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When Galectins Recognize Glycans: From Biochemistry to Physiology and Back Again

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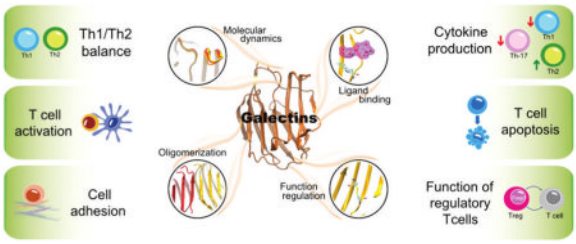
Abstract



In the past decade, increasing efforts have been devoted to the study of galectins, a family of evolutionarily conserved glycan-binding proteins with multifunctional properties. Galectins function, either intracellularly or extracellularly, as key biological mediators capable of monitoring changes occurring on the cell surface during fundamental biological processes such as cellular communication, inflammation, development, and differentiation. Their highly conserved structures, exquisite carbohydrate specificity, and ability to modulate a broad spectrum of biological processes have captivated a wide range of scientists from a wide spectrum of disciplines, including biochemistry, biophysics, cell biology, and physiology. However, in spite of enormous efforts to dissect the functions and properties of these glycan-binding proteins, limited information about how structural and biochemical aspects of these proteins can influence biological functions is available. In this review, we aim to integrate structural, biochemical, and functional aspects of this bewildering and ancient family of glycan-binding proteins and discuss their implications in physiologic and pathologic settings.

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In the postgenomic era, effort spent decoding the biological information encoded by the "glycome" has revealed substantial diversity and dynamic modulation of carbohydrate structures on cell surface or intracellular glycoconjugates.[1] Endogenous lectins are responsible for converting glycan-containing information into cell biological programs.[2,3] In the early 1970s, Nathan Sharon and colleagues were pioneers in demonstrating that lectins function as cell-agglutinating and sugar-specific proteins that regulate different biological events,[4] yet in spite of considerable advances over the four subsequent decades, the mechanisms linking glycan recognition to cellular signaling still remain poorly understood. Different glycan-binding proteins play vital roles in immunity, including C-type lectins (MGL, DC-SIGN, and Dectin-1), siglecs, and galectins.[1] Their essential, nonredundant biological functions have been demonstrated in vivo using mice engineered to lack endogenous lectins or specific glycosyltransferases.[1]

Galectins (formerly "S-type lectins"), an evolutionarily conserved family of endogenous lectins, share unique features, including their highly conserved structure, exquisite carbohydrate specificity, and ability to differentially regulate a myriad of biological responses.[5] Given the localization of these proteins within the cytoplasmic and nuclear compartments, multiple intracellular functions have been proposed for this protein family such as modulation of signaling pathways, regulation of RNA splicing, and intracellular control of apoptotic signaling, endocytic machinery, and trafficking. In spite of their lack of the typical leader peptide required for secretion, galectins are released to the extracellular environment through unusual, mostly unresolved, mechanisms, where they play key roles as soluble mediators of various cell functions. In fact, galectins have been proposed to exert biological activities by forming supramolecular structures, termed "lattices" with cell surface *N*-and *O*-glycans.[6–8] For example, the ability of galectin-1 to induce the separation of specific glycoprotein receptors is a consequence of the binding to multivalent carbohydrates that results in the formation of specific two- and three-dimensional supramolecular lattices.[9] In spite of a growing body of studies focused on the biochemistry and physiology of this bewildering family of glycan-binding proteins, limited information about how biochemical and structural aspects of galectins overall influence their biological activities in physiologic or pathologic settings is available.

With the main goal of fostering interdisciplinary collaborating projects bridging biochemistry and physiology, here we aim to summarize and integrate biochemical, biophysical, and functional aspects of this multitalented family of animal lectins, as schematically summarized in Figure 1.

BIOCHEMISTRY OF GALECTIN–GLYCAN INTERACTIONS

Among the multiple animal lectin families identified so far, galectins are distinctively characterized by their affinity for β-galactosides, no divalent cation requirement for binding, a shared primary structure motif, and a unique structural fold.[10] Although most galectins bind *N*-acetyllactosamine [Galβ1–4-NAcGlc] units, important differences in glycan binding preferences among different members of the family have been reported.[7] Interestingly, unfolding intermediates of galectins that retain carbohydrate binding specificity have been detected.[11] Structurally, the carbohydrate recognition domain (CRD) is a globular region with a jellyroll topology that contains the ligand binding groove (LBG) that displays key features that define its specificity.[12]

To date, 15 members of the galectin family have been identified in vertebrates. On the basis of their molecular architecture, galectins have been classified into three main types: (a) "proto-type" galectins, comprising a single polypeptide chain that is able to dimerize (galectin-1, -2, -5, -7, -10, -11, -13, -14, and -15); (b) "tandem repeat-type" galectins,

composed of a single polypeptide chain presenting two CRDs connected by a linker peptide (galectin-4, -6, -8, -9, and -12); and (c) the "chimera-type" galectin-3, which consists of one C-terminal CRD linked to an N-terminal peptide.[13] A more recent classification based on gene sequence comparisons and intron–exon position analysis has classified galectins' CRDs into two distinct subgroups (F3 and F4). Peculiarly, bi-CRD galectins usually display one domain of each type, F3 and F4.[14]

Regardless of its architectural type, a main feature of any given galectin is represented by its ability to recognize nonreducing terminal or internal galactosyl residues. Exceptions of this rule are the GRP (galectin-related protein; previously known as HSPC159, hematopoietic stem cell precursor), which shows no lectin attributes,[15] the mammalian GRIFIN (galectin-related interfiber protein) with no carbohydrate binding activity,[16] and galectin-10 (CLC; Charcot-Leyden crystal), which recognizes mannosyl residues with higher affinity than galactosyl residues.[17] Thus, in spite of a highly similar CRD, each individual galectin displays substantial differences in its saccharide specificity that arises from the unique architecture and dynamics of the LBG. Recently, ligand binding affinity studies have been extensively performed for several members of the galectin family, and clues about structure–function relationships have been disclosed by means of experimental and computational approaches.[18,19]

While some galectins (e.g., galectin-1 and -3) are widely expressed among different tissues of various species,[14,20,21] other family members have a more restricted tissue localization and compartmentalization (e.g., galectin-7 is preferentially found in the skin,[22–24] galectin-12 abundantly expressed in adipose tissue,[25,26] galectin-5 restricted to rat reticulocytes,[27,28] and galectin-10 strongly represented in human but not mouse eosinophils[17]), where they play essential roles in the control of cell fate[29] (which will be discussed in detail in subsequent sections).

In addition to glycan binding preferences, an interesting feature of the galectin family is the wide spectrum of glycoreceptors (glycoproteins or glycolipids) that serve as potential galectin binding partners, suggesting preferences of some galectins for certain protein or lipid frameworks where specific glycans are attached.[7] The molecular nature of this recognition, the specificity of intracellular signals delivered, and the different functions elicited by these molecular interactions represent major open questions in this rapidly growing field.

STRUCTURAL ASPECTS OF GALECTIN–GLYCAN INTERACTIONS

Following the identification of crystal structures for many members of the galectin family, there have been growing efforts aimed at understanding structural aspects of these glycan-binding proteins. From a total of 16 known human sequences, only eight galectins have been structurally described. A global structural overlook and an integral comparison of carbohydrate-binding sites across the whole family of galectins have recently been provided.[19,30] In addition, crystals have been resolved for galectins under different conditions, i.e., ligand-bound or unbound states and/or wild-type or mutant variants, generating even more complexity in structure–function analysis.[12,31–34] Herein, we will provide a general description of galectins using galectin-1 as a representative member and mentioning only briefly selective features of other family members.

Galectin-1 is usually illustrated as a paradigmatic model for the comparison of the ligand binding properties of lectins. Thus, further description of its structure will help in understanding the rules that govern general interactions between carbohydrates and ligand binding grooves within each particular CRD. The structure of galectin-1 consists of 135 amino acids arranged on two antiparallel β-sheets of five and six strands each (S1–S6 and

F1–F5), constituting the carbohydrate recognition domain.[32] All β-strands are connected by short loop regions, a structure similar to that usually described for a legume lectin fold.[32] Each monomer, hereafter termed the CRD, is implicated in a dimerization equilibrium, with a reported K_d of 7 μM.[31] As shown by crystallographic data,[12] the integrity of the galectin-1 dimer is mainly sustained through interactions held at the monomer interface and by well-conserved residues that are part of a hydrophobic core. Of note, many hydrogen bonds contribute to the maintenance of the two faces in contact, favoring the dimeric form in solution. Elegantly, a monomeric form of galectin-1 has been constructed by site-directed mutagenesis of these key residues.[35]

In wild-type galectin-1,[12] the two monomers are oriented with respect to each other in a 2-fold rotation axis approximately perpendicular to the plane of the β-sheets. In this way, the corresponding F1 and S1 strands of each monomer dynamically interact, yielding a dimer of continuous 10- and 12-stranded antiparallel β-sheet. In each subunit, the carbohydrate-binding site is located in a groove composed of strands S4–S6, localized on the opposite side of the dimerization surface, in the concave side of the β-sandwich of the CRD, forming the LBG. Importantly, the loops connecting both strands also determine the carbohydrate-binding cassette. Several residues, highly conserved among galectins, have been reported to be crucial in establishing the key interactions that determine binding specificity, namely, histidine 44, asparagine 46, arginine 48, histidine 52, asparagine 61, tryptophan 68, glutamic acid 71, and arginine 73.[32] In the search for potential determinants of specificity, several studies have been conducted to describe in detail these interactions using computational, biochemical, and biophysical approaches.[32,36–42] One notable example of a critical residue in a galectin-1 molecule is tryptophan 68, which has been found to be instrumental for stacking of the lactose moiety.[16,32]

From a global point of view, binding of galactosyl-terminal residues to a galectin CRD involves at least two major interactions: hydrophilic, through an extensive complementary hydrogen bond network, and hydrophobic, between the sugar rings and aromatic amino acid side chains in the CRD. As mentioned above, tryptophan 68, a highly conserved residue present in the LBG, appears to be critical for distinguishing the galactose ring from the glucose one, because of its preference for the axial C4-OH group that allows intimate C–H–π-cloud interactions, as described previously.[37,43] This key interaction may also be reproduced by other aromatic groups, as substitution of tryptophan for tyrosine maintains lectin activity.[44] Interestingly, in the apo form of galectins, it has been reported that water molecules can mimic the ligand hydrogen bond network, thus emphasizing the hydrophilic interactions held between protein and carbohydrate molecules.[34,38,44]

A distinctive characteristic of some mammalian galectins is the requirement of a reducing environment for carbohydrate binding activity. The rationale behind this biochemical property is based, with the exception of galectin-6, on the presence of a variable number of cysteine residues. Perhaps the most striking example is galectin-1 that contains six cysteines, some required in the reduced state for binding activity, in a sequence of 135 amino acids.[31] Recent studies have established a critical interplay between ligand binding and dimerization equilibria,[45] and the oxidation state of cysteine sulfhydril groups.[31,46] Cysteine-to-serine mutants have been shown to preserve lectin activity and were suggested as long-lasting functional substitutes of wild-type galectins.[44,47] This issue is particularly relevant given the sensitivity of galectin-1 to oxidative inactivation and the functional relevance of this lectin within inflammatory microenvironments where the risk of oxidation is extremely high,[48] which will be discussed in detail in subsequent sections.

As stated above, the structure of galectin-1 is representative of the proto-type subfamily and is closely related to that reported for galectin-2 and -7, which are also proto-type

galectins.[49,50] Particularly interesting is the structure of galectin-3, presenting tandem repeats of short polypeptide segments rich in proline, tyrosine, and glycine, integrating an extended N-terminal region.[12,33] Although the structure of the galectin-3 CRD shares a high degree of similarity with that of galectin-1 and -2, it should be noted that no dimerization equilibrium has been reported for this lectin. Nevertheless, the N-terminal region is determinant for the self-association capability reported for this lectin.[51]

The structure of the mouse galectin-4, a member of the tandem repeat-type subfamily, has been recently resolved by crystallization of the N-terminal CRD,[52] revealing two lactose-binding sites with different affinities. In contrast, the structure of the human C-terminal galectin-4 CRD was obtained by nuclear magnetic resonance (NMR) spectroscopy,[53] but no further structural analysis relating structure and function has been performed. Moreover, galectin-8 exhibits two CRDs joined by a short peptide, with a folding completely different from that reported for other galectins. Structures of the N-terminal CRD (Gal-8N) are available as carbohydrate-free or complexed with lactose,[54] and the structure of the C-terminal domain has been determined by NMR.[55] It has been reported that the two β-galactoside-binding CRDs of galectin-8 display a preference for larger saccharides, including glycosphingolipids, rather than simple disaccharides such as lactose, [56] mostly attributed to the N-terminal CRD. The structural and functional aspects of galectin-9, also a tandem repeat-type galectin, have been characterized in detail through the crystal structure of the N-terminal CRD in the presence of poly-*N*-acetyllactosamine.[34] Recently, the structure of the C-terminal conserved domain of human GRP (hGRPC) was resolved in its free form, and consistent with prior reports, no apparent lectin activity was identified within this protein structure.[15]

Although galectins share a high degree of similarity in their fold, their quaternary association may certainly differ. In this regard, most galectins are either bivalent or multivalent. While proto-type galectins can dimerize, tandem repeat-type galectins are at least bivalent, and galectin-3 can form oligomers upon binding to multivalent glycoproteins.[57] Multivalent oligomerization is therefore essential for attaining stability and biological functionality.[58] This feature endows galectins with the capacity to form ordered arrays of galectin–glycan clusters, often termed lattices, on the cell surface and signal through engagement of specific cell surface glycoconjugates (glycoproteins or glycolipids).[7] Thus, the interaction of multivalent galectins with multivalent glycans may allow the formation of distinct types of lattices, which might provide an explanation for their divergent biological functions. Examples of lattices formed between galectins and bivalent, trivalent, and tetravalent ligands have been provided elsewhere.[59] Particularly interestingly, it has been described that while the common lattices described for galecin-1 are homogeneous, organized crossed-linked complexes, galectin-3 forms heterogeneous disorganized cross-linking structures, with multivalent carbohydrates.[57]

It has been hypothesized that the oligomerization equilibrium may play a key role in delineating different functions of galectins.[46,60–62] Although limited information about this is available, recent studies put forward the notion that carbohydrate binding may, in fact, influence galectin structure. Interestingly, unfolding studies revealed how ligand binding induces protein stabilization,[45] and loss of entropy,[63] providing rational explanations for the observed interactions between monomers. The process of galectin oligomerization appears to be unique among distinct lectin families[18,64] and represents a key event for signaling and function. For many systems, the clustering of surface protein receptors and ligands is required for optimal initiation and transmission of signals into a cell. The assembly of highly ordered arrays of lectins and saccharides on the cell surface may thus be critical for cellular signaling and adhesion.[5] Lectin multivalency allows recognition of multiple binding partners, allowing glycan-binding proteins to play leading roles in signal transduction in

different biological processes as well as in cell–cell and cell–pathogen interactions.[65,66] In fact, linker structures between monomers have been reported to regulate lattice formation in galectin-1 mutant constructs.[35] Likewise, a number of elegant studies that aimed to evaluate the impact of protein structure and microenvironmental conditions on the monomer–dimer equilibrium and galectin biology have been conducted. In this regard, an important aspect of this growing field involves the design of selective galectin inhibitors for research and therapeutic purposes.[67] As we will discuss below, galectins play multifaceted roles in modulating cellular functions specific to particular physiologic and pathologic settings[5] and have been proposed as attractive therapeutic targets. Thus, unraveling key features of structure and ligand binding activity of galectins is essential for the design of novel strategies for interfering with lectin–glycan cluster formation.[68,69] Much effort is being invested in this direction, particularly, in the design of oligosaccharide derivatives and synthetic glycomimetics that show promising activity for the treatment of different inflammatory and neoplastic conditions.[70]

PHYSIOLOGY OF GALECTIN–GLYCAN INTERACTIONS

In the late 1990s, a growing body of experimental evidence emerged, revealing novel roles for galectins in the modulation of physiological and pathological processes. Although galectins have been implicated in many biological activities, most of the functional studies reported to date link galectins to early developmental processes, neovascularization and regulation of immune cell homeostasis, and inflammation.[7,8,71] In addition, it has been proposed that these endogenous lectins function as soluble pattern recognition receptors playing vital roles in pathogen recognition and killing or in facilitating entry of microbial pathogens and parasites into the host.[65,72–75] Through deciphering glycan-containing information about host immune cells or microbial structures, galectins can modulate a diversity of signaling events that lead to cellular proliferation, survival, chemotaxis, trafficking, cytokine secretion, and cell–cell communication.[7] In the next sections, we aim to summarize the immunomodulatory properties of different members of the galectin family, with particular emphasis on the most extensively studied galectin-1, -3, and -9, as summarized in Figure 2.

GALECTINS IN INNATE IMMUNITY

Either through extracellular or intracellular mechanisms, galectins can influence the capacity of innate immune cells (e.g., neutrophils, dendritic cells, monocytes/macrophages, eosinophils, and mast cells) to respond to chemotactic gradients, migrate across endothelial cell surfaces, synthesize and release both pro- and anti-inflammatory mediators, and recognize, engulf, and kill microbes and damaged cells.[65] In this regard, some members of the galectin family contribute to trigger innate immune responses, while others influence the resolution of acute inflammation.

Galectin-1

Despite significant advances in elucidating the role of galectin-1 within the T- and B-cell compartments (see below), its potential effects on cells of the innate immunity have not been studied in much detail. An essential role of galectin-1 in controlling neutrophil adhesion and trafficking has been proposed by in vitro experiments showing that exposure to recombinant galectin-1 impairs chemotaxis and reduces rolling and firm adhesion of neutrophils to activated endothelial cell monolayers.[76,77] Moreover, knocking down endothelial galectin-1 results in an increased number of extravasating neutrophils,[77] suggesting a critical role for endogenous galectin-1 in leukocyte transmigration across endothelial cell layers. In addition, binding of galectin-1 to neutrophils induces exposure of phosphatidylserine (PS), a ligand that targets these cells for phagocytic removal.[78–80] This phenomenon, however, does not

involve DNA fragmentation, changes in mitochondrial membrane potential, or caspase activation, suggesting an effect of galectin-1 in regulating leukocyte turnover in a manner independent of apoptosis. Although these results support an essential role for galectin-1 in the termination of neutrophil function and resolution of acute inflammation, this lectin has been originally reported to promote the release of superoxide from neutrophils,[81] suggesting that galectin-1 may either activate or dampen acute inflammatory mechanisms through different mechanisms. Whether the reduced versus oxidized galectin-1 variant or the dimeric versus monomeric form of the protein is involved in these different functions still remains to be elucidated.

As mentioned above, galectin-1 exists as a monomer that noncovalently dimerizes in solution; the dimeric form is required for effective binding and signaling through cell surface glycoproteins.[82,83] Indeed, galectin-1 dimerization was found to be required for high-affinity binding to immobilized glycan ligands, as determined by comparing wild-type dGal-1 with a permanent monomeric Gal-1 mutant (mGal-1) and a covalently dimeric form of this lectin (cd-mGal-1).[84] Interestingly, it was found that only dimeric galectin-1, but not its monomeric variant, induces cell surface exposure of PS and favors phagocytic removal by leukocytes.[85]

In addition to modulation of neutrophil function, galectin-1 was also shown to influence monocyte and macrophage physiology.[86–88] In general, galectin-1 has been proposed as a regulatory signal to deactivate inflammatory macrophages.[87] Consistent with its anti-inflammatory functions, this lectin inhibited interferon (IFN)-γ-induced Fcγ receptor type 1-dependent phagocytosis and major histocompatibility complex (MHC) II-dependent T-cell stimulation.[87] In addition, galectin-1 inhibits arachidonic acid release,[89] blocks nitric oxide synthesis,[86] and increases arginase activity,[86] thereby modulating alternative activation of cells of the monocyte/macrophage lineage. As in the case of neutrophil functions, interactions between galectin-1 and monocytes do not always lead to anti-inflammatory effects. In fact, a recent report demonstrated that galectin-1 stimulates monocyte migration in a dose- and saccharide-dependent manner through mechanisms involving mitogen-activated proten kinase (MAPK) pathways.[88] Again, future work should aim to elucidate the biochemical requirements of stimulatory versus inhibitory functions of this lectin.

Galectin-1 also influences dendritic cell (DC) physiology. DCs treated with recombinant galectin-1 showed enhanced migratory capacity through the extracellular matrix along with an increased level of phenotypic maturation.[90] This effect appears to be mediated by coclustering of CD43 and CD45 and engagement of Syk and protein kinase C (PKC) signaling pathways.[91] Moreover, in vivo administration of recombinant galectin-1 favored the recruitment of a population of DCs with a regulatory cell surface phenotype to the uterine mucosal tissue.[92] In addition, DCs engineered to overexpress galectin-1 could stimulate naïve T-cells and induce apoptosis in activated T-cells.[93] More recently, we described an immunoregulatory circuit involving DCs and T-cells that is mediated by galectin-1, IL-27, and IL-10.[94] After exposure to galectin-1, DCs acquired an IL-27-dependent regulatory function, and when transferred in vivo, these DCs promoted IL-10-mediated T-cell tolerance, blunted T helper (Th)1 and Th17 responses, and suppressed autoimmune neuroinflammation.[94]

Finally, evidence of a role for galectin-1 in mast cell physiology has also been provided. In a model of phospholipase A2-induced inflammation, intrafootpad injection of galectin-1 resulted in a reduced level of mast cell degranulation, yet the underlying mechanisms remain uncertain.[89] This inhibitory effect does not seem to involve induction of cell death as only galectin-3, but not galectin-1, triggers mast cell apoptosis.[95]

Galectin-3

Galectin-3 has been widely studied in the context of acute inflammatory responses.[8] Through intracellular or extracellular mechanisms, this lectin controls inflammatory responses by modulating cell adhesion and migration of various innate immune cells.[96] Its unique structure allows its oligomerization through the N-terminal domain upon recognition of the ligand by its C-terminal CRD,[97] thereby favoring cross-linking of ligands on the cell surface.[51] In vitro, recombinant galectin-3 promotes adhesion of human neutrophils to laminin[98] and endothelial cells.[99] In vivo, galectin-3 also serves as an adhesion molecule for neutrophils[51,98,99] by favoring neutrophil recruitment during *Streptococcus pneumoniae* lung infection via β_2-integrin-independent mechanisms.[51,99] Moreover, in naïve and primed neutrophils, exogenous galectin-3 induces L-selectin shedding and IL-8 production[100] and promotes neutrophil activation and degranulation,[101] as well as the synthesis of reactive oxygen intermediates.[102,103]

Interestingly, galectin-3 facilitates adhesive interactions between T-cells and DCs or macrophages[104] and induces CD13-mediated homotypic aggregation of monocytes.[105] Alternatively, galectin-3 can also promote migration of human monocytes/macrophages[106] and induces IL-1 production by human monocytes.[107] The impact of endogenous galectin-3 in phagocytosis has been demonstrated by comparing macrophages from *Lgals3*$^{-/-}$ and wild-type mice.[108] *Lgals3*$^{-/-}$ macrophages were found to be defective in phagocytosis of opsonized erythrocytes and apoptotic thymocytes.[108] Interestingly, intracellular galectin-3 was found to be confined around phagosomes.

Galectin-3 can also modulate survival of several innate immune cells. In vitro exposure of neutrophils to exogenous galectin-3 induces apoptosis, although the implicated glyco-receptors have not been identified.[101] In contrast, peritoneal macrophages from *Lgals3*$^{-/-}$ mice were much more prone to apoptosis than those from *Lgals3*$^{-/-}$ mice when they were treated with apoptotic stimuli, suggesting that intracellular expression of galectin-3 may lead to longer survival of inflammatory cells and persistence of inflammation.[109]

Furthermore, endogenous galectin-3 also affects DC function because *Lgals3*$^{-/-}$ DCs secrete smaller amounts of IL-12 than wild-type cells in response to microbial challenge.[110] Similarly, *Lgals3*$^{-/-}$ mast cells synthesized and released smaller amounts of inflammatory mediators when activated by cross-linkage of cell surface immunoglobulin (Ig) E receptor, as compared to their wild-type counterparts.[111]

Galectin-8

Galectin-8 is an atypical tandem repeat-type galectin formerly thought to occur as a monomer with each CRD joined by a common linker region, providing functional bivalency. Nevertheless, recent findings demonstrated that it exists as a dimer, likely through homodimeric interactions of the N-terminal domain that result in four CRDs, allowing functional bivalency at each separate domain.[112] Cross-linking of cell surface receptors must rely on recognition by a functionally bivalent C-terminal domain. Therefore, galectin-8 dimerization promotes functional bivalency of each CRD, which allows this lectin to signal PS exposure in leukocytes entirely through C-terminal domain recognition of poly-LacNAc glycans.[112] Thus, like the effects observed for galectin-1[84,85] and galectin-3,[21] dimerization appears to be a general requirement for inducing cross-linking of functional counter-receptors in galectin-8 signaling.[112] In addition, through binding to α_M integrin on neutrophils, recombinant galectin-8 enhances the adhesive properties of these cells and induces superoxide production.[113]

Galectin-9

Galectin-9, another tandem repeat-type member of the galectin family, has been shown to induce apoptosis in both monocytic (THP-1) and myelocytic (HL-60) cell lines through carbohydrate-dependent mechanisms.[114] In searching for potential cytokine genes involved in galectin-9′s immunoregulatory effects, Matsura and collaborators[115] demonstrated that intracellular galectin-9 activates two transcriptional factors, nuclear factor (NF) IL6 (C/EBPβ) and activator protein-1 (AP-1), and induces transcription of the pro-inflammatory cytokines IL-1α, IL-1β, and IFN-γ in human monocytes. On the other hand, exogenously added galectin-9 does not promote the synthesis of these cytokines. The authors concluded that galectin-9 transactivates inflammatory cytokine genes in monocytes by functioning intracellularly, possibly through direct interaction with NF-IL6. These findings represent the first example of an intracellular function of galectin-9.[115]

On the other hand, this lectin has been demonstrated to promote the maturation of DCs at levels similar to those of lipopolysaccharides.[116] Galectin-9-matured DCs secreted IL-12 but not IL-10 and selectively elicited the production of Th1 cytokines (IL-2 and IFN-γ) by allogeneic $CD4^+$ T-cells. This effect does not appear to be dependent on the lectin properties of this protein as a galectin-9 mutant lacking β-galactoside binding activity retains its immunostimulatory properties. Moreover, the effect of galectin-9 on DC maturation was only slightly inhibited by lactose.[116] This effect appears to be mediated by activation of the p38 MAPK.[116] These results, suggesting a critical role of galectin-9 in the initiation of innate immune responses, are consistent with a recent report showing that the T-cell immunoglobulin- and mucin domain-containing molecule-3 (TIM-3) acts as a galectin-9 ligand, is expressed on innate immune cells, and promotes tissue inflammation.[117]

Interestingly, human galectin-9 was originally identified as a potent specific eosinophil chemoattractant (so-called “ecalectin”) produced by T-cells.[118–120] It has been proposed that the N- and C-terminal CRDs of galectin-9 interact with the same or closely related ligands on the eosinophil cell surface.[121] Paradoxically, this lectin has been found to significantly inhibit apoptosis of eosinophils from allergic patients but enhances apoptosis in cells from healthy individuals.[122] Whether this effect relies on differential “glycosylation signatures” of eosinophils isolated from allergic versus healthy individuals remains to be investigated. In this regard, IFN-γ-induced galectin-9 expression on fibroblasts has been shown to mediate eosinophil–fibroblast interactions,[123] suggesting a crucial role for this galectin in modulating allergic acute inflammation.

Other Galectins

Like galectin-1, galectin-2 and galectin-4 also induce PS exposure in a carbohydrate-dependent fashion in activated but not resting human neutrophils,[78] suggesting a contribution of these lectins to leukocyte turnover. On the other hand, galectin-10 is present in early undifferentiated HL-60 cells, and its level of expression increases considerably during eosinophilic and neutrophilic differentiation, suggesting its potential role in myeloid cell specification.[124] Thus, different members of the galectin family act either through an intracellular or an extracellular fashion to regulate multiple signaling pathways and biological responses in innate immune cells.

GALECTINS IN ADAPTIVE IMMUNITY

Through binding to polylactosamine-enriched glycoconjugates, secreted galectins can shape adaptive immunity by influencing T-cell signaling and activation, modulating T-cell survival, altering the cytokine balance, and regulating the B-cell compartment. Additionally, research over the past few years has shed light on a previously unappreciated role for endogenous galectins as crucial mediators of the suppressive function of T-regulatory (T_{reg})

cells. [7] As individual members of the galectin family selectively influence different biological processes, the final balance of their synchronized effects contributes to orchestration of the activation, polarization, and resolution of adaptive immune responses.

Galectin-1

Galectin-1 has been shown to regulate T-cell homeostasis by modulating cytokine production, proliferation, and apoptosis.[7] Compelling evidence demonstrated that this lectin induces inhibition of cell growth and promotes apoptosis of human and murine T-cells, not only during development in the thymus but also following stimulation in the periphery.[125–133] Expression of galectin-1 is found at sites of immunological synapse in both primary and secondary lymphoid organs, where it interferes with early T-cell signaling processes.[134–137] In the thymus, expression of galectin-1 by thymic epithelial cells can shape the T-cell repertoire by differentially modulating positive or negative selection through the control of extracellular signal-regulated kinase (ERK) activation.[134] Once in the secondary lymphoid organs, galectin-1 regulates the T-cell fate by modulating T-cell receptor (TCR)/costimulator-dependent clustering and signaling, thus establishing the appropriate T-cell activation thresholds.[135]

The activity of galectin-1 in modulating T-cell viability has been extensively studied by several research groups,[29,138] yielding in some cases controversial results. This lectin has been shown to induce apoptosis of activated but not resting T-cells through cross-linking specific glycoreceptors, promoting their segregation into membrane microdomains and selectively triggering pro-apoptotic signaling pathways.[29] Remarkably, T-cell susceptibility to galectin-1-induced cell death is controlled through the selective expression and assembly of cell surface glycoprotein receptors (such as CD45, CD43, CD2, and CD7),[139–142] and by the coordinated activity of a set of glycosyltransferases that are responsible for exposing or masking specific cell surface carbohydrate moieties.[141,143–145] In addition, galectin-1 triggers the activation of selected intracellular pathways (e.g., p56 and ZAP-70 activation, modulation of Bcl-2 expression, involvement of the AP-1 transcription factor, activation of executor caspases, and sphingomyelinase-mediated release of ceramide).[131,132,146–148] In spite of single studies reporting modulation of a variety of intracellular signals, a hierarchical dissection of signaling pathways implicated in galectin-1-induced T-cell death is still lacking.

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.[7] In vitro exposure of activated T-cells to 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.[130,149–154] In searching for potential mechanisms that could explain this Th1/Th17-specific immunoregulatory effects, we have reported a link among differential glycosylation of T-helper cells, susceptibility to galectin-1-induced cell death, and termination of the inflammatory response.[143] 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 masking exposed galactosyl moieties by differential α2–6 sialylation of cell surface glycoproteins. 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 contrast to the pro-apoptotic effects of galectin-1 on activated T-cells, secretion of this protein by stromal cells was capable of supporting the survival of naïve T-cells without promoting their proliferation.[136] Whether glycan-dependent mechanisms also regulate this effect remains to be investigated.

Importantly, at least part of the immunosuppressive function of T_{reg} cells is mediated by galectin-1 as this lectin is overexpressed in T_{reg} cells as compared to effector T-cells.[155,156] Strikingly, the inhibitory effects of human and mouse $CD4^+CD25^+FoxP3^+$ T_{reg} cells were significantly reduced following galectin-1 blockade.[156] In addition, in vitro exposure of T-cells to galectin-1 resulted in considerable expansion of a population of $CD4^+CD25^{high}$ T_{reg} cells with strong expression of the FoxP3 transcription factor that is a hallmark of these cells.[153] In addition, galectin-1 interferes with T-cell trafficking by blocking adhesion of T-cells to the extracellular matrix[150] and suppressing transendothelial migration of T-cells through mechanisms involving CD43 clustering.[157] Using siRNA-mediated silencing strategies, it was found that galectin-1 limits T-cell capture, rolling, and adhesion to activated endothelial cells under flow.[158] This evidence suggests that different mechanisms may contribute to the anti-inflammatory and immunosuppressive activities of galectin-1 in experimental models of chronic inflammation and autoimmunity (previously reviewed[138]) and tumor immune escape.[153,159]

In addition to its well-established role within the T-cell compartment, galectin-1 has been shown to regulate B-cell function by influencing B-cell development, differentiation, and survival. Within the bone marrow, galectin-1 is strongly expressed by stromal cells surrounding pre-B-cells, where it binds to the pre-B-cell receptor (pre-BCR) and contributes to the formation of synapses between pre-B-cells and stromal cells,[160] to influence pre-BCR signaling and activation.[161] Indeed, galectin-1-deficient ($Lgals1^{-/-}$) mice evidenced an arrest of B-cell development in the pre-BII cell stage.[162] Once in the periphery, galectin-1 is upregulated by activation signals[163] and contributes to differentiation of activated B-cells into antibody-secreting plasma cells.[164] Nevertheless, galectin-1 was also shown to negatively regulate B-cell proliferation and BCR-mediated signal transduction.[165] In addition, recent work demonstrated that overexpression of galectin-1 can facilitate death of memory B-cells,[166] thus confirming the role of this protein in favoring the plasma cell phenotype.

Galectin-2

Galectin-2 promotes T-cell apoptosis probably via glycan-dependent binding to cell surface β-integrins, and involving caspase-3 and -9, cytochrome *c* release, disruption of the mitochondrial membrane potential, and an increase in the Bax/Bcl-2 ratio.[167] Like galectin-1, galectin-2 can also modulate T-cell-derived cytokines in vitro and shift the balance toward a Th2 profile.[167] It is noteworthy that expression of galectin-2 inversely correlates with the severity of colitis in a mouse model,[168] and treatment of the mice with recombinant galectin-2 induced apoptosis in mucosal T-cells and reduced the severity of inflammatory colitis.[168] Interestingly, galectin-2 has been shown to control lymphotoxin secretion and influence the severity of inflammation during myocardial infarction.[169] Therefore, it seems apparent that galectin-2, as well as galectin-1, influences T-cell survival and differentially controls the balance of pro- and anti-inflammatory cytokines.

Galectin-3

Accumulating evidence indicates that galectin-3 can act in a dual manner either protecting T-cells from apoptosis or stimulating T-cell death, depending on whether the protein acts within the intracellular or extracellular compartment.[140,170,171] T-Cell transfectants overexpressing galectin-3 are protected from apoptosis induced by a variety of agents, including Fas ligand and staurosporine.[170] Investigation of potential mechanisms underlying this effect revealed that intracellular galectin-3 may confer resistance to apoptosis by engaging apoptosis regulation pathways inside the cells[172,173] or by modulating mitochondrial homeostasis.[174] In contrast, extracellular galectin-3 has been shown to induce T-cell apoptosis,[140] through mechanisms that involve caspase-3 but not caspase-8

activation.[171] While some studies proposed CD7 and CD29 as possible mediators of galectin-3-induced T-cell apoptosis,[171] others demonstrated that CD45 and CD71 but not CD29 and CD43 are involved in this function.[140] On the other hand, intracellular expression of galectin-3 inhibited galectin-1-induced cell death,[132] demonstrating how different members of the galectin family might cross-regulate each other to modulate T-cell survival and regulate the inflammatory response. In addition to modulation of apoptosis, T-cell proliferation has also been shown to be affected by galectin-3. Cells treated with galectin-3-specific antisense oligonucleotides had a reduced rate of proliferation.[175] In contrast, exogenously added galectin-3 appears to have a negative effect on T-cell growth, as it inhibits mitogen-induced proliferation of peripheral blood T-cells.[176]

Very elegant studies demonstrated that galectin-3 can also modulate T-cell activation and signaling. By forming multivalent complexes with *N*-glycans on the TCR, galectin-3 potentially restricts the lateral mobility of TCR complexes, raising the threshold for ligand-dependent receptor clustering and signal transduction, thus preventing uncontrolled activation of T-cells.[177] A further mechanistic analysis revealed that *N*-glycan branching coordinates homeostatic set points in T-cell activation and signaling to modulate TCR clustering.[178] Recent evidence further demonstrated that endogenous galectin-3 can directly control T-cell activation at sites of immunological synapse.[179]

With regard to the ability of galectins to modulate the T-helper cytokine profile, the impact of galectin-3 is still controversial. While administration of a galectin-3-encoding plasmid inhibits the synthesis of IL-5, a typical Th2-type cytokine,[180] studies using *Lgals3*$^{-/-}$ mice indicated an essential role for endogenous galectin-3 in downregulating Th1 cell responses in experimental models of allergic inflammation and parasite infection,[110,181,182] indicating that reinforced expression and genetic delivery of galectin-3 may not exactly reproduce the function of the endogenous lectin. Moreover, endogenous galectin-3 might differentially regulate cytokine production in different pathophysiological settings, as it favors Th2 responses in allergic inflammation but amplifies Th1- and Th17-type cytokine responses in autoimmune settings.[183]

Galectin-4

Although most of the studies performed to date have focused on galectin-1 and -3, Hokama and collaborators demonstrated, using in vivo and in vitro strategies, that galectin-4 expressed by intestinal epithelial cells favors CD4^{+} T-cell activation and induces IL-6 production through a PKCΦ-dependent pathway.[184] These findings were confirmed in an experimental model of intestinal inflammation indicating that galectin-4 may function as a T-cell activator by favoring secretion of pro-inflammatory cytokines.[184] Nevertheless, other studies suggested that galectin-4 induces apoptosis of mucosal T-cells and promotes resolution of the inflammatory disease.[185] In a model of experimental colitis, galectin-4 ameliorated mucosal inflammation, induced apoptosis of mucosal T-cells, and decreased the level of secretion of pro-inflammatory cytokines.[185] The definitive role of galectin-4 in vivo remains to be established in galectin-4-deficient mice when they become available.

Galectin-8

In searching for differential expression of glycosylation-related genes on different mouse thymic cell populations, Tribulatti and co-workers demonstrated sustained intrathymic expression of galectin-8 and proposed an active role for this lectin in modulating the survival of developing thymocytes and shaping the mature T-cell repertoire. Addition of recombinant galectin-8 to thymocyte cultures in vitro induced apoptosis only in CD4^{high}CD8^{high} thymocytes.[186] Within peripheral tissues, galectin-8 has also been shown to function as a potent pro-apoptotic agent. In Jurkat T-cells, galectin-8 triggers death by

eliciting a phospholipase D/phosphatidic acid signaling pathway, robust ERK1/2 activation, and expression of Fas ligand and caspase-mediated cell death. Moreover, in freshly isolated human peripheral blood mononuclear cells previously stimulated with anti-CD3 and anti-CD28, galectin-8 proved to be pro-apoptotic in a subpopulation of activated T-cells.[187] However, recent findings suggested that galectin-8 provides proliferative and costimulatory but not apoptotic signals in peripheral T-cells, suggesting a dual role for this lectin, like other members of the family.[188] Finally, through specific binding to α_4 integrins, galectin-8 can also modulate the adhesive properties of T-cells without modulating their survival or proliferative activity.[189] The mechanisms underlying these different outcomes still remain to be explored in galectin-8-deficient mice.

Galectin-9

A growing body of experimental evidence supports a critical role for galectin-9 in T-cell function. An early study demonstrated that this lectin is strongly represented in the mouse thymus and induces apoptosis of developing thymocytes in a carbohydrate-dependent manner.[190] Furthermore, this protein also promotes death of peripheral fully activated $CD4^+$ and $CD8^+$ T-cells.[114] The proposed mechanisms involve activation of caspase-1 but not caspase-8, -9, and -10.[114] More recently, in elegant studies, Zhu et al. demonstrated that galectin-9 acts as a binding partner for Tim-3 to induce apoptosis of Th1 cells.[191] The association of this effect with attenuation of autoimmune inflammation in a model of experimental autoimmune encephalomyelitis,[191] as well as with prolongation of allograft survival,[192] confirmed the pathophysiologic relevance of these findings. In addition, galectin-9 inhibits the development of Th17 cells and increases the frequency of T_{reg} cells in an experimental model of autoimmune arthritis,[193] supporting the anti-inflammatory function of the galectin-9–Tim-3 axis.

Galectin-10

Kubach and co-workers identified galectin-10 as being constitutively expressed in human $CD4^+CD25^+Foxp3^+$ T_{reg} cells, with restricted intracellular expression. In contrast, expression of this lectin was almost absent in resting and activated $CD4^+$ T-cells. siRNA-mediated silencing of endogenous galectin-10 in T_{reg} cells dramatically restored their proliferative capacity and abrogated their immunosuppressive activity, indicating an essential role for intracellular galectin-10 in controlling T_{reg} cell function.[194]

LINKING GALECTIN STRUCTURE TO FUNCTION

Despite the original excitement caused by linking the structure and function of these evolutionarily conserved glycan-binding proteins, relatively few studies have integrated structural and functional approaches to address biological questions, suggesting the need for fostering interdisciplinary projects bridging the gap among biochemistry, biophysics, and immunology. Here we illustrate how galectin structure affects target cell preference, signaling pathways, cellular function, and overall biological responses.

Several studies have independently shown dissimilar potencies of different members of the galectin family when they trigger a particular cellular response. For example, in eliciting signaling on T-cells and neutrophils, tandem repeat-type galectin-4, -8, and -9 are much more potent than the chimera-type galectin-3, which is in turn more potent than proto-type galectin-1.[78,112,114,140,149,167,186,195] The ability of tandem repeat-type galectins to induce cell signaling at concentrations lower than those of proto-type galectins has been proposed to result from the constitutive bivalency of the former.[196–198] Indeed, a covalently linked form of the galectin-1 dimer was found to be a potent pro-apoptotic agent on mouse thymocytes and mature T-cells at concentrations 10-fold lower than that of wild-type

galectin-1.[62] Furthermore, only dimeric galectin-1, but not its monomeric mutant form, induces PS exposure in leukocytes.[85] Notably, the orientation, rotational flexibility, and spacing of the CRDs present in bivalent or multimeric galectins may also determine differences in the recognition of specific glycan ligands on different cell types.[5,6,198,199]

In an elegant study aimed at dissecting the structural features of galectin-1 and galectin-9 that contribute to their function, Bi and co-workers[198] designed a series of hybrid proteins that combined CRDs of these two lectins, connected with different peptide linkers. Using these constructs, the authors found that while the N-terminal CRD and linker peptide contributed to the potency of the lectins, the C-terminal CRD was the primary determinant of receptor recognition, death pathway signaling, and target cell susceptibility.[198] Importantly, not only the higher valency of multimeric galectin-9, compared with that of dimeric galectin-1, but also the increased spacing and rotational flexibility of the CRDs accounted for the differences in the potency displayed by these two lectins.[198]

To specifically investigate the contribution of the linker region, in a manner independent of the CRD specificity, to the increased potency of tandem repeat-type galectins, Baum and colleagues[199] created three tandem repeat-type galectin constructs with different linker regions joining identical galectin-1 CRDs. The authors found that random-coil or rigid α-helical linkers that permit separation of the two galectin-1 CRDs facilitated the formation of higher-order galectin multimers. These galectins were considerably more potent in binding to glycan ligands and cell surface glycoprotein receptors, as well as in triggering T-cell death, than native galectin-1 or a construct with a short rigid linker.[199] Thus, the increased potency of tandem repeat-type galectins compared with that of proto-type galectins is likely due to the ability of the linker domain to permit intermolecular CRD interactions, thus promoting higher-order multimerization and an increased level of lattice formation on the cell surface. Interestingly, from a pathophysiologic standpoint, in vivo formation of higher-order multivalent multimers by tandem repeat-type galectins may allow these lectins to exhibit significant biological effects at tissue concentrations much lower than those observed for proto-type galectins. Moreover, the length and nature of specific linker regions may contribute to differences in the function and potency among different galectin isoforms, thus allowing fine-tuning of signaling thresholds.

In addition to directly regulating signaling by modifying valency and lattice formation, monomer–dimer equilibrium may also modulate other aspects of galectin-1 activity. Interestingly, this lectin displays exquisite sensitivity to oxidative inactivation.[31,44,200,201] During oxidation, each subunit of galectin-1 forms three distinct intramolecular disulfide bridges that result in profound conformational changes, thereby preventing galectin-1 dimerization and ligand recognition.[202] Stability under nonreducing conditions is considerably improved in cysteine-to-serine mutants, while sugar binding specificity and affinity are barely affected.[44,47] It was demonstrated that ligand engagement partially protects galectin-1 from oxidation.[46] Stowell and co-workers[46] found that binding to specific ligands may control the sensitivity of galectin-1 to oxidation by shifting the monomer–dimer equilibrium toward dimerization, suggesting that glycan binding protects galectin-1 from oxidative inactivation. Dimerization may limit the conformational freedom needed to successfully form intramolecular disulfide bonds, thereby protecting galectin-1 from oxidation. Supporting these findings, a mutant form of galectin-1 (mGal-1) that exhibits impaired dimerization, showed enhanced sensitivity to oxidation and failed to induce cell surface PS exposure.[46] Thus, binding to specific glycan ligands enhances dimerization and reduces sensitivity to oxidative inactivation. Accordingly, mutations that impair dimerization and therefore increase the level of monomer formation favor oxidation of the protein. Although protein oxidation was originally believed to be part of an inactivating process to attenuate or eliminate galectin functions, recent studies suggested

that oxidized galectin-1 may display alternative functions that are independent of glycan ligand recognition, including enhancement of peripheral nerve regeneration.[202–204]

Particularly interesting is the unique structure of galectin-3, which is monovalent in the absence of ligands and can oligomerize through the N-terminal domain upon ligand recognition by its C-terminal CRD.[57] This oligomerization process leads to cross-linking of receptor ligands on the cell surface,[51] which is essential for the majority of galectin-3 functions, including cellular activation and cell adhesion (reviewed in refs 173 and 203). Using site-directed fluorescence labeling of galectin-3 and fluorescence resonance energy transfer (FRET) detection, Nieminen and colleagues directly visualized galectin-3 oligomerization at the cellular level in biological settings.[51] At physiological concentrations, oligomerization of galectin-3 was detected during galectin-3 lattice formation on neutrophils and endothelial cells, galectin-3-mediated signal transduction in neutrophils, and galectin-3-mediated adhesion of neutrophils to an endothelial cell layer.[51] In all these experimental settings, galectin-3–glycan lattices were found to be robust and resistant to lateral movement once lectin multimers were formed. Finally, a recent study demonstrated that galectin-9 can bind to cell surface protein disulfide isomerase on Th2 cells, where it regulates the redox environment to enhance T-cell migration and HIV entry.[72] This study clearly illustrates how the formation of galectin–glycoprotein lattices can control the biochemical status of T-cells with direct functional consequences, including enhanced viral entry. Further studies aimed at connecting the structure, biochemistry, and biology of this protein family are expected to improve our understanding of their intracellular and extracellular functions.

CONCLUSIONS AND PERSPECTIVES

Several critical cell processes that are instrumental for immune cell homeostasis are regulated by cellular galectins.[7] This protein family was originally defined by a common structural fold, homologous carbohydrate recognition domains (CRDs), and specificity for β-galactoside-containing glycoconjugates,[10] yet emerging information indicates that different members of this protein family can recognize unique glycan structures, signal through different intracellular pathways, and trigger divergent cellular responses. In addition, mice engineered to lack expression of individual galectins display different phenotypic abnormalities, suggesting nonredundant functions of these proteins in vivo. Why can different members of this evolutionarily conserved family of proteins display such divergent functions? Can biochemical and structural studies contribute to the solving of this mystery? As mentioned above, recent efforts toward dissecting the structure–function relationship of galectin-1, -3, and -9 have provided potential explanatory mechanisms for the different potencies, target cell susceptibilities, and binding specificities of these lectins, yet more studies are needed to bridge the gap among biochemistry, glycobiology, and immunology to improve our understanding of the biology of these proteins.

After all, most galectins are functionally multivalent, by being structurally composed of two CRDs or as the result of a monomer–dimer or –oligomer equilibrium. In the proto-type subfamily, which includes galectin-1, the CRD exists as a monomer that noncovalently dimerizes in solution, and the dimeric form is required for effective binding and signaling through cell surface glycoproteins.[82,83] In tandem repeat-type galectins, there are two distinct CRDs joined by a random-coil linker, which is required for its typical function(s).[196] Functional multivalency, which allows the cross-linking of multivalent glycan ligands, is a common feature of galectins and is required for many of their biological effects.[59] Regulation of the monomer–dimer or monomer–oligomer equilibrium appears to be an important mechanism for controlling galectin functions. In addition, oxidation may also represent another biochemical mechanism by which galectin-1 activity is physiologically regulated. Interestingly, both mechanisms appear to be interconnected as shifting the

monomer–dimer equilibrium in favor of dimerization favors ligand binding, which protects galectin-1 from oxidative inactivation.[46] Sensitivity to oxidative inactivation might have evolved as an intrinsic regulatory means of limiting galectin-1 activity once it is secreted outside the cells. Thus, the distribution and duration of galectin-1 signaling may be regulated by the environmental redox potential.

A more complete understanding of the connection between structure and function of this multifunctional protein family might allow us to capitalize on this information for the implementation of novel therapeutic strategies, including the design of selective galectin inhibitors and stable galectin analogues for the treatment of neoplastic and inflammatory conditions.

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DEDICATION

This article is dedicated to the memory of Prof. Nathan Sharon who was the pioneer of lectin research and has inspired the new generations in the field of glycobiology.

ABBREVIATIONS

Gal	galectin
CRD	carbohydrate recognition domain
LBG	ligand binding groove
GRP	galectin-related protein
GRIFIN	galectin-related interfiber protein
CLC	Charcot-Leyden crystal
DC	dendritic cell
PS	phosphatidylserine
PKC	protein kinase C
IL	interleukin
IFN	interferon
Th	T-helper
CD	cluster of differentiation
Ig	immunoglobulin
MAPK	mitogen-activated proten kinase
TCR	T-cell receptor

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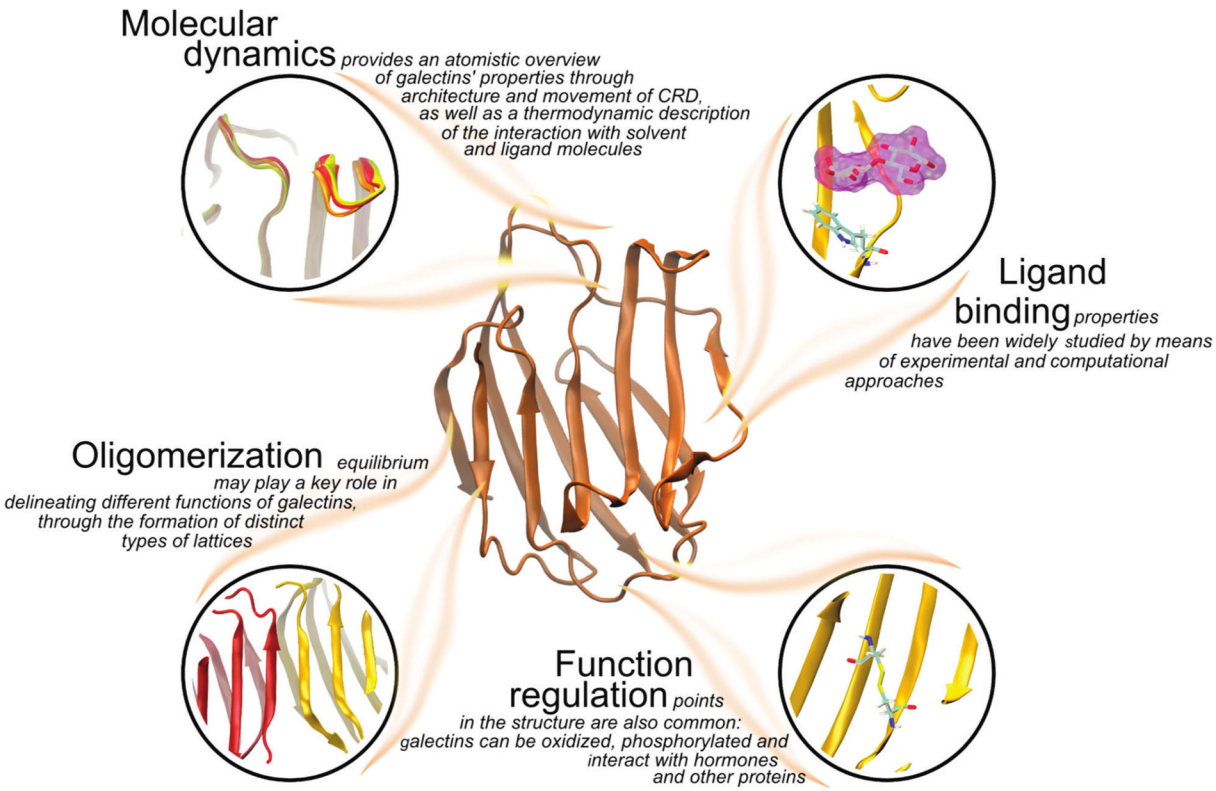

Figure 1.
Typical biochemical features of galectins that are critical for dissecting the structure–function relationship of this distinctive family of animal lectins.

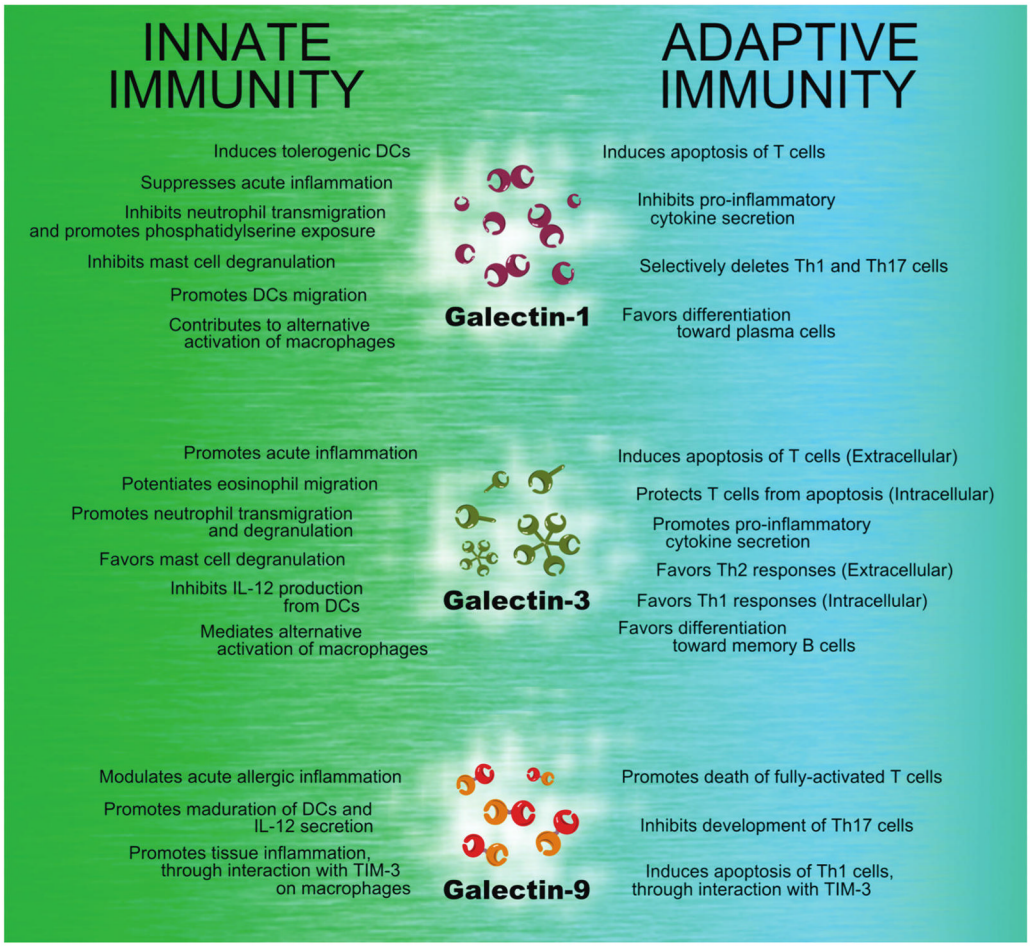

Figure 2.
Typical functions in innate and adaptive immunity of the most widely studied members of the galectin family belonging to the proto-type (galectin-1), chimera-type (galectin-3), and tandem-repeat type (galectin-9) classes.