in T cells in co-culture experiments through expression of this protein.[165] Thus, galectin-1 may contribute to delineate the magnitude and quality of the inflammatory infiltrates at sites of tumor growth and metastasis, suggesting its potential use as therapeutic targets in cancer.[166,167]

Galectin-3. The effect of galectin-3 derived from tumor cells on tumor-infiltrating T cells has also been studied.[168] Galectin-3 induces apoptosis in tumor-reactive T cells. In a human melanoma tumor model in immunodeficient mice, treatment of mice harboring tumor with galectin-3 resulted in suppressing the tumor-killing effect of tumor-reactive T cells.[168] Additional studies indicated that galectin-3 expressed by the tumor is insufficient to affect the functionality of tumor-reactive T cells, which is different from the case of galectin-1.

Galectin-9. Administration of recombinant galectin-9 prolonged the survival of Meth-A tumor-bearing mice, and this effect was a result of T-cell-mediated immune responses.[169] While galectin-9 induced apoptosis in $CD4^+$ T cells, it increased both the number of IFN-γ-producing Tim-3^+ $CD8^+$ T cells and the number of Tim-3^+ $CD86^+$ dendritic cells. The induction of such dendritic cell activity was suppressed by Tim-3-Ig fusion protein, suggesting that galectin-9 potentiates $CD8^+$ T-cell-mediated antitumor immunity via interaction between galectin-9 on dendritic cells and Tim-3 on $CD8^+$ T cells.[169]

Adminstration of galectin-9 also considerably prolonged the survival of mice bearing B16F10 melanoma.[170] The authors found that the response was associated with increased numbers of NK cells, $CD8^+$ T cells, and macrophages and proved that the effect of galectin-9 was dependent on these individual cell types. Furthermore, galectin-9 treatment substantially enhanced the cytolytic activity of NK cells; this was not because of galectin-9 acting directly on NK cells but rather functioning through macrophages. They further identified a subpopulation of macrophages with a plasmacytoid cell-like phenotype, which was affected by galectin-9 and conveyed signals for enhancing NK activity.[170]

Infection-related inflammation

There are numerous studies on the role of galectins in host–pathogen interactions and infection. We will discuss here only those that investigated the associated inflammatory responses.

Galectin-1. Although still not demonstrated *in vivo*, galectin-1 treatment enhanced parasite growth in macrophages infected with *Trypanosoma cruzi* by modulating cell survival and IL-12 production.[22]

Galectin-3. *Lgals3*$^{-/-}$ mice were found to be more susceptible to LPS-induced shock associated with excessive induction of inflammatory cytokines and nitric oxide production; however, this strain of mice had greater resistance to *Salmonella* infection. In the same study, the authors found *Lgals3*$^{-/-}$ macrophages had a higher response to LPS.[32] They confirmed that galectin-3 binds to LPS in a carbohydrate-dependent manner and acts as a negative regulator of the LPS response through its ability to sequester this endotoxin.[32]

Previously, galectin-3 was found to accumulate in the alveolar space of *Streptococcus pneumoniae*-infected lungs, and the amount of the accumulated protein strongly correlated with the onset of neutrophil extravasation.[15] Together with *in vitro* data, these results suggest that galectin-3 contributes to extravasation of neutrophils by promoting adhesion to endothelial cells.[15] In a subsequent work, the authors found lower numbers of neutrophils accumulated in the lungs of *Lgals3*$^{-/-}$ mice infected with *S. pneumoniae* compared to wild-type mice. Thus, galectin-3 plays a key role in the recruitment of neutrophils to lungs infected by *S. pneumoniae*.[171] In addition, *Lgals3*$^{-/-}$ mice developed more severe pneumonia after infection with *S. pneumoniae*, as demonstrated by increased bacteriemia and lung damage compared to wild-type mice.[171] Administration of recombinant galectin-3 protected *Lgals3*$^{-/-}$ mice from developing severe pneumonia. The authors also found that recombinant galectin-3 activated neutrophils and enhanced their phagocytosis. They concluded that galectin-3 protects the host from this infection by augmenting the function of neutrophils.[171]

Compared to wild-type mice, *Lgals3*$^{-/-}$ mice developed lower inflammatory response in the intestines, liver, and brain but not in the lungs

when infected by *Toxoplasma gondii*.[38] The brain of *Lgals3*$^{-/-}$ mice displayed a significantly lower number of infiltrating monocytes/macrophages and CD8^{+} T cells but a higher parasite burden. Furthermore, *Lgals3*$^{-/-}$ mice mounted a higher Th1-polarized response. Despite these differences, these mice had comparable survival rates when compared with wild-type mice following perioral *T. gondii* infection, although they were more susceptible to intraperitoneal infection. This study also revealed a tendency of galectin-3 to promote Th2 responses as splenocytes from *Lgals3*$^{-/-}$-infected animals secreted higher levels of IFN-γ and IL-12 than those obtained from wild-type animals.[38] This may be related to the ability of galectin-3 to suppress IL-12 production by dendritic cells. Thus, galectin-3 suppresses Th1 response but promotes the development of an inflammatory response, which is consistent with the number of *in vitro* and *in vivo* findings described above. As a net result, galectin-3 deficiency does not lead to a significant change in the susceptibiity of mice to this parasite.

However, when infected by *Schistosoma mansoni*, *Lgals3*$^{-/-}$ mice developed lower numbers of T and B lymphocytes in the spleen as well as a lower extent of liver granulomas and mounted a Th1-polarized response relative to wild-type mice.[40] As mentioned above, galectin-3 deficiency in dendritic cells resulted in the cells being able to induce a stronger T-cell response. Whether the *in vivo* findings are related to altered dendritic cell function remains to be determined.

On the other hand, *Lgals3*$^{-/-}$ mice were found to be considerably more resistant to the lethal effect of *Rhodococcus equi*, a facultative intracellular bacterium of macrophages compared to wild-type mice.[172] *Lgals3*$^{-/-}$ mice exhibited delayed but higher inflammatory responses, including higher production of the Th1 cytokines IL-12 and IFN-γ. Interestingly, *Lgals3*$^{-/-}$ macrophages exhibited higher levels of Toll-like receptor (TLR)2 mRNA and protein compared to wild-type cells. The authors also found that *Lgals3*$^{-/-}$ mice mounted higher IL-1β serum levels after infection compared to wild-type mice.[172] Thus, galectin-3 may contribute to the sensitivity of mice to *R. equi* infection by suppressing IL-1β production.

Finally, *Lgals3*$^{-/-}$ mice were found to be more susceptible to infection by *Paracoccidioides brasiliensis* and developed a Th2-polarized immune response compared to wild-type mice. The authors also found that *Lgals3*$^{-/-}$ macrophages exhibited higher levels of TLR2 mRNA and IL-10 production compared to wild-type macrophages after stimulation with *P. brasiliensis* antigens.[173] The reason for galectin-3 expression to favor a Th1 rather than a Th2 response as developed in other infections mentioned above is unknown.

Galectin-9. Administration of recombinant galectin-9 in mice resulted in a decreased sensitivity to LPS-induced vasculitis, the Shwartzman reaction, while *Lgals9*$^{-/-}$ mice were more susceptible to this reaction.[174] Thus, galectin-9 appears to exert an anti-inflammatory activity against LPS-induced inflammation. Galectin-9 treatment together with LPS suppressed production of these pro-inflammatory cytokines and LPS-induced elevation of these cytokines was not observed in galectin-9 transgenic mice. Finally, the authors provided evidence that the effects of galectin-9 are a result of its attraction of neutrophils. These results imply that galectin-9 has suppressive effects on bacterial infection-induced inflammation.

The suppressive role of galectin-9 in viral infections has also been demonstrated. In a model of corneal herpes simplex virus infection, administration of recombinant galectin-9, either systemically or locally, diminished the severity of eye lesions.[175] The mechanisms underlying this suppressive functions include apoptosis of the effector T cells along with a reduction in pro-inflammatory cytokines. Moreover, galectin-9 treatment resulted in expansion or induction of FOXP3^{+} Treg cells and expansion of myeloid-derived suppressor cells.[175]

General comments

Numerous functions have been demonstrated by administration of recombinant proteins to experimental animals. While many studies confirmed the functions demonstrated *in vitro*, whether they are indeed related to the *in vitro* responses remains to be definitively established. This is because galectins bind to many cell types and can exert a variety of functions when acting on these cells. Conversely, there are studies demonstrating *in vivo* functions by using specific neutralizing antibodies. There are also an increasing number of studies demonstrating *in vivo* functions of galectins, using mice deficient in a specific galectin. Here the remaining challenge

is the linkage of the phenotypes to the specific functions of that galectin demonstrated *in vitro*. This is especially true for galectins that are expressed by a large number of different cells and tissues.

It also remains to be determined whether the phenotypes are a result of extracellular or intracellular functions of an individual galectin. Future studies using mutant mice with a given galectin deleted conditionally in a tissue- and cell-type-specific manner are important for definitively identifying the *in vivo* functions of galectins. In addition, the approach would be strengthened by additional use of specific neutralizing antibodies or other specific inhibitors administered to wild-type mice to reproduce the phenotypes demonstrated with mutant mice.

Conclusions

Extensive *in vitro* and *in vivo* functional studies have yielded significant insights into the functions of galectins in cultured cells and in the whole organisms. While galectin research has spread into many fields, the role of galectins in immune and inflammatory cells has been particularly studied.

Many of the *in vivo* studies have established the use of recombinant galectins as therapeutics for a number of different diseases. For example, recombinant galectin-1 and -9 are used in the suppression of autoimmune diseases, such as rheumatoid arthritis, autoimmune hepatitis, autoimmune uveitis, diabetes, inflammatory bowel disease, graft versus host disease, and multiple sclerosis.

The studies have also identified some galectins as targets for the treatment of certain diseases. This is exemplified by galectin-3 being a target for treatment of allergic inflammation. It is interesting that for allergic inflammation, administration of transgenic galectin-3 has also been shown to be effective in suppressing the disease activity in an animal model. These seemingly paradoxical results can be explained by the difference in the functions of an exogenously introduced galectin and an endogenous one. Galectin-4 has been shown to have a contributory role in colitis, and neutralizing antibody has been shown to suppress colitis in a mouse model. Thus, targeting galectin-4 may be useful for treatment of inflammatory bowel disease.

Finally, with regard to antitumor immunity, there is evidence that galectin-1 produced by tumor contributes to tumor-immune escape; thus galectin-1 inhibitors could be used to counteract this effect and enhance antitumor immunity. Galectin-3 may have similar functions. Moreover, in view of the involvement of various galectins in the inflammatory responses and the known contribution of inflammation to tumor development and progression, targeting galectins may be useful for the treatment of cancer.

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Conflict of interest

The authors declare no conflicts of interest.

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