Trends in Biochemical Sciences

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Feature Review

Translating the 'Sugar Code' into Immune and Vascular Signaling Programs

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The vast range and complexity of glycan structures and their dynamic variations in health and disease have presented formidable challenges toward understanding the biological significance of these molecules. Despite these limitations, compelling evidence highlights a major role for galectins, a family of soluble glycan-binding proteins, as endogenous decoders that translate glycan-containing information into a broad spectrum of cellular responses by modulating receptor clustering, reorganization, endocytosis, and signaling. Here, we underscore pioneer findings and recent advances in understanding the biology of galectin–glycan interactions in myeloid, lymphoid, and endothelial compartments, highlighting important pathways by which these multivalent complexes control immune and vascular programs. Implementation of novel glycoanalytical approaches, as well as the use of genetically engineered cell and organism models, have allowed glycans and galectins to be explored across a range of cellular processes.

Trends

Galectins initiate, amplify, or attenuate immune and vascular signaling programs by reprogramming the function of glycosylated receptors in the absence or presence of their canonical ligands.

Galectin–glycan interactions act by modulating clustering, reorganization, distancing, internalization, and signaling of relevant receptors in myeloid, lymphoid, and endothelial compartments.

A dynamic glycosylation signature on target cells controls the immunoregulatory and proangiogenic activities of galectins.

Galectins have emerged as novel therapeutic targets in autoimmunity, inflammation, fibrosis, and cancer, and their expression may confer resistance to anticancer therapies.

Glycans: The Power of Diversity

Glycans have fundamental roles in most biological processes, from cellular communication to differentiation, immunity, and vascularization [1–3] (Box 1). The most common **glycosylation** (see Glossary) pathways, *N*-linked and *O*-linked glycosylation, are finely regulated at several levels, including: (i) transcriptional and/or epigenetic control of **glycosyltransferases** and **glycosidases**; (ii) steric hindrance or modification of the access of glycosyltransferases or glycosidases to specific substrates; and (iii) spatial compartmentalization of components associated with the glycosylation machinery. Programmed remodeling of glycosylation by environmental stimuli, including nutrients, hypoxia, and cytokines, or by pathological conditions, such as inflammation and cancer (Box 2), has been shown to have a critical influence on cellular processes by masking or unmasking specific glycoepitopes for endogenous lectins [4–8].

Galectins: Professional Decoders of Glycan-Containing Information

Three major families of endogenous glycan-binding proteins have key roles in immune and vascular signaling programs: the C-type lectins, sialic acid-binding immunoglobulin-like lectins (siglecs), and galectins [6–8] (Box 3). Whereas C-type lectins and siglecs act mainly as transmembrane proteins associated with the surface of immune and endothelial cells (ECs), galectins are soluble proteins that function in the extracellular milieu by interacting with myriad glycosylated receptors or intracellularly by controlling signaling pathways through protein–glycan or protein–protein interactions [7,8] (Figure 1). Although binding of galectins to single saccharides leads to low-affinity interactions, multivalency of galectins and glycan ligands results in high-avidity binding promoting cross-linking, reorganization, and clustering of a preferential set

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Box 1. Glycosylation Pathways Governing the Synthesis of Galectin Ligands

The cellular 'glycome', a term that defines the full repertoire of glycan structures present in cells and tissues, is dynamically regulated by the coordinated action of hundreds of glycosyltransferases and glycosidases. Thus, protein and lipid glycosylation adds to the diversity created by the genome, proteome, and lipidome together with all other post-transcriptional and post-translational modifications, bestowing cells with essential biological information properly stored in saccharide units and their specific linkages [158]. Unlike DNA, RNA, and proteins, in which sequence can be predicted, the process of glycosylation is not template driven, is heterogeneous in nature, and varies among different cell types, a fact that has hindered our understanding of the biological significance of glycans for many decades [158]. However, the implementation of modern glycoanalytical technologies, as well as the use of genetically engineered cells and organism models lacking components of the glycosylation machinery, have contributed to circumvent these limitations, allowing glycans to be explored at structural and functional levels across a range of cellular processes [158–161]. The coordinated action of glycosyltransferases and glycosidases leads to the generation or masking of specific galectin glycan ligands. The scheme is simplified showing only relevant glycan-modifying enzymes discussed in this review (Figure I). These include the α1,2-mannosidase II (αM-II), a Golgi mannose-hydrolyzing enzyme that catalyzes the first committed step in the biosynthesis of complex *N*-linked oligosaccharides, the *N*-acetylglucosaminyltransferases (Mgat1; 2; 4 and 5), which act sequentially to generate the GlcNAc branch complex-type *N*-glycans (preferred ligands for galectins), and the α2,6 sialyltransferase 1 (ST6Gal1), which incorporates sialic acid in α2,6 linkages to *N*-glycans preventing galectin (Gal)-1 binding. Other glycosyltransferases shown are: galactosyltransferases (GalTs), which catalyze the transfer of galactose to glycoprotein-bound *N*-acetylglucosamine; T-synthase [core-1 β(1,3)-galactosyltransferase], which is essential for the biosynthesis of core 1 *O*-glycans (Galβ1,3GalNAcα1-Ser/Thr); α2,3 sialyltransferase 1 (ST3Gal1), which adds sialic acid to the galactose residue of core 1 *O*-glycans; core 2 *N*-acetylglucosaminyltransferase 1 (C2GnT1), which generates *O*-glycans containing a GlcNAc branch connected to GalNAc (core 2 *O*-glycans); and fucosyltransferase-3 (Fut3), which transfers an L-fucose residue to *N*-acetylglucosamine, creating Lewis^X antigen. For detailed information, the reader is referred to [1].

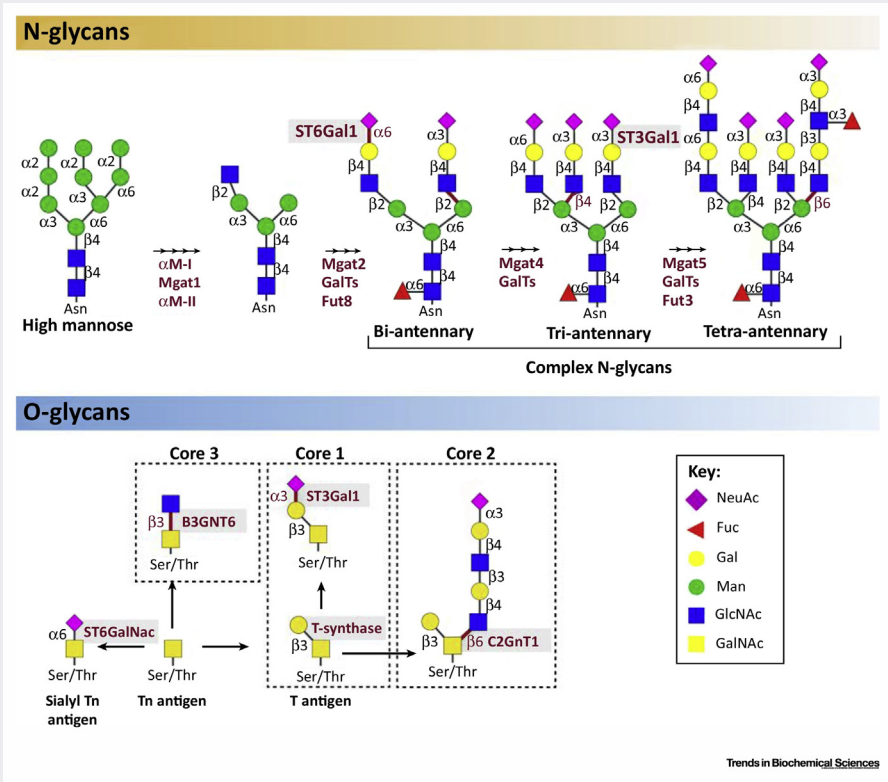

Figure I. Simplified Scheme Showing Only Relevant Glycan-Modifying Enzymes.

of glycoproteins and glycolipids. These multivalent complexes (often termed 'lattices') can segregate into scaffolds that regulate receptor signaling, endocytosis, and activation [9]. Within immune and vascular compartments in particular, galectin–glycan complexes may control signaling thresholds of relevant receptors, including: the **T cell** receptor (TCR) [10]; pre-**B cell** receptor (pre-BCR) [11]; cytokine receptors, such as the transforming growth factor-β receptor

Glossary

B cells: bone marrow-derived lymphoid cells that have essential roles in antibody-mediated responses. Beyond their traditional functions as antigen-presenting cells and precursors of antibody-secreting plasma cells, B lymphocytes display multiple roles, including secretion of relevant cytokines that tailor adaptive immunity and suppression of T cell responses.

Dendritic cells (DCs): bone marrow-derived myeloid cells known for their ability to present antigens to naïve T cells and orchestrate adaptive immunity. However, DCs can also trigger inhibitory circuits that ensure immunological tolerance and tissue homeostasis. Due to their remarkable plasticity, these cells respond to a plethora of environmental inputs that signal the occurrence of pathogens, tumors, or tissue inflammation by migrating from peripheral tissues into and within secondary lymphoid organs and by instructing T cells with stimulatory or regulatory potential.

Eosinophils: bone marrow-derived myeloid cells involved in allergic reactions, tissue inflammation, and defense against helminth parasites. They are capable of undergoing activation and recruitment into tissues in response to appropriate stimuli, including IL-5 and eotaxin. These cells express surface receptors, including the IL-5 receptor, CC chemokine receptor 3 (CCR3), Siglec-8/Siglec-F, and several integrins, including very late antigen-4 (VLA-4; $\alpha_4\beta_1$), which support the adhesion, trafficking, and degranulation of these cells.

Glycosidases: enzymes that catalyze the hydrolysis of glycosidic linkages, thereby degrading oligosaccharides and glycoconjugates.

Glycosylation: common post-translational modification that includes: *N*-glycosylation, in which the glycan is attached to an asparagine (Asn) residue present in the consensus sequon Asn-X-Ser/Thr (where X can be any amino acid except proline), and *O*-glycosylation, in which the glycan is attached to a serine (Ser) or threonine (Thr) residue. Other types of glycosylation are summarized in [1].

Glycosyltransferases: enzymes that catalyze the transfer of a sugar moiety from an activated donor sugar

Box 2. Aberrant Glycosylation in Cancer

Aberrant glycosylation often occurs in pathological conditions, particularly in cancerous tissues [4,5]. These include, among other changes: truncated glycans, such as the Thomsennoveau antigen (Tn: GalNAc-O-Ser/ Thr) and the sialyl-Tn antigen (STn); augmented expression of *N*-glycan structures, including β1,6 branching of complex *N*-glycans, resulting from enhanced expression of *N*-acetylglucosaminyltransferase 5 (Mgat5); augmented expression of the bisecting GlcNAc branch generated by the *N*-acetylglucosaminyltransferase 3 (Mgat3); increased frequency of α2,6-linked sialic acid attached to terminal *N*-acetyllactosamine (Gal-β1,4GlcNAc) units; and enhanced synthesis of core-2 *O*-glycans by the core 2 β1,6-N-acetylglucosaminyltransferase 1 (C2GnT1). These glycan structures can themselves control receptor–ligand interactions or are specifically recognized by endogenous glycan-binding proteins (Figure I) [6,7].

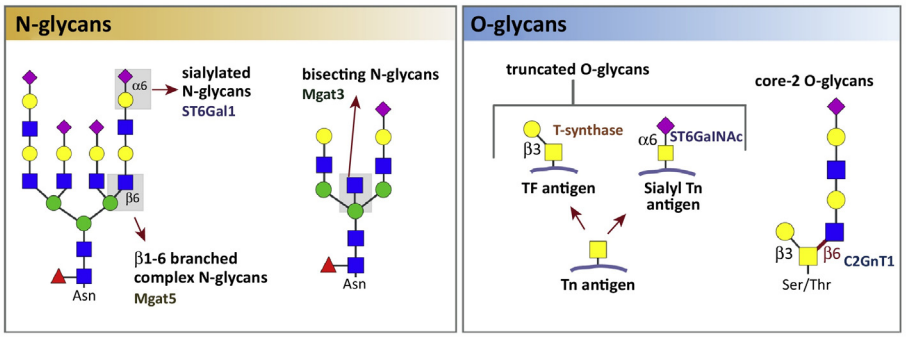

Figure I. Glycan Structures.

Box 3. The Galectin Family

Galectins are defined by a common structural fold and a conserved carbohydrate recognition domain (CRD) that recognizes glycans containing the disaccharide *N*-acetyllactosamine (Galβ1,4GlcNAc or LacNAc) [7,8], although emerging evidence disclosed substantial differences in glycan-binding preferences among individual members of this family. As many as 15 galectins have been identified to date in different tissues and species. Whereas some members of the family (Gal-1, 2, 5, 7, 10, 11, 13, 14, and 15) are traditionally classified as 'proto-type' galectins having one CRD, others, called 'tandem-repeat' galectins, contain two homologous CRDs in tandem in a single polypeptide chain separated by a linker of up to 70 amino acids (Gal-4, 6, 8, 9, and 12). Gal-3 is unique in that it contains a CRD connected to a nonlectin N-terminal region that is responsible for oligomerization of the lectin and ligand cross-linking (Figure 1, main text). Although galectins do not have a classical signal sequence, they are secreted to the extracellular milieu through a endoplasmic reticulum (ER)–Golgi-independent mechanism that is poorly understood [7]. Some galectins (i.e., Gal-1 and Gal-3) are present in a range of cell types, with notable expression in macrophages, DCs, and eosinophils [8,48,55,71,162–164], regulatory T cells (Tregs) [142], as well as endothelial cells (ECs) [165], whereas others have a more restricted localization, with Gal-5 preferentially expressed in rat reticulocytes, Gal-7 in skin and other stratified epithelia, Gal-12 in adipocytes, Gal-13 in placental tissue, and Gal-10 in human eosinophils and Tregs [7,8].

(TGF-βR) [12]; phosphatases, including CD45 [13]; **immune checkpoint** molecules, including lymphocyte-activation gene 3 (LAG-3) [14], cytotoxic T lymphocyte antigen 4 (CTLA-4) [15], and T cell immunoglobulin and mucin-domain containing 3 (TIM-3) [16,17]; tyrosine kinase receptors, such as vascular endothelial growth factor receptor 2 (VEGFR2) [18,19]; and integrins, including $\alpha_1\beta_1$ and $\alpha_5\beta_1$ [20].

How do multivalent galectin–receptor complexes control immunological and vascular programs? What is the functional relevance of these signaling complexes in physiological and pathological settings? In this review, we discuss the role of galectin–glycan interactions in reprogramming the function of relevant receptors associated with myeloid, lymphoid, and endothelial compartments, stressing structural requirements, ligand dependency, and the potential of these complexes to initiate, amplify, or mitigate signaling programs that govern inflammation, immunity, and angiogenesis.

onto saccharide and nonsaccharide acceptors.

Immune checkpoints: inhibitory pathways crucial for maintaining self-tolerance and modulating the amplitude of physiological immune responses in peripheral tissues to minimize collateral tissue damage. Tumors may coopt certain immune-checkpoint pathways (CTLA-4, PD-1, LAG-3, TIM-3, among others) as major mechanisms to evade T cell responses.

Macrophages: bone marrow-derived myeloid cells that have multiple roles in immunity, including phagocytosis, antigen presentation, and immunoregulation. Whereas classically activated 'M1-type' macrophages arise in response to IFN-γ and lipopolysaccharides and promote antimicrobial, proinflammatory, and antitumor responses, alternatively activated M2-typé macrophages are induced in the presence of IL-4 and favor tumor escape, wound healing, neuroprotection, and angiogenesis.

Mast cells (MCs): bone marrow-derived myeloid cells that have key roles via release of preformed granule mediators, such as histamine, and *de novo* synthesis of lipid mediators, cytokines, and chemokines. Although best known for their activities in innate immunity and allergy, mast cells may also have roles in angiogenesis, immune tolerance, and pregnancy.

Microglia: glial cell located throughout the brain and spinal cord; they have homeostatic roles in the central nervous system (CNS) as a defense mechanism against pathogens, by scavenging the CNS for plaques, damaged or unnecessary neurons, or by amplifying or resolving CNS inflammation depending on whether they display an M1 (classically activated) or M2 (alternatively activated) phenotype.

Natural killer (NK) cells: a prototypical group of innate lymphoid cells (ILCs) that have crucial roles in the control of infections and malignancies. They express a portfolio of inhibitory and activating receptors that facilitate fine discrimination between damaged and healthy cells.

Neutrophils: the most abundant type of myeloid cells traditionally conceived as innate immune cells with a restricted set of

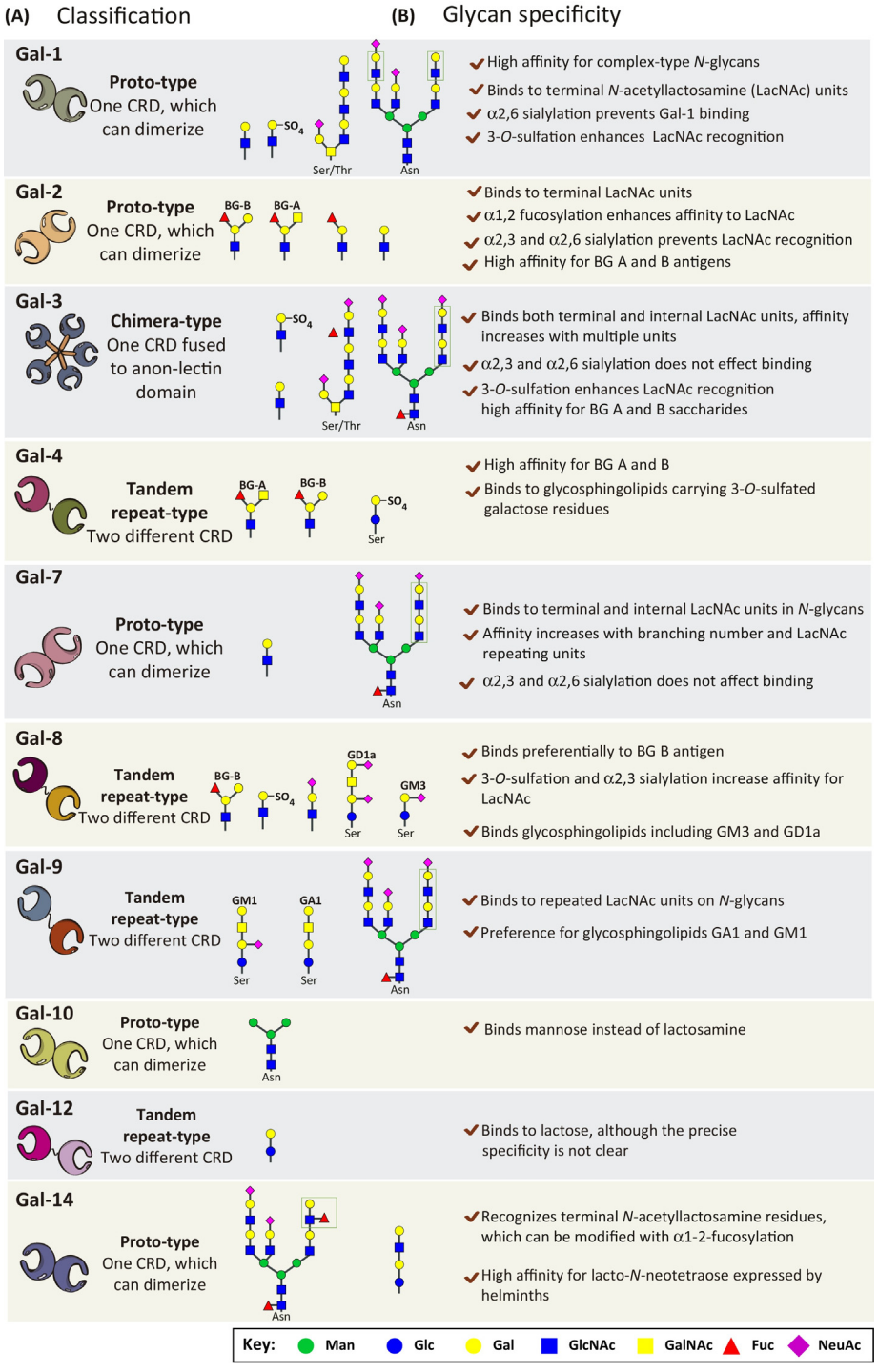

Figure 1. Structure, Function, and Glycan Specificities of Galectins. (A) Structure and classification of the most widely studied galectins. Galectins are subdivided into three groups: prototype galectins, which contain one carbohydrate recognition domain (CRD) and can dimerize; tandem-repeat type galectins, which contain two distinct CRDs in a single polypeptide chain and are inherently bivalent; and the unique chimera-type galectin 3 (Gal-3), which contains a CRD connected to a non-lectin N-terminal region responsible for oligomerization. (B) Glycan structures preferentially recognized by each individual galectin. Abbreviation: BG, blood group.

proinflammatory activities. However, it has become increasingly apparent that neutrophils display a vast array of specialized functions at the frontiers of innate and adaptive immunity to promote protective immunity or dampen tissue inflammation.

Regulatory T cells (Tregs): T lymphocytes that suppress or downregulate activation and proliferation of effector T cells. They are grouped into: (i) naturally occurring Tregs generated in the thymus and expressing the biomarkers CD4, FoxP3, and CD25; (ii) inducible Tregs generated in peripheral tissues and sharing the biomarkers CD4, FoxP3, and CD25; and (iii) IL-10-producing CD4 T (Tr1) cells lacking the transcription factor FoxP3, among others. They have key roles in preserving tissue homeostasis, safeguarding against the detrimental effects of inflammation and thwarting antitumor immunity.

T cells: lymphoid cells bearing a T cell receptor (TCR) that can be grouped into various subsets based on their functions and molecular signatures: (i) cytotoxic CD8 T cells kill infected and malignant cells; (ii) T helper (Th)1 cells control intravesicular pathogens and contribute to autoimmune disease pathogenesis; (iii) Th follicular (Thf) cells cooperate with B cells to elicit antibody-mediated responses; (iv) Th2 cells are critical in allergic reactions and parasite immunity; 9 v) Th17 cells control fungi and extracellular bacteria and sustain chronic inflammation; and (vi) regulatory T (Tregs) cells dampen excessive inflammation.

The Galectin–Glycan Axis in the Control of Myeloid Cell-Dependent Regulatory Programs

Myeloid cells not only have an important role in orchestrating and resolving immunity, but can also foster blood vessel formation, suggesting an intertwined relation between immune and vascular networks [21]. In this section, we provide selected examples that illustrate the role of galectin-driven regulatory circuits within myeloid compartments (Figure 2).

Monocytes, Macrophages, and Microglia

Monocytes, **macrophages**, and **microglial** cells have multifaceted roles in the regulation of host defenses, tissue homeostasis, and angiogenesis, depending on their activation status and polarization profiles. Macrophages respond to inflammatory stimuli by secreting galectin 1 and 3 (Gal-1 and Gal-3), suggesting that these endogenous lectins have key roles as threat-associated molecular patterns, either evoking or resolving immune responses [8,22,23]. Interestingly, galectins can also target monocyte and macrophage functions, including phagocytosis, antigen presentation, and immunoregulation. Whereas low concentrations of galectin 1 increased Fcγ receptor (FcγR)I-dependent phagocytosis, higher levels of this lectin (typically found in inflammatory microenvironments) inhibited phagocytosis by downregulating FcγRI expression [24]. Moreover, both exogenous and endogenous galectin 1 inhibited major histocompatibility complex (MHC)-II expression and MHC-II-dependent antigen presentation in a dose- and glycan-dependent manner by regulating extracellular-regulated kinase (ERK1)/2 signaling [24], suggesting that this lectin critically influences the magnitude of innate and adaptive immune responses. Moreover, Gal-1 reduced nitric oxide production while promoting arginase expression [25], indicating an extra role in tailoring macrophage polarization toward an M2 phenotype. This galectin 1-driven anti-inflammatory phenotype characterized by low IL-12 production favored parasite replication upon macrophage infection with *Trypanosoma cruzi* [26]. Accordingly, within the central nervous system (CNS), astrocyte-derived Gal-1 contributed to turn off classically activated microglia, inducing a phenotype of alternative activation through modulation of p38-MAPK, cAMP response element-binding (CREB), and nuclear factor-κB (NF-κB) signaling. This effect involved binding of Gal-1 to core 2 *O*-glycans on CD45, which promoted retention of this glycoprotein on the microglial cell surface and augmented its phosphatase activity. *In vivo*, Gal-1 tempered microglia activation and prevented inflammation-induced neurodegeneration [13]. Interestingly, M1- but not M2-type microglia exhibited the repertoire of glycans that mediate Gal-1 binding, supporting a major role for this glycan-binding protein in skewing the balance toward an M2 anti-inflammatory phenotype [13]. Accordingly, in acute inflammatory settings, Gal-1 promoted the conversion of macrophages toward a proresolving profile, characterized by reduced expression of CD11b, upregulation of the activity of the proresolving enzyme 12/15-lipoxygenase and downregulation of proinflammatory cytokines [27]. However, despite its anti-inflammatory effects, other studies revealed that Gal-1 stimulates chemotaxis of monocytes via a p44/42 MAPK-mediated and a pertussis toxin-sensitive pathway [28], highlighting a combined promigratory and proresolving function of this lectin.

The first functional reports of Gal-3 within the immune system were mainly focused on its proinflammatory activities toward macrophages and monocytes, as evidenced by increased chemotaxis, phagocytosis, and release of reactive oxygen species upon exposure to this lectin [29–31]. Accordingly, targeted disruption of the gene encoding Gal-3 attenuated macrophage-dependent peritoneal inflammation [32]. Moreover, several subsequent studies demonstrated pronounced microglial activation in response to Gal-3 [33–36]. This effect involved Gal-3 binding to IGF receptor (IGFR)1 [34] or to Toll-like receptor (TLR)-4 [35] and was confirmed in models of neuroinflammation and stroke [35]. Accordingly, in a model of cuprizone-induced demyelination, Gal-3-deficient mice displayed reduced microglia activation and lower expression of the phagocytic receptor TREM2b [36]. Thus, a delicate balance between stimulatory signals triggered by Gal-3–TLR4 or Gal-3–IGFR1 interactions and the inhibitory activity elicited by the Gal-1–CD45

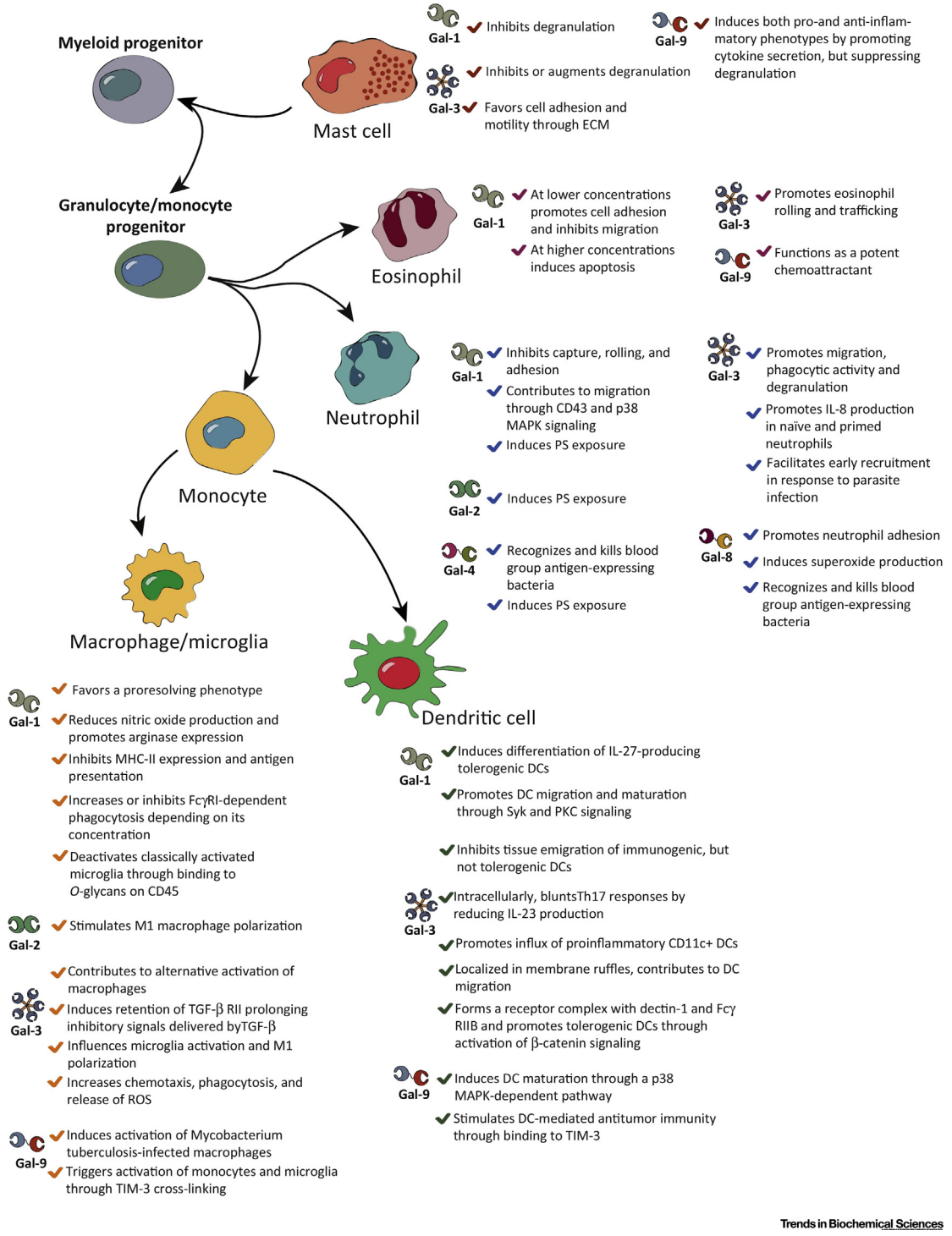

Figure 2. The Galectin–Glycan Axis in the Control of Myeloid Cell-Dependent Regulatory Programs. Galectins control the fate and function of myeloid cells, including monocytes, macrophages, dendritic cells (DCs), mast cells (MCs), eosinophils, and neutrophils, through modulation of several processes, including adhesion, trafficking, activation, differentiation, polarization, chemotaxis, phagocytosis, and degranulation. Some relevant examples are illustrated. Abbreviations: ECM, extracellular matrix; PKC, protein kinase C; PS, phosphatidylserine; TGF-β, transforming growth factor-β; TIM-3, T cell immunoglobulin and mucin-domain containing 3.

axis could reciprocally tune microglia activation and control inflammation-induced neurodegeneration. However, despite the well-known proinflammatory roles of this lectin, other studies revealed that, in specific circumstances, Gal-3 could also display anti-inflammatory activity. Gal-3-mediated cross-linking of complex branched *N*-glycans on TGF-βRII augmented its surface retention by macrophages and prolonged inhibitory signals delivered by TGF-β [12]. Likewise, disruption of the gene encoding Gal-3 specifically blunted IL-4/IL-13-induced alternative activation of macrophages without altering IFN-γ-induced classical activation [37]. Moreover, tumor-derived Gal-3 also contributed to alternative macrophage activation, an effect that facilitated tumor angiogenesis through VEGF-dependent pathways [38].

Despite the well-established roles of Gal-1 and -3 in macrophage and/or microglia physiology, the roles of other members of the family are poorly understood. Recent studies described the proinflammatory activity of Gal-2 and its ability to stimulate M1 macrophage polarization via CD14/TLR4 cross-linking [39]. By contrast, interaction of Gal-9 with TIM-3 led to activation of *Mycobacterium tuberculosis*-infected macrophages and stimulation of their bactericidal activity via induction of caspase 1-dependent IL-1β secretion [40,41]. Accordingly, within the CNS, Gal-9 triggered activation of monocytes and microglia through cross-linking TIM-3, leading to exacerbation of autoimmune neuroinflammation [42]. Consistent with this proinflammatory activity, recent evidence revealed that TLR4 and TLR7/8 ligation facilitated *cis* association of Gal-9 and TIM-3 within monocytes and/or macrophages, which differentially controlled the expression of IL-12 and IL-23, leading to M1 polarization [43]. These results suggest a major stimulatory role of the Gal-9–TIM-3 axis within the macrophage and/or microglia compartment, as opposed to its inhibitory activity in the context of T cell biology. Importantly, TIM-3-independent functions of Gal-9 have also been reported [44,45]. More recently, Gal-12, a lectin abundantly expressed in adipose tissue, was shown to polarize macrophages toward an M1 profile, an effect accompanied by enhanced inflammation and reduced insulin sensitivity [46]. Thus, although results are highly dependent on biochemical and pathophysiological conditions, Gal-1 appears to have predominant proresolving and anti-inflammatory roles, whereas Gal-2, -9, and -12 mostly elicit proinflammatory activities and Gal-3 promotes dual phenotypes.

Dendritic Cells

Despite their ability to orchestrate immune responses, **dendritic cells** (DCs) can also trigger inhibitory circuits that ensure immunological tolerance. Analysis of the 'glycosylation signature' of DCs during differentiation and maturation revealed dramatic changes, including upregulation of LacNAc residues, which serve as critical ligands for most galectins [47]. Through binding to CD43, a highly glycosylated mucin, Gal-1 activates a tolerogenic circuit involving differentiation of IL-27-producing DCs and expansion of IL-10-secreting type 1 T regulatory (Tr1) cells. This immunosuppressive circuit contributes to the resolution of autoimmune inflammation and thwarts T cell-mediated antitumor responses [48]. Interestingly, Gal-1-educated DCs mediated the tolerogenic effects of intravenously injected encephalitogenic peptides [49], conferred immune privilege during pregnancy [50], and blunted antiparasite immunity during *T. cruzi* infection [51]. DCs lacking Gal-1 were consistently more immunogenic than wildtype DCs, favored polarization toward T helper type 1 (Th1) and Th17 profiles, and mitigated **regulatory T cell** (Treg) responses [48,51,52]. Given that Gal-1 also promotes DC migration and maturation through mechanisms involving Syk and protein kinase C signaling [53], it appears likely that this lectin may impart a distinctive immunoregulatory program, characterized by both migratory and tolerogenic profiles. In fact, recent studies revealed that Gal-1 inhibited tissue emigration of immunogenic, but not tolerogenic DCs through a mechanism involving differential core 2 *O*-glycosylation of CD43, and selective inhibition of the protein tyrosine kinase Pyk2 [54]; these results suggest additional pathways by which Gal-1 sustains immune tolerance *in vivo*. Accordingly, Gal-1 produced by inflammatory DCs, synergized with IL-6 to create an

immunosuppressive microenvironment in ovarian cancer through mechanisms involving the special AT-rich sequence-binding protein-1 (Satb1) transcription factor [55]. Alternatively, tumor-derived Gal-1 may also instruct DCs with tolerogenic potential through mechanisms involving the inhibitor of DNA binding 3 (Id3) transcription factor [56]. Thus, Gal-1–*O*-glycan interactions selectively activate tolerogenic and trafficking DC programs, effects that facilitate tumor escape, promote resolution of autoimmune inflammation, and tailor the course of infections.

In contrast to the broad tolerogenic activity of Gal-1, Gal-3 showed paradoxical effects in shaping the DC compartment. Intracellular Gal-3 blunted Th17 responses by reducing IL-23 production in response to ligation of the C-type lectin dectin 1 [57]. Moreover, in a model of *Leishmania major* infection, absence of the gene encoding Gal-3 led to augmented, although mixed, DC-driven Th1/Th2 responses via modulation of the Notch pathway [58]. Similarly, during *Schistosoma mansoni* infection, Gal-3 deficiency in DCs increased T cell cytokine responses, without skewing the immune response toward Th1 or Th2 profiles [59]. By contrast, in a model of liver injury, targeted disruption of the gene encoding Gal-3 alleviated influx of inflammatory $CD11c^+$DCs and favored a tolerogenic microenvironment [60]. Moreover, Gal-3, localized in membrane ruffles, also contributed to DC migration [61]. Interestingly, a recent study exploring the mechanisms underlying oral tolerance and gut homeostasis showed that MUC-2, a highly glycosylated mucin, endowed DCs with anti-inflammatory activity by assembling a trimeric complex comprising Gal-3, dectin 1, and FcγRIIB, which rapidly activated β-catenin signaling [62]. Thus, Gal-3 may differentially control the inflammatory, tolerogenic, and migratory phenotypes of DCs in a context- and tissue-dependent manner. Unlike other galectins, Gal-9 almost exclusively induced DC maturation through a p38 MAPK-dependent pathway [63,64]. Accordingly, Gal-9 stimulated DC-mediated antitumor immunity through mechanisms involving TIM-3 cross-linking [65]. Thus, synchronized expression of Gal-1, -3 and -9 during the course of inflammatory, neoplastic, and infectious diseases could differentially control immunogenic and migratory patterns of DCs.

Mast Cells

Early studies revealed impaired **mast cells** (MCs) degranulation following Gal-1 treatment in a model of phospholipase A_2-induced edema [66]. More recently, studies showed that uterine MCs contribute to pregnancy-related events, including trophoblast placentation, via secretion of Gal-1 [67], suggesting alternative, nonimmunological functions of these cells. By contrast, other studies revealed major proinflammatory effects of Gal-3 within the MC compartment. Upon cross-linkage of high-affinity IgE receptor (FcεR)I, bone marrow-derived MCs lacking Gal-3 secreted lower amounts of histamine and IL-4 compared with wildtype MCs through mechanisms involving c-Jun N-terminal kinase (JNK)-1 [68]. Recently, dual roles for Gal-3 in FcεRI signaling in MCs have been identified. Whereas this lectin promoted internalization of IgE–FcεRI complexes, favored FcεRI ubiquitination, and inhibited antigen-induced chemotaxis, its presence facilitated MC adhesion and motility through the extracellular matrix [69]. Moreover, Gal-9 exhibited both pro- and anti-inflammatory roles by promoting cytokine secretion, but suppressing MC degranulation in human MC lines lacking FcεRI [70]. Further studies are warranted to elucidate the roles of these lectins in primary MCs and basophils and to identify possible counter-receptors and signaling pathways.

Eosinophils

Recent studies revealed dose- and *N*-glycan-dependent effects of Gal-1 on **eosinophils**. At low concentrations, Gal-1 induced redistribution and clustering of the CD49d integrin, promoted eosinophil adhesion, and inhibited ERK1/2 activation and eotaxin-1-induced migration. However, exposure to higher concentrations of this lectin resulted in ERK1/2-dependent apoptosis and disruption of the F-actin cytoskeleton [71]. *In vivo*, allergen-challenged Gal-1-deficient mice

showed increased recruitment of eosinophils to the airways and developed airway hyperresponsiveness relative to wildtype mice [71]. Moreover, in an IgE-mediated model of allergic conjunctivitis, administration of recombinant Gal-1 resolved clinical signs of disease and diminished Th2 cytokines, and eotaxin and ERK1/2 signaling, although this effect was associated with increased eosinophilia in the conjunctiva [72]. These divergent effects could be related to different concentrations or tissue-specific reactions induced by this lectin. By contrast, Gal-3 has been proposed to function as an adhesion molecule supporting eosinophil rolling and trafficking through glycosylation-dependent binding to $\alpha_4\beta_1$ integrin [73], whereas Gal-9 (originally called 'ecalectin') functions as a potent eosinophil chemoattractant [74]. Notably, eosinophils express and/or release several members of the family, including Gal-1 [71], Gal-3 [73], Gal-10 [75], and Gal-14 [76], in response to allergenic challenges, suggesting still unrecognized roles for endogenous galectins in eosinophil biology with critical implications in airway inflammation and parasite immunity.

Neutrophils

Early studies in a model of acute inflammation demonstrated a central role for Gal-1 in inhibition of **neutrophil** recruitment to sites of inflammation [66]. This inhibitory effect was untangled at the molecular level showing that both exogenous and endogenous Gal-1 decreased capture, rolling, and adhesion of neutrophils on activated endothelial monolayers in response to inflammatory stimuli by preventing inducible CD11b expression [77]. However, in the absence of additional inflammatory stimuli or tissue injury, Gal-1 contributed to neutrophil migration through mechanisms that were independent of G-protein-coupled receptor, but involved glycosylation-dependent binding of this lectin to CD43 and signaling via p38 MAPK [78]. Thus, Gal-1 may exert dual effects depending on the inflammation status of injured tissues, either inhibiting or promoting neutrophil migration. Consistent with its proresolving function, other studies demonstrated the ability of Gal-1 to induce reversible phosphatidylserine (PS) exposure in neutrophils independently of cell death, leading to their subsequent removal by phagocytes [79]. This effect was not restricted to Gal-1 because human Gal-2 and Gal-4 also contributed to PS-mediated neutrophil clearance upon excessive or inappropriate activation [80].

Conversely, Gal-3 has been shown to orchestrate innate immunity by promoting L-selectin shedding and IL-8 production in naïve and primed neutrophils. Interestingly, upon Gal-3 binding, primed neutrophils cleaved Gal-3 through the release of elastase, leading to a truncated lectin lacking the N-terminal domain [81]. Further experiments, using fluorescence resonance energy transfer, allowed visualization of Gal-3 oligomerization on the surface of neutrophils and establishment of potential galectin–receptor lattices [82]. Accordingly, it has been demonstrated that Gal-3 can act as a damage-associated molecular pattern or alarmin to facilitate early neutrophil recruitment in response to microbial infection [83,84]. However, Gal-3 not only promoted migration of neutrophils, but also increased their phagocytic activity and degranulation [85].

Although little is known about other members of the galectin family, Gal-8 has demonstrated glycan-dependent proadhesive activity toward circulating neutrophils. A mechanistic analysis identified binding of the N-terminal carbohydrate recognition domain (CRD) of this lectin with proMMP-9 and preferential interaction of the C-terminal CRD with both α_M/CD11b integrin and proMMP-9 [86]. Given the emerging role of Gal-9–TIM-3 interactions in neutrophil-mediated Gram-negative bacterial killing [87] and the selective recognition and killing activities of Gal-4 and Gal-8 toward human blood group antigen-expressing bacteria [88], it appears that two-CRD galectins are endowed with antibacterial functions through direct bactericidal activity or neutrophil-mediated proinflammatory mechanisms. Thus, neutrophils may respond to tissue inflammation, tumor growth, or microbial infection by releasing and/or sensing individual members of the galectin family, which may act in concert to tailor the course of innate and adaptive immunity.

The Galectin–Glycan Axis in the Control of Lymphoid-Dependent Programs

Natural Killer Cells

Galectins have demonstrated **natural killer** (NK) cell regulatory activity through modulation of their portfolio of stimulatory or inhibitory receptors. Targeting Gal-1 in Kaposi's sarcoma led to enhanced recruitment of NK cells to tumor microenvironments [89]. Accordingly, NK cells successfully eradicated Gal-1-deficient but not Gal-1-sufficient gliomas, an effect that required the presence of Gr-1^{+}CD11b^{+} myeloid cells [90]. By contrast, Gal-3 suppressed NK cell function by acting as a soluble inhibitory ligand of the NKp30 receptor [91] or by reducing the affinity of MHC I-related chain A (MICA) for the NKG2D receptor, an effect that involved binding of Gal-3 to LacNAc residues present in core 2 *O*-glycans on MICA [92]. Interestingly, Gal-9 induced divergent effects on NK cells depending on whether this lectin signaled via TIM-3. Whereas Gal-9 binding to TIM-3 resulted in enhanced IFN-γ production by human NK cells [93], this lectin impaired the function of human and mouse NK cells through a TIM-3-independent pathway [94]. Thus, galectins may control the function of NK cells through glycosylation-dependent mechanisms that target stimulatory or inhibitory receptors.

B Cells

Research over the past few years has identified Gal-1 as an essential component of the synapse established between stromal bone marrow cells and pre-B cells [95]. Notably, ligand-induced pre-BCR activation relied upon interactions among pre-BCR, Gal-1, and $\alpha_4\beta_1$, $\alpha_5\beta_1$, and $\alpha_4\beta_7$ integrins, leading to pre-BCR clustering and signaling and generation of pre-BII/stromal cell niches [96]. Further structural analysis revealed binding of Gal-1 to the pre-BCR via the unique motif of λ5 (λ5-UR), which adopts a stable helical conformation that docks onto a Gal-1 hydrophobic surface adjacent to its carbohydrate-binding site [97]. Gal-1 binding to the pre-BCR did not involve protein–glycan interactions, although, surprisingly, this glycan-independent complex reduced Gal-1 affinity for LacNAc epitopes by inducing local conformational changes in the CRD [11]. Thus, protein–protein interactions established between Gal-1 and particular receptors could directly influence glycosylation-dependent interactions by altering the affinity of lectin–glycan lattices.

Within the mature B cell compartment, Gal-1 amplified B cell activation by augmenting the strength of BCR signaling. Recombinant Gal-1, in the presence of suboptimal concentrations of anti-IgM, fully activated BCR signaling in chronic lymphocytic leukemia (CLL) B cells, as assessed by Syk and Erk1/2 phosphorylation [98]; these results were substantiated by phosphoproteomic analysis revealing selective activation of Syk, Btk, and phosphoinositide 3-kinase (PI3K) pathways upon exposure of mature B cells to Gal-1 [99]. By contrast, Gal-3 bound to a highly *O*-glycosylated CD45 isoform in an autocrine manner, resulting in increased survival of malignant diffuse large B cell lymphoma (DLBCL) cells [100]. Notably, Gal-1, -3, and -9 increased substantially during B cell activation and their expression influenced commitment toward memory B cell or plasma cell phenotypes [101–105], suggesting both extracellular and intracellular roles of these endogenous lectins during the lifespan of B cells.

T Cells

Galectins have emerged as key players that shape the profile of individual T cell subsets by controlling T cell activation, differentiation, and survival [8]. An outstanding example showed that multivalent interactions between Gal-3 and Mgat5-modified *N*-glycans can impair T cell receptor (TCR) clustering and alter the T cell signaling threshold by restraining lateral TCR movement [10]. A closer examination of these effects revealed that galectin–*N*-glycan complexes prevent filamentous actin-dependent redistribution of the TCR, CD4, and Lck tyrosine kinase to GM1-enriched membrane microdomains, limiting spontaneous TCR activation by favoring Lck inactivation and retaining the CD45 phosphatase at these membrane domains [106]. Moreover, in human tumor-infiltrating lymphocytes (TILs), Gal-3 functions by keeping the

TCR away from CD8 molecules, thereby promoting receptor exclusion and contributing to T cell anergy [107]. This deactivation process involved marked changes in the *N*- and *O*- cell surface glycome [108]. Interestingly, Gal-3 association with TILs led to diminished cytokine secretion, reduced adhesion to target cells, and impaired recruitment of lymphocyte function-associated antigen-1 (LFA-1) through mechanisms involving defective actin rearrangements at sites of immune synapse [109]. Interestingly, Gal-4 also contributed to T cell anergy and/or exhaustion during CNS inflammation via specific interactions with sulfatide-enriched brain glycolipids [110]. Thus, galectin–glycan interactions can control T cell function by modulating TCR clustering and signaling, distancing relevant glycosylated receptors, and controlling cytoskeleton rearrangements.

Galectin–glycan interactions may also act by trapping relevant receptors, preventing endocytosis, and promoting their retention on the membrane of T cells. Illustrating this concept, interactions between Gal-3 and complex *N*-glycans on CTLA-4 may delay the endocytosis of this co-inhibitory receptor and prolong delivery of arrest signals upon T cell activation [15]. Similarly, multivalent complexes formed between Gal-9 and the protein disulfide isomerase on the surface of Th2 cells hindered internalization of this enzyme, altering the redox state of the plasma membrane, and potentiating infection by HIV [111]. Although these functions have been mainly attributed to extracellular roles of galectin–glycan lattices, Gal-3 promotes TCR down-modulation and destabilizes the immunological synapse by acting intracellularly through interaction with the adaptor protein Alix [112]. Notably, galectins may also tune T cell activation by directly engaging negative or positive co-stimulatory molecules. While Gal-3 delivers inhibitory signals by modulating CTLA-4 recycling [15] or coopting the LAG-3 pathway [14], Gal-9 promotes glycosylation-dependent aggregation of the co-stimulatory molecule 4-1BB [113] and cross-links the inhibitory receptor TIM-3 [16]. Moreover, Gal-1 has been identified as a novel ligand for CD69, which contributes to inhibition of Th17 differentiation [114]. Thus, galectin binding to selected glycoproteins (TCR, CTLA-4, LAG-3, 4-1BB, TIM-3, and CD69) and subsequent formation of multivalent glycan–galectin complexes, leading to modulation of receptor clustering, segregation, endocytosis, and signaling, may explain the broad immunoregulatory activities of galectins in autoimmunity, infection, and cancer [8].

Interestingly, galectin–glycan complexes may also control T cell viability by interacting with different components of the cell death machinery. Extracellularly, galectins may coopt particular glycosylated receptors and transduce intracellular signals, directly leading to T cell apoptosis or may deliver 'find me' or 'eat me' signals required for the removal of dying cells. Intracellularly, galectins can interfere with signaling pathways that control T cell viability [115]. In particular, Gal-1 can engage apoptotic programs through binding to *N*- and *O*-glycans present in CD45, CD43, and CD7 [116] or by sensitizing T cells to the Fas-mediated pathway [117]. Of note, Gal-1 activity is primarily controlled by biochemical factors, such as the redox status of cells and tissues, and its monomer–dimer equilibrium; in fact, high-affinity ligand–receptor interactions are facilitated when the dimeric form of the protein occurs and when reducing conditions prevail [118]. Moreover, Gal-1 binding can be influenced by the dynamic remodeling of surface glycans and the regulated expression of glycosyltransferases in target cells. Whereas Th1- and Th17-polarized cells express the repertoire of glycans that are critical for Gal-1 binding and apoptosis, Th2 cells are resistant to the effects of this lectin due to increased α2,6-sialylation of surface glycoproteins [119] (Box 1). This effect correlated with the augmented frequency of Th1 and Th17 cells in antigen-challenged Gal-1-deficient mice [119]. This selective proapoptotic activity may contribute to the Th2-skewed immunosuppressive microenvironment induced by Gal-1 in models of autoimmunity and cancer [119–127]. Notably, other studies suggested that, in the absence of a reducing microenvironment, Gal-1 is unable to engage a full apoptotic program in nondifferentiated T cells, although it can prepare cells to apoptosis by inducing early PS exposure [79]. By contrast, Gal-3 can trigger pro- or antiapoptotic signals depending on whether

it functions extracellularly or acts within intracellular compartments. Whereas extracellular Gal-3 signals apoptosis by cross-linking CD45/CD71 or CD7/CD29 [116,128], intracellular Gal-3 protects T cells from Fas-induced death by interacting with the Bcl-2 protein family members [129]. This paradoxical effect differs from intracellular Gal-1, which sensitizes T cells to apoptosis induced by extracellular Gal-1 [130]. In addition, T cells may also engage apoptotic programs in response to tandem-repeat galectins, including Gal-4, -8, and -9 [16,131–133]. Whereas Gal-8 promotes T cell death through phosphatidic acid-mediated ERK1/2 activation via an unrecognized receptor [131], Gal-9 kills Th1 cells through binding to TIM-3 [16]. This proapoptotic effect could be rescued by human leukocyte antigen B (HLA-B)-associated transcript 3 (Bat3), which

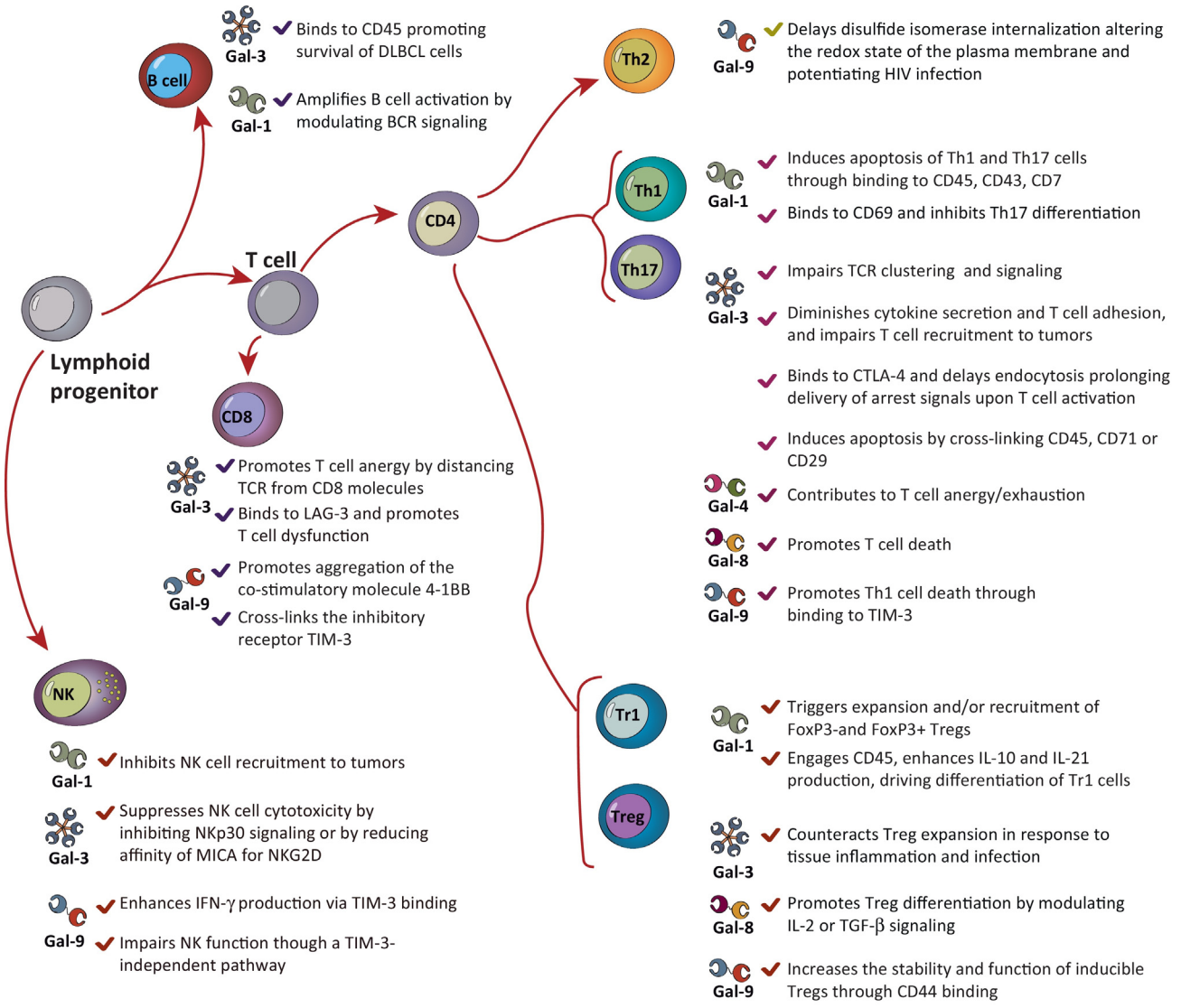

Figure 3. The Galectin–Glycan Axis in the Control of Lymphoid Cell-Dependent Regulatory Programs. Within the B–cell compartment, galectins can affect immature and mature B cells by influencing B cell signaling, immune synapse, and differentiation. Within the T cell compartment, galectins regulate a range of processes, including T cell receptor (TCR) signaling, activation, apoptosis, cytokine secretion, and regulatory T (Treg) cell expansion. Moreover, galectins can negatively or positively control the function of natural killer (NK) cells through glycosylation-dependent interactions with stimulatory or inhibitory receptors. Some relevant examples are illustrated. Abbreviations: CTLA-4, cytotoxic T lymphocyte antigen 4; DLBCL, diffuse large B cell lymphoma; Gal, galectin; LAG-3, lymphocyte-activation gene 3; MICA, major histocompatibility complex I-related chain A; TIM-3, T cell immunoglobulin and mucin-domain containing 3.

binds to and represses TIM-3 [17]. However, an increasing number of studies have revealed TIM-3-independent mechanisms that contribute to the immunoregulatory effects of Gal-9 [134,135]. Interestingly, the paradoxical immune stimulatory or inhibitory effects of this lectin have been scrutinized at the molecular level, revealing a potent role for the C-terminal domain in inducing T cell death, whereas its N-terminal region was more effective in activating DCs [136]. Thus, by coopting a distinct set of glycosylated receptors, individual members of the galectin family may cooperate to control cell death programs and influence T cell-dependent tolerogenic circuits (Figure 3).

Given their key roles in preserving tissue homeostasis, safeguarding against the detrimental effects of inflammation, and thwarting antitumor immunity, the role of galectins on Tregs has been explored. Whereas Gal-1 triggered the expansion and/or recruitment of FoxP3^{-} and FoxP3^{+} Tregs in models of pregnancy, parasite infection, autoimmunity, and breast cancer [50,51,121,124], Gal-3 expression counteracted Treg expansion in response to tissue inflammation and infection [137,138]. Furthermore, Gal-8 promoted Treg differentiation by modulating IL-2 and TGF-β signaling [139]. Finally, Gal-9 increased the stability and function of inducible Tregs by directly binding CD44, which, upon association with TGF-βRI, triggered Smad3 signaling, ultimately altering the CNS1 region of the FoxP3 transcription factor [140]. By contrast, direct engagement of CD45 by Gal-1 enhanced IL-10 and IL-21 expression in T cells through the c-Maf/aryl hydrocarbon receptor pathway, thus providing a rational explanation for Gal-1-driven

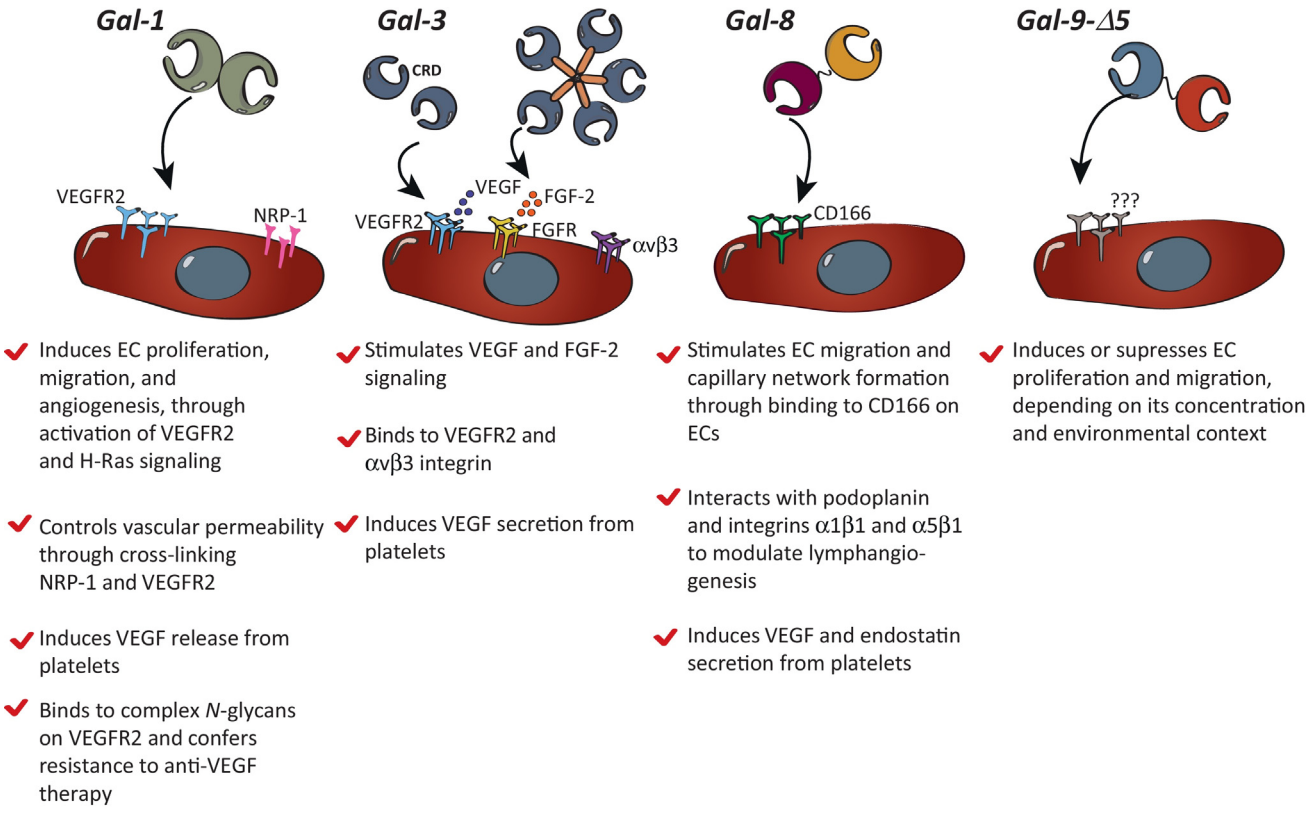

Figure 4. The Galectin–Glycan Axis in the Control of Vascular Signaling Programs. Galectin–glycan interactions can modulate angiogenesis and lymphangiogenesis through various mechanisms, receptors, and signaling pathways. Galectins (Gal) -1, -3, -8, and -9 (Δ5 isoform) exert partially overlapping functions in the control of endothelial cell (EC) biology by acting independently or interconnected with canonical proangiogenic pathways. Some relevant examples are illustrated. Abbreviations: NRP-1, neuropilin 1; VEGFR2, vascular endothelial growth factor receptor 2.

Box 4. Roles of Galectins in Angiogenesis and Lymphangiogenesis

Gal-1 induces EC proliferation, migration, and capillary tube formation *in vitro* and promotes angiogenesis *in vivo* [18,89,147,148,151,152]. At the molecular level, this lectin binds and cross-links neuropilin 1 (NRP-1) and VEGFR2, leading to receptor phosphorylation and signaling via ERK1/2 and Akt [18,153,154]; these signaling events recapitulate those triggered by VEGF, the master VEGFR2 ligand. Gal-1 recognizes complex *N*-glycans present in immunoglobulin domains-3, 4-, and -7 of VEGFR2, leading to glycosylation-dependent segregation and retention of this receptor on the surface of ECs [18]. Interestingly, exposure of ECs to hypoxic conditions led to significant changes in the EC glycome, inducing lower expression of α2,6-linked sialic acid, increased branching of β1,6 *N*-glycan structures, and elongation of poly-LacNAc residues [18], which facilitated Gal-1 binding and angiogenesis. In a highly vascularized model of Kaposi's sarcoma, interaction of Gal-1 with complex *N*-glycans on ECs linked tumor hypoxia to angiogenesis through mechanisms involving reactive oxygen species and NF-κB [89]. By contrast, Gal-3 displays proangiogenic activity through binding to Mgat5-modified *N*-glycans on integrin $\alpha_v\beta_3$ and facilitating retention of VEGFR2 on the surface of ECs [19,149,155], whereas Gal-8 triggers angiogenesis through binding to the activated leukocyte cell adhesion molecule (ALCAM; CD166) [150]. More recently, Gal-8 has been shown to have key roles in lymphangiogenesis through mechanisms involving interactions with podoplanin and integrins $\alpha_1\beta_1$ and $\alpha_5\beta_1$ [20]. Moreover, the Gal-9Δ5 isoform exhibit dual effects, either promoting or suppressing EC function, depending on its concentration and environmental context [156]. Alternatively, galectins may control angiogenesis via induction of platelet-derived proangiogenic mediators [166]. Interestingly, profiling galectin expression in ECs revealed abundant expression of Gal-1, -3, -8, and -9 and low expression of Gal-2, -4, and -12 [167], suggesting a possible autocrine effect of these lectins. These findings highlight different, although partially overlapping, roles of distinct members of the galectin family in the control of EC biology (Figure 4, main text).

Given the striking similarities of VEGF and Gal-1 signaling, it has been proposed that glycosylation-dependent binding of Gal-1 to VEGFR2 might preserve angiogenesis in settings of VEGF blockade, particularly in tumors resistant to anti-VEGF treatment [18]. Accordingly, anti-VEGF resistant tumors produced high amounts of Gal-1 and their associated vasculature displayed glycosylation patterns that facilitated Gal-1–EC interactions. By contrast, vessels associated with anti-VEGF-sensitive tumors displayed high amounts of α2,6-linked sialic acid, which prevented Gal-1 binding and suppressed angiogenesis [18]. These findings highlight the relevance Gal-1–*N*-glycan interactions as a potential target to overcome anti-VEGF compensatory programs.

Outstanding Questions

Is it possible to portray, by super-resolution imaging or other biochemical strategies, a typical 'galectin–glycan lattice' that might in turn delineate unique and discernible pro- or anti-inflammatory patterns?

Why do galectins preferentially coopt specific glycosylated receptors, given that glycan ligands are ubiquitously expressed in an assortment of possible receptor targets?

Do protein–protein interactions, in addition to protein–glycan interactions, have a significant role in galectin–receptor selectivity?

Is it possible to define a typical 'signalosome' associated with particular functions of individual galectins?

Do phenotypes of galectin-knockout mice reflect complete independence, partial redundancy, or complementary functions of distinct members of the family?

Is it possible to target individual galectins selectively, thereby providing therapeutic benefits and avoiding undesired 'off-target' effects?

differentiation of Tr1 cells [141]. Finally, Foxp3$^+$ Tregs expressed high amounts of Gal-1 and -10, which contributed to the immunosuppressive function of these cells [124,142,143]. At the molecular level, Treg-derived Gal-1 inhibited effector T cell function by cross-linking the GM1 ganglioside and activating the TRPC5 channel [144]. Interestingly, Gal-1 also conferred immunosuppressive activity to regulatory γδ-T cells, which were expanded in response to systemic inflammation and contributed to distant tumor growth [145]. Thus, galectins may sculpt the biology of Tregs by supporting their differentiation, expansion, stability, and immunosuppressive potential.

The Galectin–Glycan Axis in the Control of Vascular Signaling Programs

Vascular programs leading to the development of blood vessels (angiogenesis) and lymphatic vessels (lymphangiogenesis) entail the coordinated action of different cell types, including ECs, pericytes, and myeloid cells, and the integration of a network of stimulatory and inhibitory factors [146]. Galectins participate in these processes by acting independently or interconnected with canonical angiogenic pathways, and their expression may confer resistance to antiangiogenic therapies [18,20,89,147–156] (Box 4 and Figure 4).

Concluding Remarks and Future Perspectives

Research over the past decade has shed light on the broad immunomodulatory activities of galectins in several models of cancer, autoimmunity, allergy, and infection [7,71,157]. Given the importance of translating these basic discoveries into galectin-based therapeutic modalities, an improved understanding of the molecular mechanisms underlying these multifunctional activities is essential. In this review, we discuss and integrate pioneer and emerging findings highlighting the relevance of galectin–glycan interactions in myeloid, lymphoid, and vascular signaling programs.

A few important patterns emerge from the aforementioned discussion. First, galectins can sense and translate glycan-containing information into functional responses by modulating cellular

processes, including assembly, reorganization, endocytosis, and clustering of numerous glycosylated receptors. Second, while some members of the galectin family typically evoke proinflammatory responses acting as danger-associated molecular patterns, others contribute to resolution of inflammation and function as alternative immunological checkpoints. Third, galectins contribute to angiogenesis and lymphangiogenesis by coopting canonical receptors and signaling pathways. Fourth, a given galectin can have opposing roles depending on its concentration, oligomerization status, cellular localization, and redox state of the microenvironment. Fifth, the glycosylation pattern of target cells and specific receptors control their sensitivity to immunoregulatory and proangiogenic functions of individual galectins. Although exciting, this new wealth of information opens several questions (see Outstanding Questions).

Further studies should be aimed at defining tissue-specific roles of galectins *in vivo*, identifying their preferred receptors, and providing stronger mechanistic insights that could further support the implementation of galectin-based treatments. Whereas several galectin-tailored agents have been designed for the treatment of cancer, fibrosis, and autoimmunity, including synthetic glycan inhibitors, natural polysaccharides, peptidomimetics, and biological agents, progress thus far might represent only the 'tip of the iceberg' of a therapeutic potential that awaits future discovery.

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