Immunity
Review

Regulatory Circuits Mediated by Lectin-Glycan Interactions in Autoimmunity and Cancer

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Numerous regulatory programs have been identified that contribute to the restoration of homeostasis at the conclusion of immune responses and to safeguarding against the detrimental effects of chronic inflammation and autoimmune pathology. Malignant cells may usurp these pathways to create immunosuppressive networks that thwart antitumor responses. Herein we review the role of endogenous lectins (C-type lectins, siglecs, and galectins) and specific N- and O-glycans generated by the coordinated action of glycosyltransferases and glycosidases that together promote regulatory signals that control immune cell homeostasis. We also discuss the mechanisms by which glycan-dependent regulatory programs integrate into canonical circuits that amplify or silence immune responses related to autoimmunity and neoplastic disease.

Merging Glycobiology and Immunology to Understand Immune Tolerance

The immune system faces the difficult challenge of discerning between "self" and "non-self" and simultaneously defending against microbial pathogens. Although self-reactive T cells can be deleted in the thymus, the elimination process is often incomplete, and therefore activation of regulatory mechanisms in peripheral tissues is required in order to dampen potentially harmful responses. Homeostatic signals delivered in the form of immunosuppressive cytokines or inhibitory receptors are integrated into tolerogenic circuits that sustain peripheral tolerance mechanisms. These mechanisms, including T cell deletion, anergy, and expansion of regulatory T (Treg) cells, serve to prevent collateral tissue damage resulting from overexuberant immune responses to pathogens or seemingly innocuous environmental stimuli (Bluestone et al., 2010). At a more intimate cellular level, regulatory signals can influence trafficking, clustering, and endocytosis of signaling receptors, thereby modulating the threshold of immune cell activation, differentiation, and survival and ultimately dictating immune cell responsiveness or tolerance. Although there is still no integrated portrait of these regulatory circuits, disruption of single pathways may result in substantial and unpredictable inflammatory and autoimmune states. Conversely, their aberrant activation represents a hurdle for the development of antitumor immunity. Although underappreciated for many years, exciting findings underscore the essential contribution of cell surface glycosylation and lectin-glycan signaling to regulatory circuits operating in immune tolerance and homeostasis.

Glycosylation is a common posttranslational modification to secretory and membrane-anchored proteins and lipids that become altered in this fashion when crossing the lumen of the endoplasmic reticulum and the Golgi apparatus. The glycosylation machinery is responsible for assembling a diverse and abundant repertoire of glycan structures, collectively termed the glycome, through the synchronized action of a portfolio of glycosyltransferase and glycosidase enzymes. To create the large repertoire of glycan structures, each of these glycosyltransferases uses a single nucleotide-sugar substrate and forms specific linkages between one monosaccharide and a glycan precursor. The nature and extent of glycosylation of a given protein depends on the presence of N- and O-linked glycosylation sites in the protein backbone, as well as on the activities of particular glycosyltransferases and glycosidases within a specific cell or tissue (Marth and Grewal, 2008). The diversity of the glycome (which adds to the diversity already introduced by the genome and proteome) and the pronounced immunological phenotypes observed upon disruption of glycosyltransferases or lectin genes reflect the central role played by glycosylation in the establishment or disarmament of immune cell networks. Combinatorial possibilities of glycan presentation during immune cell activation, differentiation, and signaling and their aberrant expression during the transition from normal to inflamed or neoplastic tissue provide a vast potential for information display (Dube and Bertozzi, 2005). Although deciphering this information has proven to be challenging because of the non-template nature of carbohydrate synthesis and the heterogeneity of glycosylation patterns, endogenous glycan-binding proteins or lectins can specifically decode saccharide structures and glycosidic linkages and convey this structural information into functional cellular responses (van Kooyk and Rabinovich, 2008).

Despite phylogenetic conservation of many genes encoding components of the glycosylation machinery, considerable intra- and interspecies variations have been detected among glycans, suggesting a prominent role as danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) that are crucial for discrimination of self from non-self (Varki, 2011). The contribution of lectin-glycan systems to pathogen recognition and immune cell trafficking has recently been reviewed (Sato et al., 2009; Osorio and Reis e Sousa, 2011; Sperandio et al., 2009). Herein we focus on the mechanisms by which lectin-glycan interactions control regulatory

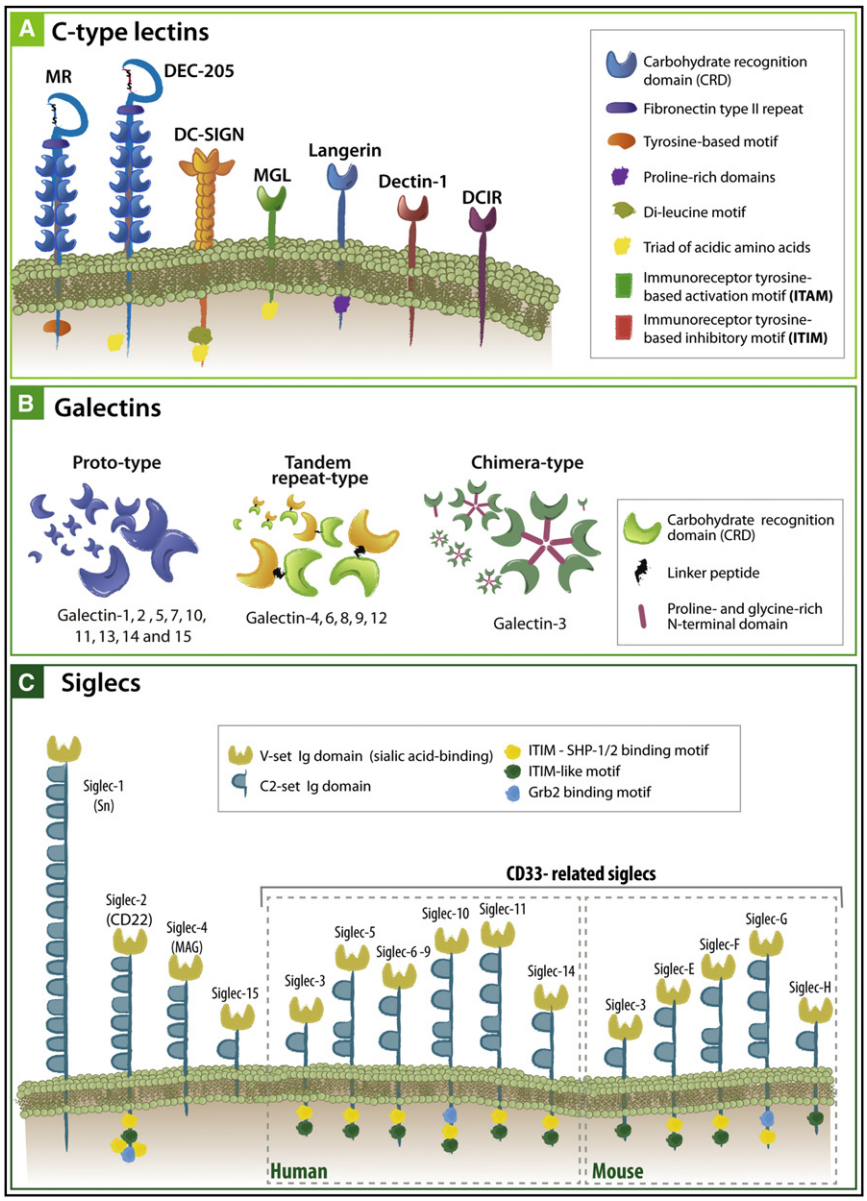

Figure 1. Schematic Representation of the Structure of Glycan-Binding Proteins Involved in Immune Cell Homeostasis

(A) Most CLRs are glycan-binding receptors that contain one or more carbohydrate-recognition domains (CRDs) (although exceptions exist like NK cell surface-associated CLRs that are not implicated in saccharide recognition). Selected members (MR, DEC-205, DC-SIGN, MGL, Langerin, Dectin-1, and DCIR) that play key roles in APC regulatory programs are shown.

(B) Galectins are subdivided into prototype galectins, which contain one carbohydrate recognition domain (CRD) and can form homodimers; tandem-repeat galectins that contain two distinct CRDs in tandem connected by a linker peptide; and the unique chimera-type Galectin-3, which consists of unusual tandem repeats of proline and glycine-rich short stretches fused onto the CRD.

(C) Siglecs are grouped into the most distantly interrelated members such as sialoadhesin (Sn; Siglec-1), CD22 (Siglec-2), and myelin-associated glycoprotein (MAG; Siglec-4) and CD33-related siglecs that share sequence similarity.

cell programs and activate tolerogenic circuits that temper autoimmune inflammation and shape antitumor immunity.

Deciphering the "Glyco-Code:" Lectin Recognition Systems in Immunity

Lectins recognize complex glycan determinants with relatively high affinity in the submicromolar range. In fact, it is the structure, number, and density of glycan epitopes in multivalent glycoproteins, as well as the density of the glycoproteins expressed on the cell surface and the multivalent nature of some lectins, which together determine the avidity of lectin-glycan interactions (Cummings and Esko, 2009; Dam and Brewer, 2010). Modeling of these interactions, based on the analysis of structural determinants of purified molecules, revealed the formation of two- and three-dimensional arrangements of multivalent lectins and glycans often termed "lattices" (Dam and Brewer, 2010). Yet, these high-ordered supramolecular complexes need to be further characterized at the cellular level by both in vitro and in vivo visualization approaches. Nevertheless, multivalent lectin-glycan complexes have been proposed to serve as scaffolds for organizing plasma membrane domains, which in turn modulate the signaling threshold of relevant surface glycoproteins including the T cell receptor (TCR), B cell receptor (BCR), and specific cytokine receptors (Dennis et al., 2009).

We focus here on three lectin families that are involved in glycan recognition and signaling in the immune system: C-type lectins, siglecs, and galectins (Figure 1). C-type lectin receptors (CLRs) are a heterogeneous family of Ca^{2+}-dependent glycan-binding proteins that can be divided into two categories on the basis of an amino acid motif involved in glycan recognition and coordination of the Ca^{2+} ion. Most CLRs are glycan-binding receptors that contain one or more carbohydrate-recognition domains (CRDs) and are expressed on the surface of numerous cell types including macrophages and dendritic cells (DCs). CLRs that contain an EPN (Glu-Pro-Asn) amino acid motif typically have specificity for mannose- or fucose-containing glycans ($Lewis^{a,b,x,y}$). These include the DC-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), mannose receptor (MR), and langerin. In contrast, galactose-specific CLRs, such as macrophage galactose lectin (MGL), contain a QPD (Gln-Pro-Asp) sequence and recognize N-acetylgalactosamine (GalNAc)-terminated glycans (Drickamer, 1999). However, CLRs displaying Ca^{2+}-independent glycan recognition also exist, including Dectin-1, which specifically recognizes β-glucan

structures on yeasts (Rogers et al., 2005). The role of CLRs as endocytic pattern recognition receptors and their ability to influence Toll-like receptor (TLR) signaling is well established (Osorio and Reis e Sousa, 2011); however, their function as signaling receptors capable of delivering tolerogenic signals in response to antigen recognition is not as clearly understood.

Sialic acid-binding immunoglobulin-like lectins (siglecs) constitute a major group of the immunoglobulin type (I-type) lectins. These lectins are well known for their specificity for sialic acid-containing glycans. They can be subdivided into two subsets: the most distantly interrelated group (25%–30% sequence identity), which includes Sialoadhesin (Sn; Siglec-1), CD22 (Siglec-2), and myelin-associated glycoprotein (MAG; Siglec-4), and the rapidly evolving group of CD33-related siglecs that share high sequence similarity (50%–99%) (Siglec-3, -5, -6, -7, -8, -9, -10, -11, and -14 in humans and Siglec-E, -F, -G, and -H in mouse) (Crocker et al., 2007). Siglecs are unique in their dual ability to mediate *cis* and *trans* interactions with sialylated glycans. Although in *cis* interactions the siglec is often masked by low-affinity ligands on neighboring membrane receptors, these linkages do not prevent *trans* interactions with other cell types. Although some siglecs have a restricted expression pattern, others are more widely expressed among hematopoietic cell lineages. For example, Sn is mainly expressed by macrophages, CD22 by B cells, and Siglec-8 and Siglec-F by eosinophils. Interestingly, Siglec-9 and Siglec-E are selectively expressed on myeloid DCs, whereas Siglec-5 and Siglec-H are expressed on plasmacytoid DCs. For most CD33-related siglecs and CD22, ligand engagement results in phosphorylation of ITIMs by Src family tyrosine kinases and recruitment of SHP phosphatases, which attenuate tyrosine phosphorylation (Crocker et al., 2007).

In contrast to CLRs and siglecs, galectins are soluble proteins that function in the extracellular milieu by interacting with a myriad of cell surface glycosylated ligands (Rabinovich and Toscano, 2009). However, these lectins also play roles inside the cells including modulation of signaling and splicing machineries (Liu et al., 2002). Among the various lectin families, galectins are probably the most conserved and ubiquitous with members identified in most animal taxa examined so far (Vasta, 2012). Although galectins do not have the signal sequence required for the classical secretory pathway, most of them are externalized through a nonclassical mechanism that is still poorly understood. "Prototype" galectins (Galectin-1, -2, -5, -7, -10, -11, -13, -14, and -15) have one CRD that can dimerize, whereas "tandem-repeat" galectins (Galectin-4, -6, -8, -9, and -12) contain two homologous CRDs in tandem in a single polypeptide chain. Galectin-3 is unique as it contains a CRD connected to a non-lectin N-terminal region that is responsible for oligomerization (Figure 1).

Although originally defined by their ability to recognize the disaccharide N-acetyllactosamine [Galβ(1–4)-GlcNAc; LacNAc], substantial differences exist in glycan-binding preferences of individual members of the galectin family, particularly in the recognition of sialylated and sulfated glycans, which might explain differences in biological activity (Hirabayashi et al., 2002; Stowell et al., 2008; Ideo et al., 2011). Interestingly, although some galectins are widely expressed in immune cells and tissues, others have a more limited cellular distribution. For example, Galectin-1 is considerably upregulated in activated T and B cells, inflammatory macrophages, tolerogenic DCs, decidual natural killer (NK) cells, and $CD4^+CD25^+$ Treg cells, whereas Galectin-10 expression appears to be restricted to eosinophils and $CD4^+CD25^+$ Treg cells. Noteworthy, different intrinsic and extrinsic factors may control the biological activity of galectins including (1) their oligomerization status, (2) their stability in reducing or oxidative microenvironments, and (3) the active remodeling of N- and O-glycans on target cells (Rabinovich and Toscano, 2009).

How do endogenous lectins (CLRs, siglecs, and galectins) translate information encoded by glycosylated ligands into regulatory programs that control immune cell homeostasis? In the next sections we integrate pioneering work and recent findings that facilitate our understanding of the role of lectin-glycan interactions in promoting immune cell homeostasis.

Tuning Activation and Signaling Thresholds

Tunable signaling thresholds allow immune cells to interpret the context in which ligand is presented, which facilitates triggering of activation or tolerogenic programs. Multivalent interactions between endogenous lectins and glycosylated receptors profoundly affect signaling thresholds by reducing the rate of receptor trafficking, bridging association with other glycoproteins, limiting receptor clustering, and/or preventing receptor endocytosis (Figure 2). An outstanding example was provided by functional studies of the TCR which is modified by complex N-glycans generated by the enzyme N-acetylglucosaminyltransferase 5 (GnT5; encoded by the *MGAT5* gene). This enzyme generates the β1,6 N-glycan branch structure, which represents a limiting step for the generation of high-affinity ligands for galectins. Multivalent interactions between complex N-glycans and Galectin-3 can limit TCR clustering at sites of immune synapse by restricting lateral TCR movement within the plane of the membrane, thus increasing agonist thresholds for TCR signaling. Remarkably, lack of β1,6 N-glycan branching in GnT5-deficient mice lowers the threshold for T cell activation by enabling TCR clustering and signaling characterized by enhanced TCR-dependent tyrosine phosphorylation and robust proliferation. This effect results in augmented delayed-type hypersensitivity reactions and increased susceptibility to autoimmune disease (Demetriou et al., 2001). Although this study provides compelling evidence for the role of GnT5-modified N-glycans in TCR signaling, the direct involvement of endogenous Galectin-3 (or other galectins) in this effect requires further characterization. Dissection of the underlying mechanisms reveal that, in the absence of cognate ligand, cross-linking of N-glycans prevents filamentous actin-dependent targeting of the TCR, CD4, and Lck tyrosine kinase to GM1-enriched membrane microdomains. This prevents spontaneous TCR activation by favoring Lck inactivation and specifically retaining the CD45 phosphatase at these membrane domains (Chen et al., 2007). Of note, a feedback loop has been proposed because TCR signaling further stimulates transcription of N-glycan-processing enzymes including α-1,2-mannosidase II (αM-II) and GnT5, which final product contributes to T cell growth arrest and tolerance (Chen et al., 2009a). In this regard, β1,6-GlcNAc branching appears to be sensitive to the availability of metabolites of the hexosamine pathway because higher concentrations of the cellular donor UDP-GlcNAc

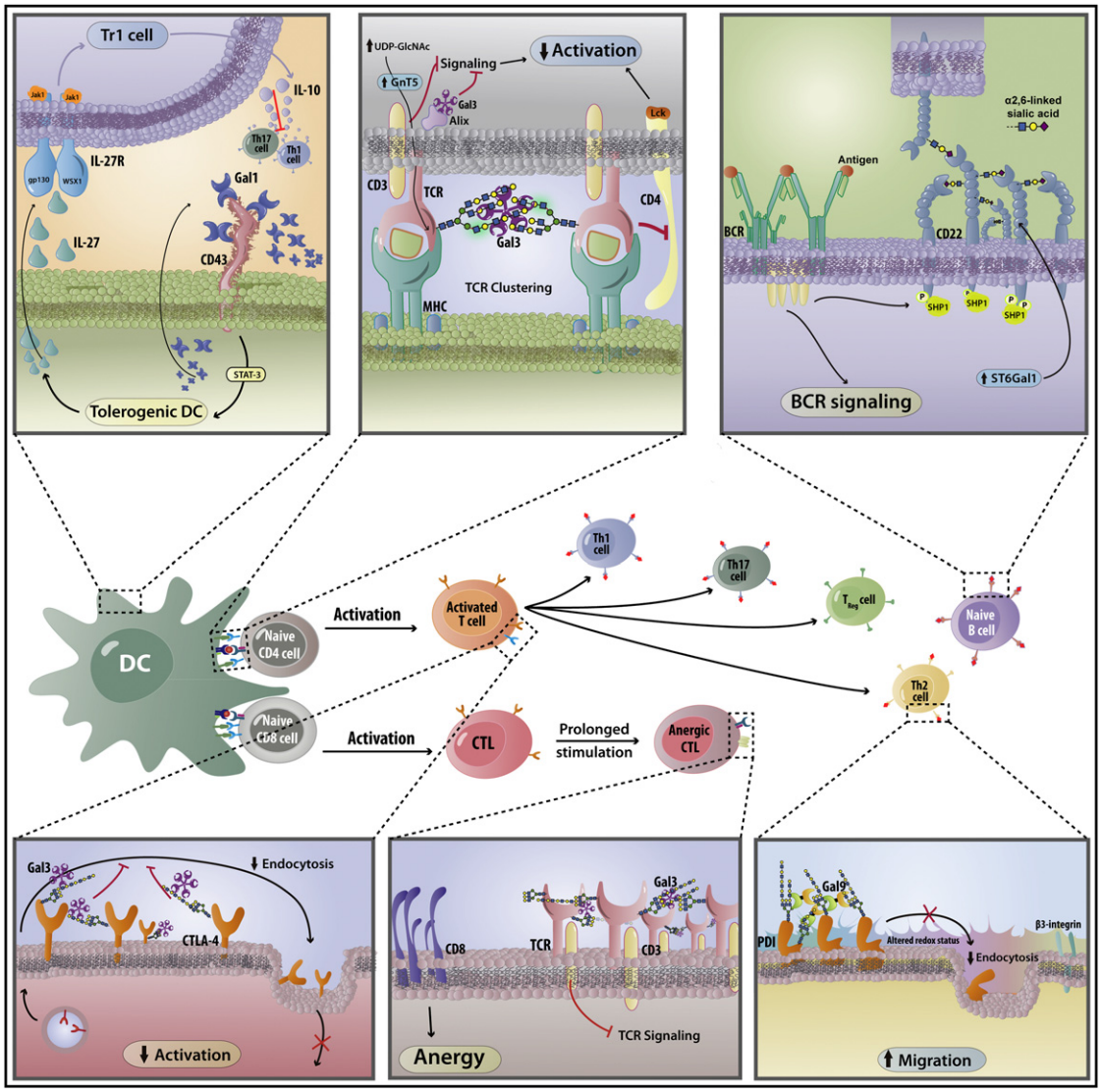

Figure 2. Homeostatic Control of Immune Cellular Programs by Lectin-Glycan Interactions

During the course of adaptive immune responses, multivalent lectin-glycan interactions profoundly affect the thresholds of immune cell activation, differentiation, and survival by modulating the organization, trafficking, clustering, and endocytosis of signaling receptors (selected examples are shown as insets). In DCs, Galectin-1 (Gal1)-glycan interactions set the threshold that controls tolerogenic or immunogenic programs by favoring differentiation of IL-27-producing regulatory DCs that promote the differentiation of IL-10-producing type-1 regulatory T (Tr1) cells. In naive T cells, functional interactions between Galectin-3 (Gal3) and β1,6-branched N-glycans limit TCR clustering and increase agonist thresholds for TCR signaling. Intracellular Gal3 also restrains TCR signaling by inducing TCR downmodulation. In activated T cells, Gal3-N-glycan interactions prevent CTLA-4 endocytosis, thus allowing sustained delivery of inhibitory signals. In effector CTLs, Gal3 binding to complex N-glycans favor a state of anergy by distancing the TCR from CD8 molecules. In Th2-differentiated cells, Galectin-9 (Gal9) increases cell surface retention of the protein disulfide isomerase (PDI), which alters the redox status of the plasma membrane, thereby facilitating cell migration. In naive B cells, the threshold of BCR signaling is governed by Siglec-2 (CD22)-BCR interactions. CD22 binding to α2,6-sialylated ligands promotes homotypic CD22-CD22 interactions and prevents CD22 association with the BCR, thereby decreasing the threshold for BCR activation.

increases the capacity of GnT5 to catalyze N-glycan branch formation. Supporting this notion, oral administration of GlcNAc enhances N-glycan branching in vivo, dampens TCR signaling, blunts Th1 and Th17 cell responses, and attenuates autoimmune neuroinflammation (Grigorian et al., 2011). Thus, GnT5-modified complex N-glycans presented on cell surface glycoprotein receptors can control T cell activation threshold and tailor adaptive immunity.

Reinforcing this notion, cell surface galectin-glycoprotein complexes can also play an integral role in the control of the effector activity of cytotoxic T lymphocytes (CTLs). After several days of antigenic stimulation, CTLs become anergic and lose colocalization of the TCR with the glycoprotein CD8. Demotte et al. (2008) found that during this anergic state, Galectin-3 plays a key role through binding to TCR N-glycans and sequestering the TCR from CD8 molecules in both mouse models and human tumor-infiltrating lymphocytes. Loss of CTL function involves major alterations of the T cell glycome including the presence of larger LacNAc oligomers and reduced abundance of α2,6-linked N-glycans (Antonopoulos et al., 2012).

In addition to their role in TCR biology, galectin-glycan complexes may also function by trapping glycoprotein receptors at the cell surface and preventing their endocytosis. Receptor retention at the cell surface increases their responsiveness to extracellular inputs, prolongs intracellular signaling, and accentuates functional outputs. In this regard, interactions between Galectin-3 and GnT5-modified N-glycans on transforming growth factor-β receptor (TGF-βR) prolong Smad-dependent signaling and increase macrophage responsiveness to TGF-β_1 (Partridge et al., 2004). Likewise, complexes formed between galectins and complex N-glycans on cytotoxic T lymphocyte antigen-4 (CTLA-4) prevent endocytosis of this inhibitory receptor and amplify homeostatic circuits after T cell activation. In contrast to growth-promoting receptors, which display a high number of N-glycosylation sites per peptide (multiplicity), arrest-promoting receptors like TGF-βR and CTLA-4 have few N-glycosylation sites and display ultrasensitive responses to metabolic flux of UDP-GlcNAc to attain the branching required for lectin binding, surface retention, and growth arrest (Lau et al., 2007). Although CTLA-4 transcription considerably increases after 4–5 days of TCR signaling, the endosomal machinery limits cell surface expression of CTLA-4, keeping it below the threshold for the induction of growth arrest. Sustained TCR signaling favors GlcNAc branching and formation of galectin-N-glycan complexes, which enables CTLA-4 surface retention and delivery of TCR stop signals (Dennis et al., 2009). Another example of a low-multiplicity glycoprotein that is highly dependent on N-glycan branching is the glucose transporter GLUT-2. In this case, the limiting enzyme is the N-acetylglucosaminyltransferase 4a (GnT4a; encoded by the *MGAT4* gene), which extends N-glycan branching and prolongs the cell surface half-life of GLUT-2, thereby preventing its premature endocytosis. Because GLUT-2 and Galectin-9 colocalize on pancreatic β cells in a GnT4a-dependent manner, it is thought that Galectin-9-N-glycan interactions may act to retain glucose receptors to prevent the development of diabetes (Ohtsubo et al., 2005). Of note, although the role of GnT5 and GnT4a has been unequivocally demonstrated, the role of endogenous galectins in these processes still needs further characterization. In addition, multivalent complexes formed between Galectin-9 and the protein disulfide isomerase on the surface of Th2 cells have been shown to hinder internalization of this enzyme. Increased surface residency of disulfide isomerase alters the redox status of the plasma membrane, increases cell migration via β_3 integrins, and potentiates HIV infection (Bi et al., 2011).

Interestingly, Chen et al. (2009b) have shown that, in addition to its extracellular activities, Galectin-3 also acts intracellularly by promoting TCR downmodulation at sites of the immunological synapse via interaction with regulatory proteins such as Alix. Likewise, studies reveal that Galectin-1 produced by antigen-experienced $CD8^+$ T cells functions as an autocrine regulator that negatively controls TCR signaling (Liu et al., 2009). Moreover, analysis of major histocompatibility complex class I (MHC I)-restricted thymocyte development reveals an opposite role for this lectin during positive and negative selection. Although Galectin-1 antagonizes positive selection driven by partial agonists through inhibition of extracellular signal-regulated kinase (ERK) activity, this lectin facilitates negative selection induced by full TCR agonists by promoting transient ERK activation (Liu et al., 2008). These results support the essential role of galectin-glycan interactions in discriminating TCR activation thresholds and tuning T cell fate. In this direction, work from several groups reveal a major role of glycosylation, particularly O-fucosylation, in ligand-mediated Notch signaling during T cell development (Stanley and Guidos, 2009), suggesting diverse roles for lectins and glycans in dictating T cell fate.

However, the regulatory role of glycan-binding proteins is not limited to the T cell compartment. During normal B cell development, pre-BCR signaling is governed by glycosylation-dependent interactions between the pre-BCR, Galectin-1, and $\alpha_4\beta_1$, $\alpha_5\beta_1$, and $\alpha_7\beta_1$ integrins (Espeli et al., 2009). Recent evidence indicates that bone marrow pre-B cells progress from IL-7^+ to Galectin-1^+ supportive stromal cell niches, which differentially control B cell maturation (Mourcin et al., 2011). Whereas Galectin-1-mediated interactions shape the immature B cell compartment, the threshold of mature B cell signaling is dictated by the association of the BCR with CD22. This siglec binds α2,6-linked sialic acid-bearing ligands (generated by α2,6-sialyltranferase 1; ST6Gal1) that are localized on contiguous glycoproteins of the same cell (*cis* ligands) and on other interacting cells (*trans* ligands). In normal settings, CD22 binding to α2,6-sialylated ligands promotes homotypic CD22-CD22 interactions and prevents CD22 association with the BCR, thereby decreasing recruitment of SHP1 phosphatase and reducing the threshold for BCR signaling. B cells lacking α2,6-linked sialic acid show increased colocalization of CD22 and BCR, which favors SHP1 recruitment to CD22 cytoplasmic tail, increases BCR endocytosis, and markedly suppresses B cell activation and antibody-mediated responses. Accordingly, ST6Gal1 deficiency prevents autoimmune pathogenesis in a model of systemic lupus erythematosus (SLE). Notably, deficiency of both CD22 and ST6Gal1 prevents BCR internalization and restores BCR tonic signaling, suggesting the requirement of CD22 for the hypoactive phenotype displayed by sialic acid-deficient B cells (Collins et al., 2006; Grewal et al., 2006). Thus, whereas β1,6 N-glycan branching controls T cell activation thresholds, α2,6 sialylation sets the threshold for B cell activation by restricting the access of the BCR to CD22 and decreasing BCR endocytosis.

"Sweetening" Cell Death Decisions

Several molecular checkpoints acting during the induction, execution, and resolution phases of cell death dictate the immunogenic or tolerogenic nature of this process. Lectin-glycan recognition systems can influence lethal signaling programs by directly transmitting death signals, by tuning the function of canonical death receptors, or by providing "find me" and "eat me" signals that promote efficient clearance of dying cells.

It has become increasingly apparent that sialylation of N- and O-glycans serves as an "on-and-off" switch that controls life and death decisions. The α2,3 sialyltransferase 1 (ST3Gal1) competes with the core-2-β1-6-N-acetylglucosaminyltransferase 1 (GCNT1; encoded by *C2GNT* gene) for core-1 O-glycan substrates and may inhibit the addition of O-linked poly-LacNAc ligands. Increased α2,3-sialylation of the CD8 glycoprotein decreases its binding to MHC I molecules and alters selection of the $CD8^+$ T cell repertoire during thymocyte development (Daniels et al., 2001). In the periphery, ST3Gal1 inactivation

renders $CD8^+$ T cells highly sensitive to apoptosis through enhanced cross-linking of O-glycoproteins in the absence of TCR signaling. This proapoptotic effect, which might involve a still unidentified lectin recognition system, promotes a dramatic contraction of the $CD8^+$ T cell compartment that compromises clearance of viral and bacterial infections (Priatel et al., 2000; Van Dyken et al., 2007).

Galectins are unique in their capacity to act both extracellularly and intracellularly to control cell death. Extracellularly, galectins cross-link a preferred set of glycosylated receptors to transduce signals that directly lead to apoptosis. Intracellularly, galectins can interfere with other signaling pathways that control cell viability. The susceptibility of T cells to the proapoptotic effects of extracellular galectins is regulated by the repertoire of glycosylated receptors and the spatiotemporal expression of selected glycosyltransferases (Rabinovich and Toscano, 2009). Challenging previous assumptions that assign redundant roles for all galectins in inducing a common death pathway, mounting evidence indicates that each galectin binds to a restricted set of glyco-receptors and triggers a distinct and unique apoptotic program. Galectin-1 directly kills immature thymocytes and activated T cells through binding and clustering of CD45, CD43, and CD7 (Stillman et al., 2006), although it can also sensitize T cells to the canonical Fas-mediated pathway (Matarrese et al., 2005). Current evidence suggests that both N- and O-glycans control the proapoptotic activity of galectins through selective usage of glyco-receptors. Expression of ST6Gal1 selectively modifies N-glycans on CD45 to promote inhibition of Galectin-1 binding and cell death. Moreover, GCNT1-dependent core-2 O-glycan branching differentially regulates Galectin-1 binding to CD45 or CD43 (Earl et al., 2010). Thus, a given glycosylation profile may have a different impact on Galectin-1-mediated effects depending on the particular glyco-receptor implicated. In addition, the extracellular milieu may also influence this proapoptotic effect because in the absence of reducing agents, Galectin-1 induces phosphatidylserine exposure but does not alter T cell viability (Stowell et al., 2009).

The ''chimera-type'' Galectin-3 acts in a dual manner and can either protect T cells from apoptosis or promote cell death, depending on whether it is functioning intracellularly or added exogenously to T cell cultures. T cells overexpressing Galectin-3 are protected from apoptosis induced by Fas ligation (Hsu et al., 2009). Moreover, endogenous Galectin-3 enhances B cell survival and favors a B cell memory phenotype (Acosta-Rodríguez et al., 2004). By contrast, extracellular Galectin-3 induces T cell apoptosis through binding to CD45, CD71, or CD29 (Stillman et al., 2006; Fukumori et al., 2003). Because Galectin-3-deficient mice frequently show attenuated T cell responses (Hsu et al., 2009), it seems that dominant antiapoptotic and proinflammatory activities of endogenous Galectin-3 prevail.

The tandem-repeat Galectin-9 has been identified as a major binding partner of the Tim-3 inhibitory receptor, which activates tolerogenic circuits that halt Th1 cell responses (Zhu et al., 2005). Mice lacking Galectin-9 display an increased $CD4^+Tim\text{-}3^+$ effector T cell population that promotes autoimmune pathology (Seki et al., 2008). However, other studies have identified Galectin-9-independent partnering processes that contribute to Tim-3 effects (Cao et al., 2007) and immunoregulatory functions of Galectin-9 that are independent of Tim-3 (Su et al., 2011), suggesting contributions from other, as-yet-unrecognized receptors to Galectin-9-mediated effects. Recently, a comparative study revealed that tandem-repeat galectins, such as Galectin-9, are much more potent than prototype galectins, such as Galectin-1, in triggering T cell death. This effect does not relate to different saccharide-binding specificities, but reflects the ability of the linker domain of tandem-repeat galectins to permit intermolecular CRD interactions that lead to the formation of higher-order multimers (Earl et al., 2011). Although their physiologic relevance awaits further examination, other family members including Galectin-2, Galectin-4, and Galectin-8 also display proapoptotic activity in in vitro settings (Loser et al., 2009; Paclik et al., 2008; Tribulatti et al., 2007; Norambuena et al., 2009), suggesting that galectins may have evolved as regulatory mediators that act in an autocrine or paracrine fashion to control thresholds of immune cell survival.

Although less well studied in comparison to galectins, glycosylation-dependent mechanisms mediated by CLRs and siglecs have also been described that function to control cell death. Macrophages and tolerogenic DCs express the CLR MGL, which interacts with GalNAc (Tn) structures decorating CD45 on human effector T cells. Binding of MGL to CD45 triggers the phosphatase activity of CD45, leading to T cell apoptosis and inhibition of proinflammatory cytokines (van Vliet et al., 2006). Moreover, Siglec-F plays a key role in modulating eosinophil apoptosis. Allergen-challenged Siglec-F-deficient mice show increased lung eosinophil infiltration and reduced peribronchial-cell apoptosis (Zhang et al., 2007). Similarly, Siglec-G has emerged as an inhibitory signal that selectively controls expansion and survival of B1 cells (Hoffmann et al., 2007).

Programmed remodelling of cell surface glycans occurs during T cell development (Daniels et al., 2001), activation (Comelli et al., 2006), and differentiation (Toscano et al., 2007). Whereas early mouse T cell activation is accompanied by a switch from N-Glycolylneuraminic acid (NeuGc)- to N-Acetylneuraminic acid (NeuAc)-terminated glycans (Redelinghuys et al., 2011), late activation involves dramatic reduction of sialylated biantennary N-glycans (Comelli et al., 2006). In this regard, the varying cell surface glycan composition of T cell subsets can differentially regulate the fate of these cells. Although human and mouse Th1 and Th17 cells express the repertoire of cell surface glycans that are critical for Galectin-1 binding and cell death (i.e., poly-LacNAc branching on core-2 O-glycans and complex N-glycans), Th2 cells are resistant to apoptosis because of increased α2,6-sialylation of membrane glycoproteins. This phenomenon is consistent with the ability of Galectin-1 to selectively antagonize Th1 and Th17 cell survival and with the enhanced number of Th1 and Th17 cells in antigen-challenged Galectin-1-deficient mice (Toscano et al., 2007; Motran et al., 2008).

However, changes in glycosylation can also modulate responsiveness of canonical death receptors. Gradual loss of α2,6-linked sialic acid on tumor necrosis factor receptor 1 (TNFR1) sensitizes activated macrophages to TNF-α-induced apoptosis, thereby limiting the lifespan of these cells (Liu et al., 2011). Moreover, decreased α2,6-sialylation can also serve as an eat-me signal that promotes engulfment of apoptotic lymphocytes, possibly through recruitment of endogenous lectins (Meesmann

et al., 2010). In this regard, recent findings identify Galectin-3 as a soluble opsonin that bridges apoptotic cells to macrophages for efficient clearance (Karlsson et al., 2009). Moreover, Stowell et al. (2009) have found that several members of the galectin family (Galectin-1, -2, -4, and -8) prepare living leukocytes for phagocytic removal by promoting reversible phosphatidylserine exposure. This N-glycan-dependent process may offer an immunologically silent mechanism to promote cellular turnover and avoid aberrant inflammation. Consistently, Galectin-1 also contributes to disarming the apoptotic machinery by inducing partial cleavage of fodrin, a linker molecule that connects CD45 to the actin cytoskeleton (Pang et al., 2009). Interestingly, CLEC9A, a CLR selectively expressed by CD8α^+ DCs, functions as a selective sensor of necrotic cells that mediates cross-presentation of dead-cell-associated antigens and controls necrosis-induced inflammation (Sancho et al., 2009). Thus, lectin-glycan systems couple cell death programs to tolerogenic circuits by directly delivering death signals, tuning the thresholds of canonical death pathways, and/or preparing dying cells for phagocytic removal.

Educating Antigen-Presenting Cells for Tolerance

Lectin-glycan interactions play fundamental roles in regulating inflammatory or tolerogenic antigen-presenting cell (APC) programs. Beyond their traditional roles in recognizing and delivering antigens to intracellular compartments, CLRs behave as signaling receptors that translate glycan-containing information into inflammatory or tolerogenic programs (van Kooyk and Rabinovich, 2008). A key role for CLRs in immune tolerance induction was initially noted in experiments involving targeting of glycosylated antigens to DCs through the CLR DEC-205 (Bonifaz et al., 2002). Subsequent findings demonstrated that delivery of mannosylated myelin-derived antigens suppresses experimental autoimmune encephalomyelitis (EAE), whereas unglycosylated peptides aggravate inflammatory disease (Kel et al., 2007). Similarly, recognition of mannosylated antigens by SIGNR1, a mouse homolog of human DC-SIGN expressed on lamina propria DCs, unleashes a cascade of tolerogenic events that stimulate the differentiation of IL-10-producing regulatory T (Tr1) cells in inflamed intestinal mucosa (Zhou et al., 2010). Interestingly, glycoantigens associated to intestinal commensals can directly amplify these circuits by promoting differentiation of Tr1 cells with gut-homing specialization (Kreisman and Cobb, 2011).

How do subtle differences in glycan recognition translate into inflammatory or tolerogenic signaling programs that tailor adaptive immunity? Gringhuis et al. (2009) have demonstrated that interactions of DC-SIGN with ligands containing mannose or fucose can elicit pro- or anti-inflammatory cytokine profiles depending on selective activation of intracellular signals. DC-SIGN binding to mannose-containing glycans results in increased production of IL-12, IL-6, and IL-10 via assembly of a signalosome complex involving different scaffolding proteins linked to the protein kinase Raf-1. However, interaction with fucose-containing ligands increases only IL-10 and favors disassembly of this complex (Gringhuis et al., 2009). Recently, a highly fucosylated form of clusterin, an enigmatic protein implicated in autoimmunity and cancer, has been identified as a soluble ligand for DC-SIGN, suggesting its potential role in tolerance induction (Sabatte et al., 2011). Likewise, engagement of Dectin-1 can signal DCs either to promote generation of Th17 cells through activation of p38 mitogen-activated protein kinase or to drive differentiation of IL-10-producing tolerogenic DCs via ERK-dependent mechanisms (Dillon et al., 2006; Osorio and Reis e Sousa, 2011). Recently, Chen et al. (2009c) have identified a glycosylation-dependent pathway that helps APCs to discriminate between infectious and noninfectious injury signals that promote inflammation. The sialoglycoprotein CD24 selectively recognizes DAMPs (HMGB1), but not PAMPs, and interacts with Siglec-10 (Siglec-G in mice) to dampen inflammatory responses (Chen et al., 2009c). These elegant studies place the Siglec-10-CD24 axis at the center of APC homeostasis, while opening avenues for selectively targeting inflammation induced by DAMPs.

Examination of the "glycosylation signature" of DCs during maturation reveal profound changes in glycan expression profiles, including upregulation of LacNAc and sialylated structures and downregulation of core-2 O-glycans (Bax et al., 2007). Through binding to poly-LacNAc-containing glycans on CD43$^+$ DCs, Galectin-1 initiates a tolerogenic circuit involving the differentiation of IL-27-producing DCs, which in turn promote the expansion of IL-10-producing Tr1 cells. This circuit operates in autoimmune and cancer settings and is interrupted in mice lacking Galectin-1, IL-27 receptor, or IL-10. Consistently, DCs lacking Galectin-1 or Galectin-3 are more immunogenic than wild-type DCs and favor polarization toward Th1 and Th17 cell profiles (Ilarregui et al., 2009; Mobergslien and Sioud, 2012). Because Galectin-1 also promotes DC maturation and migration through mechanisms involving Syk and protein kinase C (PKC) signaling (Fulcher et al., 2009), it seems likely that this lectin may impart a distinctive immunoregulatory program, characterized by either a mature or immature cell surface phenotype but increased migratory profile and enhanced tolerogenic potential. On the other hand, ligation of Tim-3 by Galectin-9 induces divergent functions on APCs and T cells, leading to initiation or termination of Th1 cell-dependent immunity (Anderson et al., 2007). These contrasting effects have been recently scrutinized, showing that the Galectin-9 C-terminal domain is more potent in inducing T cell death, whereas the N-terminal domain is more effective in activating DCs (Li et al., 2011). Interestingly, Galectin-1, Galectin-3, and Galectin-9 favor the differentiation of "alternatively activated" macrophages (Barrionuevo et al., 2007; MacKinnon et al., 2008; Arikawa et al., 2010), and poly-LacNAc-deficient macrophages are highly sensitive to agonist-induced activation (Togayachi et al., 2007), suggesting essential roles for lectin-glycan interactions in modulating APC signaling.

Tuning the Function of Regulatory T Cells

Endowed with the capacity to suppress T cell responses, Treg cells hold the promise of limiting autoimmune pathology and sustaining tolerance at the maternal-fetal interface. Conversely, they represent a considerable hurdle for successful tumor immunity. Multiple Treg cell subsets, including FoxP3$^+$ Treg, Tr1, and Th3 cells, can exert suppressive activities through mechanisms involving cell-cell interactions or release of immunosuppressive cytokines (Bluestone et al., 2010).

Analysis of gene and protein expression profiles reveal a selective upregulation of Galectin-1, Galectin-9, and Galectin-10 in

$CD4^+CD25^+$ Treg cells, which substantially contribute to the suppressive activity of these cells (Garín et al., 2007; Kubach et al., 2007; Wang et al., 2009). Dissection of the molecular mechanisms underlying this effect reveal the ability of Treg cell-derived Galectin-1 to cross-link the GM1 ganglioside and activate the TRPC5 channel, which mediates Ca^{2+} influx in effector T cells. Interestingly, effector T cells from autoimmune-susceptible mice express lower amounts of GM1 that confer resistance to Treg cell-mediated suppression (Wu et al., 2011). Future studies are needed to characterize the molecular determinants of ganglioside-galectin interactions.

Adding complexity to this picture, Galectin-1 and Galectin-9 also contribute to Treg cell expansion in vivo, whereas Galectin-3 appears to have an opposite effect (Toscano et al., 2006; Seki et al., 2008; Jiang et al., 2009). Studies in mice lacking Galectin-9 show a decreased number of $FoxP3^+$ Treg cells and exacerbation of autoimmune pathology, whereas Galectin-3-deficient mice exhibit a higher number of Treg cells and attenuated inflammatory disease. Interestingly, in a model of stress-induced pregnancy failure, treatment with Galectin-1 restores tolerance and mitigates inflammation by promoting the differentiation of uterine regulatory DCs and IL-10-producing Treg cells. These tolerogenic effects were hierarchically regulated by progesterone and abrogated in mice depleted of Treg cells or deficient in IL-10, suggesting a hormone-lectin synergism in the induction of tolerance at the fetomaternal interface (Blois et al., 2007). This immune-endocrine cross-talk was confirmed by phylogenetic footprinting studies highlighting sex-steroid-responsive elements in the *LGALS1* promoter that were gained after emergence of mammalian placentation (Than et al., 2008). These findings are consistent with the ability of human decidual NK cells to sustain fetomaternal tolerance via Galectin-1-dependent mechanisms (Kopcow et al., 2008). Moreover, recent studies supported the ability of Galectin-1 to imprint a "Tr1-like" cell immunoregulatory signature defined by IL-10 synthesis, lack of FoxP3 expression, and activation of the c-Maf and aryl hydrocarbon receptor pathways (Cedeno-Laurent et al., 2012).

Although just emerging, other lectin families also contribute to Treg cell biology. Sialoadhesin, an inhibitory siglec expressed on tissue-infiltrating macrophages, binds sialic acid-containing glycans on Treg cells and negatively regulates their expansion and suppressive activity (Wu et al., 2009). More recently, targeted deletion of Siglec-H, a particular siglec exclusively expressed by plasmacytoid DCs, revealed unique roles of these cells in differentially regulating homeostasis of effector and Treg cells (Takagi et al., 2011). Thus, different lectin-glycan recognition systems can control the expansion and suppressive capacity of Treg cells, further emphasizing the relevance of the glycosylation machinery in T cell homeostasis.

Glycosylation-Dependent Regulatory Circuits in Autoimmunity, Inflammation, and Cancer

Recent efforts involving genetic manipulation of N- and O-glycosylation pathways, as well as blockade of endogenous lectins, have illuminated essential contributions of lectin-glycan interactions to regulatory circuits that critically influence autoimmune and antitumor responses (Figure 3). A paradigmatic example is shown in mice deficient in the αM-II glycosidase. Targeted deletion of αM-II leads to diminished N-glycan branching and occurrence of an autoimmune disease similar to human SLE. This effect is independent of the adaptive immune system and is linked to chronic activation of innate immune components. A mechanistic analysis reveals that, under αM-II deficiency, an atypical exposure of cryptic N-glycans (high mannose-type N-glycans) occurs, which allows recognition by mannose-binding lectins such as MR (Green et al., 2007). Because these particular glycan epitopes are typically expressed by pathogenic bacteria, lack of αM-II recapitulates bacterial infection leading to aberrant activation of innate immunity and development of an autoimmune disease similar to that observed in MR-deficient mice (Chavele et al., 2010). Although these studies unveil the important role of innate immune mechanisms in the development of autoimmune diseases, disruption of N-glycosylation pathways may also cause T cell-mediated autoimmune responses. As mentioned above, deficiency in β1,6 N-glycan branching in GnT5-deficient mice results in unleashed T cell activation, which leads to spontaneous demyelinating disease that recapitulates multiple sclerosis (MS) (Grigorian et al., 2011). Supporting these findings, studies in MS patients reveal that dysregulated Golgi N-glycosylation is a final common pathway in which disease-associated environmental factors and multiple genetic variants (IL-7RA, IL-2RA, and CTLA-4) converge (Mkhikian et al., 2011).

Similar to N-glycans, alterations in O-glycans also promote initiation and perpetuation of inflammation. Disruption of core-3 β1,3-N-acetylglucosaminyltransferase (B3GNT6; encoded by *C3GNT* gene), an enzyme responsible for the synthesis of core-3-derived O-glycans, leads to aberrant exposure of naked Tn antigen in colonic epithelium and development of inflammatory colitis and colon adenocarcinoma, emphasizing the protective effects of core-3-derived O-glycans as essential components of intestinal mucins (An et al., 2007). Accordingly, mutations in the molecular "chaperone" Cosmc, responsible for the correct folding of the core-1 β(1-3)-galactosyltransferase (T-synthase), generates a truncated O-glycan composed of only GalNAc, which is the cause of the rare "Tn syndrome" characterized by IgA nephropathy (Ju and Cummings, 2005). Thus, alterations of N- and O-glycosylation pathways result in abnormal activation of innate and adaptive immune mechanisms and development of autoimmune disease.

Supporting these findings, mice devoid of endogenous lectins also display pronounced autoimmune phenotypes. Mice deficient in Dcir, an ITIM-associated CLR expressed on DCs, develop spontaneous autoimmune sialadenitis as a result of augmented DC activation (Fujikado et al., 2008). Moreover, mice lacking both Siglec-G and CD22 spontaneously develop autoimmune glomerulonephritis because of breakdown of B cell tolerance (Jellusova et al., 2010). Accordingly, Duong et al. (2010) demonstrated that both CD22 and Siglec-G can mediate B cell tolerance induced by sialylated "self-associated" antigens, while permitting rapid responses to unsialylated "non-self" foreign antigens. This picture appears to be more complex in the case of galectins. Mice devoid of Galectin-1 or Galectin-9 show greater susceptibility to autoimmune inflammation, augmented Th1 and Th17 cell responses, and increased T cell trafficking to inflamed tissues, compared with their wild-type counterparts (Toscano et al., 2007; Seki et al., 2008; Norling et al., 2008). Delivery of Galectin-1 or Galectin-9 to sites of

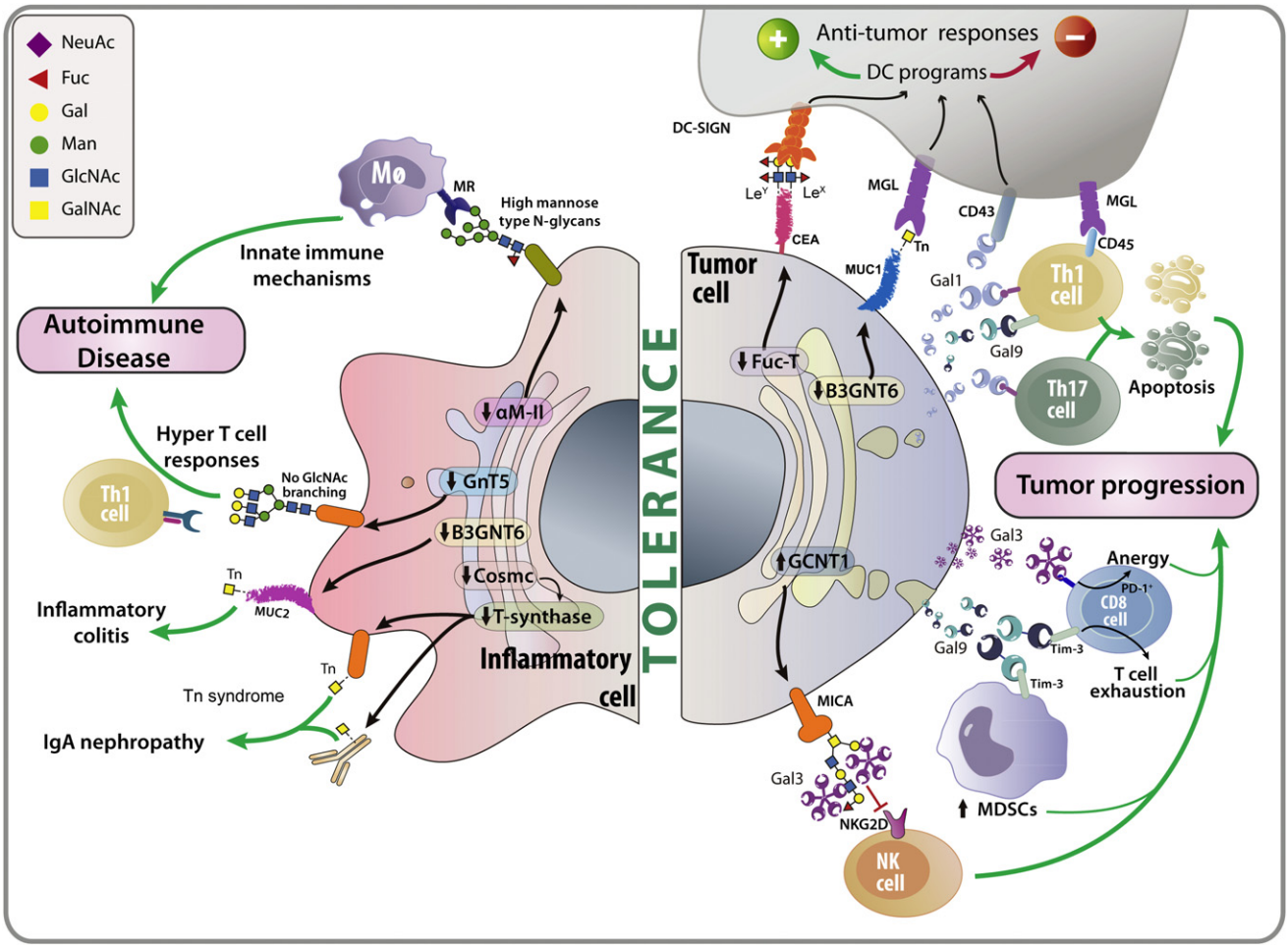

Figure 3. Glycosylation-Dependent Control of Pathogenic and Regulatory Mechanisms in Autoimmunity and Cancer

Changes in glycosylation occurring during inflammation and neoplastic transformation result in exposure of abnormal glycan structures, allowing specific recognition by endogenous lectins and modulation of innate and adaptive immune responses. Left: Deficiency in αM-II leads to exposure of high mannose-type N-glycans, recognition by mannose receptor (MR)-expressing innate immune cells, and development of a lupus-like autoimmune disease. Likewise, absence of β1,6 N-glycan branching in mice lacking GnT5 leads to hyper T cell responses and development of autoimmune neuroinflammation. Lack of core-3-derived O-glycans after disruption of B3GNT6 promotes abnormal exposure of Tn antigen and development of inflammatory colitis. Similarly, mutations in the chaperone Cosmc cause the Tn syndrome characterized by IgA nephropathy. Right: Cancer cells display upregulation of certain glycans such as Tn, sialyl-Tn, and sialyl-Lewis antigens. Binding of aberrantly glycosylated tumor antigens (CEA, MUC1) to endogenous lectins (DC-SIGN, MGL) triggers DC programs that activate or suppress antitumor responses. Alternatively, galectins synthesized by tumor cells facilitate tumor-immune escape by targeting various immune cell functions. Galectin-1 (Gal1) selectively eliminates Th1 and Th17 cells and promotes the differentiation of IL-27-producing tolerogenic DCs. Galectin-3 (Gal3) induces CTL anergy by distancing the TCR from CD8 molecules, while it impairs NK cell function by reducing the magnitude of interactions between the heavily O-glycosylated tumor-derived MICA and the activating receptor NKG2D. Galectin-9 (Gal9) selectively kills Th1 cells, favors exhaustion of CTLs, and promotes differentiation of myeloid-derived suppressor cells (MDSCs). These glycosylation-dependent mechanisms, occurring either in autoimmune (left) or tumor (right) microenvironments, contribute negatively or positively to immune tolerance (center). Understanding the complexity of these pathways will lead to development of pharmacological approaches either to restore tolerance in autoimmune diseases or to break immune tolerance in cancer.

inflammation dampens autoimmune responses through mechanisms involving selective deletion of Th1 and Th17 cell subsets, shift toward Th2 cell-type cytokine profile, expansion of Treg cells and induction of tolerogenic DCs (Rabinovich and Toscano, 2009). In contrast, endogenous Galectin-3 appears to aggravate autoimmune pathology (Jiang et al., 2009). Likewise, endogenous Galectin-4 augments T cell-mediated intestinal inflammation by driving PKC-θ-dependent IL-6 production (Hokama et al., 2004). Thus, in spite of their common structural fold, distinct members of the galectin family may exert inhibitory or stimulatory effects on autoimmune inflammation.

Similar to CLR recognition of aberrantly mannosylated glycans in both bacteria and host cells (Green et al., 2007), recent findings demonstrate the ability of Galectin-4 and Galectin-8 to specifically recognize and kill bacteria expressing common AB0(H) blood group-associated antigens. This lectin-mediated recognition event confers protective immunity against pathogens that display blood group self-antigens on their surface and cannot be eliminated by self anti-blood group antibodies (Stowell et al., 2010). Although this study provides compelling evidence for an interaction between galectins and blood group antigen-expressing bacteria, the use of the general galectin

inhibitor thiodigalactoside does not preclude the participation of other members of the galectin family in promoting this effect. Interestingly, a mechanism of host-pathogen glycan mimicry has also been observed in the case of *Campylobacter jejuni* lipooligosaccharides and host neural gangliosides, the cross-recognition of which contributes to the immunopathogenesis of Guillain-Barré syndrome. Bax et al. (2011) found that, depending on the differential sialylation of *C. jejuni* lipooligosaccharides, specific T helper cell programs are evoked through engagement of distinct DC-expressing siglecs. Thus, the immense structural diversity of pathogen glycans and their presence on both pathogens and host tissues contribute to the dual role of lectin-glycan interactions in inducing protective immunity or immunopathology.

In addition, differential glycosylation can also influence pathogenic or regulatory circuits triggered by immunoglobulins. Elegant studies demonstrate that the anti-inflammatory activity of intravenous immunoglobulins (IVIGs), commonly used for the treatment of autoimmune diseases, is triggered by a minor population of IgG Fcs expressing α2,6-linked sialic acid. The authors identified a regulatory circuit by which sialylated IgG targets myeloid regulatory cells expressing DC-SIGN, which triggers IL-33-mediated Th2 cell responses and expansion of macrophages expressing the FcγRIIB inhibitory receptor (Anthony et al., 2011). Alternatively, sialylated IVIGs can regulate BCR signaling through mechanisms involving engagement of CD22 and promotion of B cell apoptosis (Séïté et al., 2010). In this regard, agalactosyl and asialoglycoforms of IgG have been linked to the pathogenesis of autoimmune diseases, including rheumatoid arthritis and IgA nephropathy (van Kooyk and Rabinovich, 2008).

Alterations in glycosylation also occur during malignant transformation being able to generate abnormally glycosylated antigens, including carcinoembryonic antigen (CEA) and MUC-1. These atypical modifications include upregulation of Tn, sialyl-Tn, Thomsen-Friedenreich disaccharide (Gal-β(1-3)-GalNAc), and sialylated Lewis antigens (Dube and Bertozzi, 2005). Because these particular glycoforms are specifically recognized and internalized by CLRs on DCs, tumor antigens have been coupled to these glycans and used as vehicles for stimulating antitumor immunity. Illustrating this concept, GalNAc-modified tumor-associated antigens are selectively internalized by MGL, delivered to MHC II compartments for presentation to $CD4^+$ T cells, and cross-presented to $CD8^+$ T cells for stimulation of CTL responses (Napoletano et al., 2007; Singh et al., 2011a). Similarly, conjugation of ovalbumin with 3-sulfo-Lewis(A) or tri-GlcNAc specifically targets this antigen to MR that mediates internalization and cross-presentation to $CD8^+$ T cells (Singh et al., 2011b). In contrast, recognition of tumor-associated Lewis glycans expressed on CEA or CEA-related cell adhesion molecule-1 (CEACAM-1) can result in impaired DC function and inhibition of antitumor responses (Nonaka et al., 2008), suggesting that CLRs may serve as sensors that translate tumor "glycosylation signatures" into DC programs that potentiate or attenuate antitumor responses.

Additionally, cancer cells may usurp glycosylation-dependent regulatory circuits to create immunosuppressive networks that thwart antitumor responses. Tumor secretion of Galectin-1 contributes to the immunosuppressive potential of a wide range of tumors by limiting T cell survival and impairing DC function (Rubinstein et al., 2004; Juszczynski et al., 2007; Banh et al., 2011; Kuo et al., 2011; Tang et al., 2012). In addition, Galectin-3-N-glycan complexes favor anergy of tumor-specific T cells (Demotte et al., 2008), whereas Galectin-9-Tim-3 interactions increase the frequency of granulocytic myeloid-derived suppressor cells (Dardalhon et al., 2010) and favor exhaustion of PD-1^+CD8^+ T cells (Zhou et al., 2011), suggesting that distinct cellular types may serve as targets of the immunoregulatory activity of galectins. In this regard, a glycosylation-dependent pathway that targets NK cells has been identified based on the overexpression of the GCNT1 glycosyltransferase on bladder cancer cells. Interactions between Galectin-3 and poly-LacNAc-branched core-2 O-glycans decorating tumor-associated MHC I-related chain A (MICA) reduces the affinity of MICA for the activating NK cell receptor NKG2D, thereby impairing NK cell activation and antitumor activity (Tsuboi et al., 2011). Thus, changes in the glycome occurring during tumor transformation and progression contribute to the activation of regulatory circuits that profoundly influence cancer immunoediting.

Concluding Remarks

Regulatory feedback circuits are defined as physical paths, composed of intermediate switching points that convey, amplify, or attenuate input information according to tunable activation thresholds, leading to divergent functional outputs. As discussed in this review, multivalent interactions between endogenous lectins and glycans can act as tuners that, in response to antigen recognition or cytokine release (input information), can adjust thresholds of cellular activation, differentiation, and survival and critically influence inflammation or tolerance (functional outputs) by modulating the trafficking, endocytosis, and signaling of canonical receptors. However, the dramatic changes observed in the cellular glycome in physiologic and pathologic settings, the relatively high-avidity lectin-glycan interactions, and the pronounced phenotypes of mice lacking components of the glycosylation machinery suggest that glycans and lectins might act beyond their tuning function to provide hierarchical on-and-off input information that directly fuels regulatory circuits.

The current wealth of preclinical information allows the visualization of strategies through which manipulation of lectin-glycan interactions may contribute to restore tolerance and dampen pathogenic autoimmune responses or to break tolerance to promote tumor regression (Dube and Bertozzi, 2005; Ingrassia et al., 2006). However, before lectin-based therapeutic strategies can be embraced, there are still critical issues to be considered including the contribution of glycan specificity to lectin signaling activity and the visualization and characterization of the nature and signaling potency of lectin-glycan lattices. Interdisciplinary approaches that bridge immunology, glycobiology, genetics, and bioinformatics together with innovative technologies including glycan arrays, bioengineering of cell-surface glycans, glycan visualization in vivo, and tissue-specific deletion of glycosylation-related genes will promptly change the scene and create opportunities for capitalizing on the information encoded by the glycome for therapeutic purposes.

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WEB RESOURCES

The URLs for further information are as follows:

Consortium for Functional Glycomics, http://www.functionalglycomics.org
Glycoforum Japan, http://www.glycoforum.gr.jp
Lectin Database, http://www.imperial.ac.uk/research/animallectins

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