Chapter 23

Fine-Tuning Antitumor Responses Through the Control of Galectin–Glycan Interactions: An Overview

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Abstract

In recent years, we have witnessed critical advances in genomics and proteomics which contributed to delineate the "tumor progression signature". This includes the altered expression of genes and proteins not only in tumor cells, but also in tumor-associated stromal, endothelial, and immune cells. Adding more complexity to this bewildering information, efforts are being made to define the "glycosylation signature" of the tumor microenvironment, which results from the abnormal expression and activity of glycosyltransferases, glycosidases, and enzyme chaperons. The multiple combinatorial possibilities of glycan structures expressed by neoplastic versus normal tissue provide enormous potential for information display and expand potential therapeutic opportunities. The responsibility of deciphering the biological information encoded by the tumor-associated glycome is partially assigned, to distinct families of endogenous glycan-binding proteins or lectins, whose expression and function are regulated in cancerous tissues. Galectins, a family of evolutionarily conserved glycan-binding proteins, can control tumor progression by directly influencing tumor growth or by modulating cell migration, angiogenesis, and tumor–immune escape. In this review, we will highlight recent findings on how galectin–glycan lattices control the dialogue between tumor and immune cells and how these interactions could be exploited for therapeutic purposes.

Key words: Glycosylation, Cancer, Tumor microenvironment, Tumor immunity, Galectins

1. Glycomics of the Tumor Microenvironment

Glycans decorate eukaryotic cell surfaces, where they are poised to mediate a variety of cell surface recognition events including host–pathogen and host–tumor interactions, leading to a wide variety of signaling processes and cellular responses (1). Glycan structures are incorporated to macromolecules such as proteins and lipids through a coordinated process termed "glycosylation" that involves the synchronized action of glycan-modifying enzymes; namely glycosyltransferases and glycosidases.

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The expression and activity of these enzymes are exquisitely regulated according to cell fate and microenvironmental stimuli. Hence, cell surface glycosylation is altered not only during physiological processes such as immune cell activation, differentiation, and trafficking, but also during pathological settings including inflammation and cancer (1, 2). Thus, the information encoded by the "glycome" (i.e., the entire repertoire of sugar structures expressed in cells and tissues in physiological and pathological settings) may provide clues to define critical issues still unresolved by the "genome" or "proteome," including the capacity of the same cytokine receptor to trigger opposing effects, the differential trafficking patterns of immune cells, and the ability of tumors or microbes to elicit divergent signaling events (2, 3).

Studies on glycosylation have been hampered by the lack of straightforward approaches to study glycan structures in the basic "non-specialized" laboratory. However, these difficulties could be overcome in the past years by the identification of reliable and versatile strategies capable of profiling glycosylation changes; these include lectin cytometric analysis, a routine method which could be complemented by "glycan-gene" chip arrays and mass spectrometric analysis of glycan structures. Finally, definitive confirmation of the relevance of glycosylation in a given physiologic or pathologic settings may be achieved by the careful examination of mice transgenic or knockout for individual genes linked to the "glycosylation machinery" (namely glycosyltransferases, glycosidases, enzyme chaperons, or lectins) (more information is available at http://www.functionalglycomics.org).

Abnormal glycosylation tightly correlates with the development of cancer and metastasis (4). These structural alterations are often the result of changes in the activity of one or more glycosyltransferases during the process of tumor transformation or metastasis (4). Notably, changes in the glycophenotype are also apparent in the tumor-associated stroma, endothelium, and infiltrating cells (2). Abnormal expression of glycosyltransferases or glycosidases can result in the modification of *N*-linked and *O*-linked glycans (Fig. 1). An example illustrating this concept is represented by *N*-glycan elongation which is strongly linked to an increased activity of *N*-acetylglucosaminiltransferase V (Mgat5) which leads to β1,6GlcNAc branching (5). This dynamic process also creates sites for incorporation of terminal sialic acid residues by sialyltransferases which are also upregulated during tumor growth (6). Programmed remodeling of tumor-associated *N*- and *O*-glycans can influence cell–cell and cell–matrix interactions, which results in dramatic changes in cell motility, invasiveness, and metastasis. In this regard, the metastatic potential of tumor cells has been extensively correlated with increased sialylation of cell surface glycoproteins, which is consistent with the known ability of sialic acid-binding lectins, such as selectins to mediate cell adhesion and extravasation during the metastatic process (7–10).

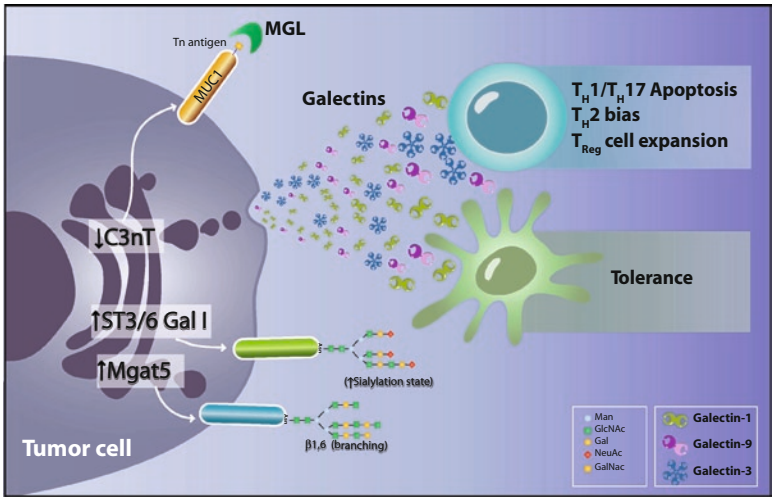

Fig. 1. Protein–glycan interactions in the tumor microenvironment. Examples illustrating glycosylation changes associated with cancer progression are shown here. Increased activity of *N*-acetylglucosaminiltransferase V (Mgat5) promotes β1,6GlcNAc branching which creates new sites for incorporation of sialic acid residues. In addition, MUC-1 binds to the C-type lectin MGL which instructs DCs to drive T_H2-mediated responses leading to anti-inflammatory responses. Galectins, a family of glycan-binding proteins, capable of recognizing multiple poly-LacNAc ligands, are secreted by tumor, stromal, and endothelial cells to modulate the survival and proliferation of effector T cells, skew the cytokine balance toward a T_H2-type profile, modulate the physiology of APCs, and/or induce the differentiation, expansion, and/or recruitment of T_{Reg} cells to favor tumor-immune escape.

The aberrant activity of glycosyltransferases not only promotes alterations in the elongation and branching of glycans structures, but also favors the incorporation of particular terminal residues in the transformed cells. For example, several carbohydrate structures, such as Tn (GalNAc-α-Ser/Thr, CD175), sialyl Tn (*N*-acetylneuraminic acid-α6-GalNAc-α-Ser/Thr, CD175s), Thomsen–Friedenreich disaccharide (Gal-β1-3-GalNAc, CD176), and (sialylated) Lewis antigens (CD15s) are highly upregulated in malignant cells and have been broadly used as diagnostic and prognostic markers (8). Interestingly, glycophenotypic differences between normal and transformed cells have been exploited in the clinics, using monoclonal antibodies as probes, to detect circulating tumor metastatic cells (2). In addition, a common feature of the tumor cell microenvironment is the abundant production of mucin glycoproteins, which are distinguished by the prevalence of high density of *O*-linked glycans. Mucins are often found in neoplastic tissues and metastatic lesions, and have been proposed as potential prognostic markers of many tumor types (11). Interestingly, Tn glycans on MUC-1 bind the C-type lectin receptor MGL and instruct dendritic cells (DCs) to drive T_H2-mediated responses, which, unlike T_H1, T_H17, or $CD8^+$ cytotoxic

T cells, do not appear to contribute to tumor eradication. For that reason, the expression of Tn epitopes on MUC-1 is considered as a poor prognosis factor in several tumor types (4). In addition, transformed cells can also express a distinct pattern of gangliosides, sialic acid-containing glycosphingolipids which play critical roles in cell recognition and immunity. In this regard, complex gangliosides are often elevated in a plethora of tumors including small-cell lung carcinomas, neuroblastomas, and melanomas (12). Altogether these examples illustrate the relevance of altered glycosylation in the tumor microenvironment and suggest the potential development of novel therapeutic and diagnostic approaches based on the positive or negative regulation of protein–glycan interactions.

2. Biochemistry and Cell Biology of Galectins

The responsibility of deciphering the biological information encoded by the "glycome" is assigned, at least in part, to a large number of endogenous glycan-binding proteins or lectins, whose expression and function are regulated during tumor progression. This includes distinct families including C-type lectins (e.g., DC-SIGN, MGL, and selectins), siglecs, and galectins which are extremely divergent from either biochemical or functional standpoints (2). Galectins are evolutionarily conserved glycans-binding proteins with emerging roles in a wide variety of physiological and pathological processes (13, 14). To date, 15 galectins have been identified in mammals, with relative homologs widely distributed in the animal kingdom. Although some galectins (e.g., galectin-5, -10, and -12) are expressed with restricted tissue specificity, most of them have a wide tissue distribution (14). Galectins share a common structure and at least one conserved carbohydrate recognition domain (CRD) of approximately 130 amino acids that mediates carbohydrate binding. Traditionally, galectins are classified based on structural similarities in "proto-type" galectins (galectin-1, -2, -5, -7, -10, -11, 13, -14, and -15), which have one CRD and exist as monomers or dimers, "tandem repeat-type" galectins (galectin-4, -6, -8, -9, and -12), which contain two different CRDs separated by a linker of up to 70 amino acids; and the "chimera-type" galectin-3, which contains one CRD connected to a nonlectin amino-terminal region (13, 14). With regard to their carbohydrate-binding activities, galectins are either bivalent or multivalent which allow the recognition of multiple binding partners and the activation of distinct signaling pathways. "Proto-type" galectins can dimerize, "tandem repeat-type" galectins are at least bivalent, and galectin-3 can form oligomers upon binding to multivalent glycoproteins (13, 14).

In spite of the absence of a classical secretory signal in their primary sequence, most galectins are exported to the extracellular milieu through an unusual route that involves the glycan-binding activity of the secreted protein (14). Upon secretion, galectins can bind multiple glycosylated partners (glycoproteins or glycolipids) and can convey glycan-encoded information into immune cell activation, differentiation, and homeostatic programs (3, 15). How do galectins decode the biological information encrypted by glycan structures? Although much remains to be learned, research over the past years put forward the idea that galectins can transduce intracellular signals by forming ordered arrays of protein–glycan structures – termed lattices – on the surface of a variety of cell types. Yet, they may also function by engaging specific cell surface glycoconjugates and forming traditional ligand–receptor interactions (14, 16, 17). However, galectins are also active within the intracellular compartment through mechanisms that remain poorly understood (13, 14). Examples illustrating this concept are galectin-3 and galectin-10, which function intracellularly either to modulate cell survival and pre-mRNA splicing or to control the immunosuppressive activity of $CD4^+CD25^+FoxP3^+$ human regulatory T (T_{Reg}) cells (14, 18).

A general consensus exists in the notion that secreted galectins, in contrast to cytokines or chemokines, do not have specific receptors, but can mediate immune cell communication through the recognition of a preferred set of cell surface glycoconjugates (3, 14). In this context, the minimal structure recognized by galectins is the disaccharide *N*-acetyllactosamine (LacNAc), which is found in *N*- and *O*-glycans and can be presented as multiple units (poly-LacNAc) on cell surface glycoproteins (19). However, research over the past few years revealed substantial differences among the glycan-binding preferences of individual members of the galectin family (3, 19, 20), which represents the basis of functional divergences in their biological activity. These variations in glycan recognition are mainly associated with the extent of *N*-glycan branching, the multiplicity of LacNAc residues, and/or the modification of terminal saccharides including sialylation or fucosylation (19, 20). Interestingly, differences in carbohydrate recognition of individual galectins can be even more pronounced, as the specific binding of galectin-10 to mannose is of much more higher affinity than its binding to LacNAc or terminal galactose (14). In addition, selective binding of galectins to different glycoproteins can result from the particular spatial orientation of individual CRDs and the unique glycoprotein topologies determined by the number of attached *N*-glycans (16). Thus, in spite of their shared sequence homology and evolutionary conservation, galectins may exhibit diverse carbohydrate specificity and play divergent roles in biology.

3. Galectin–Glycan Interactions in the Tumor Microenvironment

Although overlooked for a long time, the key importance of galectin–glycan interactions in cancer progression is now undisputed. In addition to glycophenotypic changes, gene and protein screening have repeatedly led to the identification of galectins as proteins that are up- or downregulated in neoplastic and metastatic lesions (21). Hence, given their abundant expression in tumor microenvironment, it is not surprising that the regulated expression of galectins may also contribute to delineate the "poor prognosis signature" (21). In fact, galectins can influence tumor progression through many different mechanisms, including the direct control of neoplastic transformation and/or the modulation of tumor cell survival, angiogenesis, and migration. In addition, galectins may also contribute to tumor growth by tilting the balance toward an immunosuppressive microenvironment that favors escape from T-cell-mediated immunity (21). Hence, a detailed analysis of the repertoire of galectins and their specific glycan partners may contribute to further understand the dialogue between tumor, stromal, and immune cells and delineate the potential role of protein–carbohydrate systems in cancer immunoediting.

To date, galectins-1 and -3 are the most widely studied lectins with respect to their role in tumor progression (22). Galectin-1 modulates cancer progression by influencing cell–cell and cell–matrix interactions (23), by inducing apoptosis of effector T cells (24), or by contributing to tumor cell migration and angiogenesis (25, 26). Moreover, and in keeping with its immunosuppressive functions, research from our laboratory has identified a crucial role for galectin-1 in tumor cell evasion of immune response. Interestingly, blockade of galectin-1 expression in melanoma cells resulted in heightened T-cell-mediated tumor rejection, decreased frequency of apoptotic T cells, and increased secretion of T_H1-type cytokines (27). Moreover, Reed Sternberg cells in Hodgkin lymphoma selectively overexpressed galectin-1, which contributed to the immunosuppressive activity of these cells through induction of a T_H2-type cytokine pattern, promotion of T_{Reg} cell expansion, and suppression of Epstein–Barr virus (EBV)-specific T-cell responses (28, 29). Furthermore, prostate cancer cells that had low expression of the core 2 *N*-acetylglucosaminyltransferase 1 (C2GnT1) were resistant to galectin-1-mediated cell death, although these cells expressed substantially higher amounts of this protein to selectively dampen effector T-cell responses (30). This immune inhibitory activity was further confirmed in human cancerous tissues, in which a strong inverse correlation was found between galectin-1 expression and the presence of tumor-infiltrating T cells in head and neck squamous cell carcinomas (31).

This effect appears to be a common feature of distinct members of the galectin family as local delivery of galectin-3 efficiently promoted apoptosis of tumor-reactive $CD8^+$ T cells and sustained tumor growth in a mouse model of colorectal cancer (32). Furthermore, galectin-3 expression correlated with apoptosis of tumor-associated lymphocytes in human melanoma biopsies (33). This was also true for galectin-9 as Klibi et al. recently reported the immunosuppressive effects of galectin-9-containing exosomes, which induced massive apoptosis in EBV-specific $CD4^+$ T cells from patients with nasopharyngeal carcinoma (34). This effect was prevented using an anti-Tim-3 blocking antibody, consistent with the reported role of galectin-9 in selectively eliminating Tim-3^+ T cells (35). In keeping with these findings, galectin-9 also modulates tumor immunity by facilitating Tim-3-dependent interactions between DCs and effector T cells (36). Hence, galectin–glycan interactions can influence immune tolerance in tumor microenvironments through the control of several mechanisms including the promotion of T-cell apoptosis, modulation of T-helper cytokine balance, regulation of DC physiology, and selective expansion of T_{Reg} cells. The availability of mice knock out for galectin genes as well as the possibility to manipulate galectin–glycan lattices in vivo has sparked a keen interest in studying galectin functions during tumor growth and metastasis. We will focus here on the cellular and molecular mechanisms underlying galectin-induced immunosuppression in vivo and the manipulation of galectin–glycan interactions for expanding and/or improving existing anticancer therapies.

3.1. Galectin–Glycan Interactions in the Control of T-Cell Survival

Several members of the galectin family can bind to glycoprotein receptors on the surface of mature T cells (including CD45, CD43, CD2, CD3, and CD7) and trigger distinct signaling events which act in concert to regulate T-cell survival, thus promoting contraction and/or modulation of different immune cell compartments (3). In addition, galectins can also regulate the fate of other cells in the tumor microenvironment including cancerous and stromal cells (21).

Galectin-1 can induce the upregulation of α- and β-chains of the IFN-γ receptor on activated T lymphocytes, rendering these cells sensitive to IFN-γ-induced apoptosis (37). Although the intracellular signaling pathways triggered by galectin-1 remain poorly understood, galectin-1-induced T-cell death has been shown to proceed through a caspase-independent pathway that involves rapid translocation of endonuclease G from mitochondria to the nucleus (38). However, galectin-1 was also shown to induce T-cell apoptosis through mechanisms involving sensitization to Fas (CD95) pathway and caspase-8 activation (39, 40). Moreover, exposure to galectin-1 triggers a disbalance of the Bcl-2/Bax ratio with a predominance of pro-apoptotic Bax and

activation of the ERK-1/2 and AP-1 signaling pathways (41, 42). However, and in spite of these findings, other observations suggested that galectin-1 is not capable by itself to initiate a full death program, but instead induces phosphatidylserine exposure which prepares immune cells for phagocytic removal (43). Moreover, a recent report showed that galectin-1-induced T-cell death involves degradation of fodrin, a cytoskeletal adaptor that links CD45 to actin cytoskeleton (44). More importantly, the repertoire of *N*- and *O*-glycan structures expressed by activated or differentiated T cells (T_H1, T_H2, or T_H17 cells), as well as the regulated expression and activity of particular glycosyltransferases are critical for galectin-1-induced T-cell death (45, 46). Supporting this notion, expression of the C2GnT1 can determine the susceptibility to galectin-1. This enzyme is responsible of creating and elongating the core 2 branch on *O*-glycans, thus allowing the incorporation of *N*-acetyllactosamine sequences, which are the preferred saccharide ligands of galectin-1 (47). On the other hand, the regulated expression and activity of the α2,6 sialyltransferase 1 (ST6Gal 1) can modify LacNAc ligands by the addition of sialic acid in α2-6-position of terminal galactose, which substantially blocks galectin-1 binding and abrogates galectin-1-induced cell death (45, 48). Therefore, susceptibility to galectin-1-induced T-cell death may be regulated at two distinct levels: (a) the presence of a restricted set of cell surface glycoproteins (e.g.,: CD43, CD45, or CD7) (49), whose segregation into membrane microdomains allows signaling events and activation of specific downstream effector molecules (49) and (b) the regulated expression of a set of glycosyltransferases responsible for creating or masking cell surface glycoconjugates (47, 48).

Similar to galectin-1, galectin-2 binds to β_1 integrins and triggers the death of activated T cells. This "proto-type" lectin triggers the activation of the intrinsic apoptotic pathway which involves caspases-3 and -9, cytochrome *c* release, disruption of the mitochondrial membrane potential, and increase in the Bax/Bcl-2 ratio (50). Also, the "tandem-repeat" lectin galectin-9 can induce T-cell apoptosis, an effect which is prevented by enforced expression of the anti-apoptotic protein Bcl-2 (51) and occurs via the Ca^{2+}-calpain-caspase-1 pathway (52). On the other hand, contrasting results have been reported for galectin-3 depending on its subcellular localization (14). Intracellular expression of galectin-3 has been mainly linked to an intrinsic anti-apoptotic effect of tumor and immune cells (14). In vivo evidence has been obtained through the analysis of galectin-3-deficient (*Lgals3*$^{-/-}$) mice, whose peritoneal macrophages were much more prone to IFN-γ- or LPS-induced apoptosis than with their wild-type counterpart (53). Within the tumor microenvironment, phosphorylated galectin-3 has been shown to protect BT549 human breast carcinoma cells from anoikis (a type of cell death elicited by the

loss of cell anchorage) (54, 55). Interestingly, galectin-3 can display either anti- or pro-apoptotic activities against the same tumor necrosis factor-related (TRAIL) apoptotic stimuli depending on the target cell type. Overexpression of galectin-3 in J82 human bladder carcinoma cells rendered these cells resistant to TRAIL-induced apoptosis, an effect which resulted from the activation of PI3K/Akt pathway (56), whereas galectin-3-transfected BT549 human breast carcinoma cells were paradoxically more sensitive to TRAIL-induced apoptosis through Akt inactivation (57). The mechanisms underlying these contrasting effects are still not clear. Similarly, the effects of galectin-3 on T-cell survival were found to be strongly dependent on its subcellular localization (14, 58). Exogenous galectin-3 can induce the formation of "lectin–glycoprotein lattices" which engage a T-cell apoptotic program by rising intracellular (Ca^{2+}) levels, augmenting cytochrome *c* release, and promoting caspase-3 activation (58–60). On the contrary, intracellular expression of galectin-3 conferred resistance to apoptosis induced by a variety of agents including Fas ligand (CD95L) and chemotherapeutic agents through regulation of mitochondrial integrity and reduction of reactive oxygen species (ROS) (61–63). In fact, galectin-3-transfected Jurkat T cells survive significantly longer when treated with a pro-apoptotic anti-CD95 (APO-1/Fas) monoclonal antibody (61). In this regard, two primary CD95-mediated apoptotic signaling routes have been described: (a) type I cells in which apoptosis is regulated through large amounts of caspase-8 activated by the death-inducing signaling complex (DISC) and (b) type II cells in which DISC and activated caspase 8 favor the apoptogenic activity of mitochondria through the release of cytochrome *c* and activation of caspase 3. Although type I cells express high amounts of galectin-3, type II cells appear to be negative for this protein. Notably, transfection with galectin-3 converted type II into type I apoptotic tumor cells, suggesting that galectin-3 can act directly at early signaling events of the CD95 pathway by promoting DISC formation and/or recruitment (64). An interesting observation is that galectin-3 translocates into the mitochondria when cells are exposed to apoptotic stimuli through a mechanism that involves binding to sinexin (62). Interestingly, a careful analysis of the galectin-3 primary sequence revealed the presence of four amino acids resembling the "anti-death-motif NWGR" (Asp-Trp-Gly-Arg) that is present in the BH1 domain of the Bcl-2 protein. This sequence is highly conserved among galectin-3 from different species and appears to be essential for its carbohydrate-binding activity (65). Even when galectin-3 was not capable of regulating the expression levels of any member of the Bcl-2 family (65), this lectin specifically interacted with Bcl-2 in a lactose-inhibitable fashion (61). Further studies are required to fully understand the way galectin-3 can differentially influence cell survival.

Also galectin-7 is engaged in the regulation of cell fate. Galectin-7 gene (*Lgals7*) is an early transcriptional target of the tumor suppressor p53 (66). Galectin-7 is upregulated in UVB-irradiated epidermal keratinocytes, and ectopic expression of galectin-7 made these cells more prone to undergo apoptosis compared with their normal counterpart (67). The pro-apoptotic properties of galectin-7 have been demonstrated using different cell types and distinct stimuli: a squamous cell line (67), HeLa cells, and the colon carcinoma cell line DLD-1 (68). Upon apoptosis induction, all galectin-7 transfectants displayed upregulation of c-Jun *N*-terminal kinase (JNK) activity, caspase-3 activation, and cytochrome *c* release (68). Remarkably, galectin-7-deficient keratinocytes were found to be protected from irradiation-induced apoptosis (69). Completing this picture, galectin-8 recently has been shown to modulate T-cell survival (70, 71) and even a galectin homologous isolated from liver chicken (CLL-I) can promote T-cell death (72), suggesting that galectin–glycan lattices are highly conserved molecular systems endowed with an intrinsic ability to regulate cell fate.

3.2. Galectins in the Control of T-Helper Cytokine Balance

Glycosylation can change dramatically not only in cancerous tissues, but also during physiological processes including immune cell activation, homing, and differentiation, resulting in the creation or masking of specific carbohydrate ligands for endogenous lectins (2, 73). A clear example illustrating this concept is the differential glycosylation of cell surface glycoproteins which can selectively control the survival of T-helper cells by modulating their susceptibility to galectin-1 (45). Although T_H1 and T_H17 differentiated cells express the repertoire of cell surface glycans that are required for galectin-1 binding and subsequent cell death, T_H2 cells are protected from galectin-1 through α2-6 sialylation of cell surface glycoproteins (45). In keeping with this finding, galectin-1-deficient (*Lgals1*$^{-/-}$) mice showed an increased frequency of T_H1 and T_H17 cells and enhanced the susceptibility to autoimmune neuroinflammation (45). These observations unveiled a molecular link among differential glycosylation of T-helper cells, susceptibility to cell death, and termination of the inflammatory response. Accordingly, recent studies showed that T_H2 cells promote T_H1 cell apoptosis through the secretion of galectin-1 (74), suggesting a galectin-1-dependent mechanism of counter-regulation between distinct T-helper subsets. In addition, galectin-1 can modulate the cytokine balance independently of its ability to modulate the lifespan of T cells. While exposure to galectin-1 downregulates IFN-γ production, this glycan-binding protein favors the synthesis of T-cell-derived IL-10, IL-5, and TGF-β_1 (75–78).

Tim-3 (T-cell immunoglobulin mucin 3) was identified as a T_H1-specific cell surface molecule that controls T_H1 responses and regulates T-cell tolerance. In search for specific Tim-3 ligands,

Zhu et al. identified galectin-9 as a specific binding partner capable of stimulating intracellular calcium flux, promoting aggregation, and inducing selective death of T_H1 cells (35, 79). Interestingly, galectin-9 can also suppress the differentiation of T_H17 cells both in vitro and in vivo independently of its ability to induce T-cell apoptosis (80).

Given the established role of IFN-γ-producing T_H1 cells and the controversial activity of IL-17-producing T_H17 cells in tumor growth, understanding the function of galectin–glycan lattices in the regulation of cytokine balance may contribute to define the hierarchical function of these cell subsets in controlling different stages of cancer immunoediting.

3.3. Galectins in T-Cell Signaling, Activation, and Anergy

Tumor cells use multiple tolerogenic strategies to subvert immune responses including inhibition of T-cell signaling and the promotion of T-cell anergy. Cell surface inhibitory receptors including CTLA-4, PD-1, and other molecules associated to immunoreceptor tyrosine-based inhibition motifs (ITIMs) play a crucial role in delivering negative signals that regulate the balance between T-cell activation, tolerance, and immunopathology (81). Although limited information is available on the role of galectin-1 in T-cell receptor (TCR)-mediated T-cell activation, this protein has been reported to modulate T-cell signaling at sites of immunological synapse (82). Liu et al. found that galectin-1 acts as an autocrine negative regulator of TCR binding, signal transduction, and burst size of $CD8^+$ T cells (82). Moreover, Demetriou and colleagues provided elegant evidence demonstrating that galectin-3–*N*-glycan lattices can restrict spontaneous TCR clustering and downmodulate TCR responses by interacting with *N*-glycans modified by the enzyme *N*-acetylglucosaminyltransferase 5 (Mgat5). In this regard, galectin-3 co-localized with CD45 suppressed Lck activity and TCR signaling (83). These effects may have critical implications at the cross-roads of T-cell responsiveness and tolerance during tumor progression. In keeping with this notion, delivery of high doses of recombinant galectin-3 suppressed the activation of tumor-reactive T cells and promoted tumor growth in mice receiving tumor-reactive $CD8^+$ T cells (32).

In addition, at late stages of T-cell activation, galectin–glycan lattices can contribute to the termination of immune responses by promoting cell surface retention of the inhibitory molecule CTLA-4, thus favoring T-cell growth arrest (84). In this regard, a very elegant study highlighted an essential role for galectin-3–*N*-glycan interactions in mediating anergy of tumor-specific cytotoxic T lymphocytes by favoring the segregation of CD8 from the TCR (85), suggesting the possibility to bypass T-cell anergy by interfering with lectin-glycan lattices. Supporting a role for this protein in immune cell silencing, both galectin-1 and galectin-3 were found to be upregulated in anergic B cells (86).

Therefore, galectin–glycan lattices may have evolved as endogenous homeostatic systems to prevent spontaneous T-cell activation and "turn-off" T-cell effector functions after the completion of an immune response. In turn, these interactions may contribute to delineate the typical tolerogenic microenvironment usually found at sites of tumor growth and metastasis.

3.4. Galectins and the Function of T_{Reg} Cells

In the past years, a subset of T_{Reg} cells expressing CD4 and CD25 and the transcription factor FoxP3 have gained considerable attention and popularity as key regulators of T-cell tolerance and homeostasis (87). This population of T cells is specifically engaged in the maintenance of immune self-tolerance and the control of exuberant immune responses to foreign antigens. In addition, T_{Reg} cells have been proposed to be critical obstacles that hinder antitumor immunity and favor tumor–immune escape (88, 89). Investigation of gene and protein expression profiles has shown the upregulation of galectin-1 in human and mouse $CD4^+CD25^+Foxp3^+$ T_{Reg} cells which substantially contributes to the immunosuppressive activity of these cells (90,91). Investigation of the mechanisms underlying this inhibitory activity revealed a critical function of GM1 as a potential receptor for galectin-1 capable of mediating TRPC5 channel activation on effector T cells (92). Interestingly another "proto-type member" of the galectin family, galectin-10, was also identified as a marker of human $CD4^+CD25^+FoxP3^+$ T_{Reg} cells which appeared to be essential for the suppressive activity of these cells (18).

In addition to its upregulated expression in regulatory versus effector T cells, recent findings underscored the capacity of galectin-1 to increase the relative abundance and/or expansion of peripheral T_{Reg} cells in vivo (77). Administration of recombinant galectin-1 during the efferent phase of ocular inflammatory disease resulted in a remarkable increase of the immunosuppressive cytokines IL-10 and TGF-β_1, which in turn promoted the expansion and/or activation of IL-10-producing T_{Reg} cells (77). This effect was also confirmed in a model of stress-induced fetal rejection, as injection of galectin-1 restored tolerance by promoting the expansion of IL-10-producing $CD4^+CD25^+$ T_{Reg} cells (93). Also, in Hodgkin lymphoma, tumor-derived galectin-1 could induce the differentiation of $CD4^+CD25^+$ T_{Reg} cells in vitro (28). This effect was not limited to galectin-1, as galectin-9 was also capable of modulating the T_{Reg} cell compartment. Exposure to galectin-9 in vitro induced the differentiation of $FoxP3^+$ T_{Reg} cells, while simultaneously counteracted the generation T_H17 pathogenic cells. The possible therapeutic benefits of these findings were evident in mouse model of collagen-induced arthritis (CIA) where galectin-9 administration ameliorated the arthritogenic process and induced decreased levels of the pro-inflammatory cytokines IL-17, IL-12, and IFN-γ in the joint (80). In line with these

findings, blockade of the Tim-3-galectin-9 pathway also resulted in substantial attenuation of the suppressive activity of T_{Reg} cells (94). Collectively, these findings highlight the potential role of galectin–glycan interactions in controlling T_{Reg} cell differentiation, expansion, and recruitment to the tumor microenvironments.

3.5. Galectins in the Control of Antigen-Presenting Cells

In spite of their critical role in orchestrating adaptive immunity, bone marrow-derived antigen-presenting cells (APCs), particularly DCs, are now considered the pivotal cell type involved in the induction and maintenance of T-cell tolerance in vivo (95, 96). DCs can promote peripheral tolerance by promoting the differentiation of T_{Reg} cells, including $CD4^+CD25^+FoxP3^+$ T_{Reg} cells and type-1 T_{Reg} (Tr1) cells (96, 97). Multiple factors can influence the decision of DCs to become tolerogenic, including the recognition of apoptotic cells (98), interaction with stromal cells (99), and exposure to an immunosuppressive tumor microenvironment (81). Furthermore, DCs modified by $CD4^+CD25^+FoxP3^+$ T_{Reg} cells may become tolerogenic and drive the differentiation of IL-10-producing Tr1 cells (100), suggesting a link among distinct regulatory cell populations. Given the key role of DCs at the interface of innate and adaptive immunity, it is not surprising that lectin–glycan interactions may play an important role in regulating the biological activity of these cells. In this regard, we recently identified an essential function for galectin-1 in the generation of human and mouse tolerogenic DCs. DCs differentiated in the presence of galectin-1 acquired a distinctive regulatory profile, promoted T-cell tolerance in vivo, and terminated autoimmune neuroinflammation through an immunoregulatory circuit involving IL-27 and IL-10 (101). Exposure to galectin-1 during the maturation process induced the generation of DCs with a typical mature cell surface phenotype but dominant regulatory function. In addition, we have identified a pivotal role for endogenous galectin-1 in "fine tuning" the tolerogenic function of DCs (101). Consistent with these findings, progesterone-regulated galectin-1 could restore immune tolerance in failing pregnancies and this effect correlated with the expansion of T_{Reg} cells and the appearance of uterine cells with a regulatory DC phenotype (93).

Given the plasticity of DCs, induction of a tolerogenic profile might be exploited therapeutically in order to attenuate autoimmune diseases or prevent graft rejection. On the contrary, silencing DC regulatory pathways might augment DC-cell-based vaccination efficiency or potentiate tumor immunotherapeutic strategies (81). In this regard, vaccination with tumor lysate-pulsed DCs holds a promise to treat immunogenic tumors (102). However, the protective function of DCs could be thwarted if these cells are rendered tolerogenic, as often occurs at the sites of tumor growth (81). Hence, it is the balance between immunogenic and tolerogenic signals that determines the effectiveness of immunotherapeutic strategies.

Supporting this notion, we have recently demonstrated an in vivo neoplasic setting that tumor-pulsed DCs differentiated in the presence of galectin-1 (DC_{Gal1}) may become tolerogenic and fail to suppress tumor growth or elicit effective T-cell responses (101). Surprisingly, all mice immunized with tumor-pulsed DC_{Gal1} developed progressively enlarging tumors when challenged with viable melanoma cells at a rate similar to that of mice receiving unpulsed DCs or vehicle control. Interestingly, co-injection of tumor-pulsed DCs and tumor-pulsed DC_{Gal1} resulted in accelerated tumor, confirming a dominant tolerogenic effect of tumor-pulsed DC_{Gal1}, which prevented the protective effect of tumor-pulsed control DCs. Consistently, lymph node cells from mice receiving tumor-pulsed DC_{Gal1} or a mixture of tumor-pulsed DCs, and tumor-pulsed DC_{Gal1} showed poor proliferative responses, reduced synthesis of IFN-γ, and enhanced secretion of IL-10 (101). Thus, DCs differentiated in a galectin-1-enriched microenvironment cannot elicit an effective T-cell response against tumor challenge and instead skew the cytokine balance to foster a tolerant milieu at sites of tumor growth. Adding complexity to this system, recent findings suggested that galectin-1 can also regulate DC migration through modulation of Syk and protein kinase C (PKC) signaling (103). Thus, galectin-1 may control the motility of DCs, which upon arrival to sites of inflammation or tumor growth could be endowed with tolerogenic potential.

While galectin-1 expression was found to be selectively upregulated by tolerogenic stimuli including IL-10, vitamin D3, and apoptotic cells (101), the expression of galectin-9 is peaked following exposure to maturation signals such as IFN-γ and IL-1β (104). Accordingly, galectin-9 triggered the maturation of human monocyte-derived DCs through activation of the p38 MAPK pathway (105). In this regard, Tim-3, which is expressed at high levels on human and mouse DCs, has been identified as a candidate receptor for galectin-9 (106). Ligation of Tim-3 with galectin-9 synergized with Toll-like receptors (TLRs) initiate T_H1-type immunity (106). Because Tim-3 cross-linking also dampens T_H1 responses (35, 106), it has been speculated that galectin-9–Tim-3 interactions may have different effects during the initiation and termination of immune response. In this regard, recent findings supported a critical role for galectin-9 in potentiating tumor-specific T-cell responses through enhancement of Tim-3-mediated DC–$CD8^+$ T-cell interactions (36). Finally, DCs from galectin-3-deficient (*Lgals3*$^{-/-}$) mice had decreased migratory potential, but instead secreted higher amounts of IL-12 and showed increased T-cell stimulatory capacity (107–109). Although these functions have not been studied in tumor settings, they strongly suggest an essential role for these lectins in fine-tuning DC physiology. Thus, galectin–glycan interactions may have evolved to regulate APC homeostasis and control their activation, signaling, and motility.

4. Concluding Remarks

Changes in glycosylation are a typical hallmark of pathological processes including inflammation, infection, autoimmunity, and cancer. These alterations can be of higher magnitude than those displayed by "the proteome" during cancer progression and metastasis. Galectins, a family of endogenous lectins found at sites of tumor growth and inflammation, can recognize and discriminate subtle changes on glycan structures displayed on the surface of tumor, stromal, and immune cells. Although overlooked for a long time, the key importance of galectin–glycan interactions in cancer progression is now undisputed. These interactions can lead to substantial changes in the malignant process including tumor cell adhesion, migration, angiogenesis, and immune escape. In this context, recent findings had shed light to an essential role of these lectins in the regulation of immune tolerance and inflammation. Galectin–glycan lattices can influence multiple tolerogenic mechanisms by modulating T-cell survival and signaling, controlling cytokine synthesis, promoting the differentiation and/or expansion of T_{Reg} cells, and "fine-tuning" DC physiology.

Although much remains to be learned, it is likely that galectin–glycan lattices can regulate tumor immunoediting by bridging tumor, stromal, and immune cells. Due to the recent breakthrough of "proteomics" and "glycomics," protein–glycan interactions have become more amenable of therapeutic approaches, suggesting novel anticancer strategies using small glycomimetic inhibitors, siRNA approaches, or galectin-specific blocking antibodies.

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