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T-cell growth, cell surface organization, and the galectin glycoprotein lattice

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Summary

Basal, activation and arrest signaling in T cells determines survival, coordinates responses to pathogens and when dysregulated, leads to loss of self-tolerance and autoimmunity. At the T-cell surface, transmembrane glycoproteins interact with galectins via their N-glycans, forming a molecular lattice that regulates membrane localization, clustering and endocytosis of surface receptors. Galectin – T-cell receptor (TCR) binding prevents ligand-independent TCR signaling via Lck by blocking spontaneous clustering and CD4-Lck recruitment to TCR, and in turn F-actin transfer of TCR/CD4-Lck complexes to membrane microdomains. Peptide-MHC complexes overcome galectin – TCR binding to promote TCR clustering and signaling by Lck at the immune synapse. Galectin also localizes the tyrosine phosphatase CD45 to microdomains and the immune synapse, suppressing basal and activation signaling by Lck. Following activation, membrane turnover increases and galectin binding to cytotoxic T lymphocyte antigen–4 (CTLA-4) enhances surface expression by inhibiting endocytosis, thereby promoting growth arrest. Galectins bind surface glycoproteins in proportion to the branching and number of N-glycans per protein, the latter an encoded feature of protein sequence. N-glycan branching is conditional to the activity of Golgi N-acetylglucosaminyl transferases I, II, IV and V (Mgat1,2,4 and 5) and metabolic supply of their donor substrate UDP-GlcNAc. Genetic and metabolic control of N-glycan branching co-regulate homeostatic set-points for basal, activation, and arrest signaling in T cells and when disturbed, result in T-cell hyperactivity and autoimmunity.

Keywords

CTLA-4; TCR; CD45; N-glycosylation; galectin; metabolism; hexosamine; Mgat5

Introduction

T cells play a central role in the antigen-specific adaptive immune response, but must remain tolerant of self-antigen to prevent autoimmunity. The T-cell growth cycle is divided into four temporally distinct phases: basal signaling in the absence of antigen, peptide-major histocompatibility complex (MHC) induced activation signaling, growth arrest and differentiation into effector T cells and finally, apoptosis. In the absence of antigenic stimuli, naive T cells require basal growth signaling for long-term survival. In the presence of pathogens, activation of antigen-specific T cells leads to multiple rounds of division, growth arrest, and differentiation into effector cells that clear the pathogen and finally, apoptosis, to

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prevent further unwanted inflammation and tissue damage to the host. Dysregulation at any of these four phases can lead to T-cell hyperactivity and autoimmunity.

Cell fate and function are governed by changes in gene expression and protein production. However, post-translational modifications and metabolism are increasingly recognized as major determinants of cellular function. In this review, we discuss evidence suggesting that protein N-glycosylation and metabolism coordinate basal, activation and growth arrest/differentiation signaling to control homeostasis and self tolerance in T cells (1–8). Cell surface receptors, such as the T-cell receptor (TCR), CD45, and cytotoxic T lymphocyte antigen–4 (CTLA-4), bind multi-valent galectins via their attached N-glycans, forming a molecular lattice that regulates membrane localization and concentration of glycoproteins at the cell surface. The enormous heterogeneity of N-glycans produced in the Golgi apparatus combines with genetically encoded differences in N-glycan number among cell surface glycoproteins to differentially control avidity for galectin binding and thereby, the distribution and function of surface glycoproteins (3). Galectin – glycoprotein interactions also play an important role in regulating T-cell apoptosis (9–16); however this topic has been extensively reviewed elsewhere (17).

Galectin-glycoprotein lattices

Eukaryotic cell surfaces and the extracellular matrix with which they interact are highly decorated with glycans. Cell surface topology is dominated by the glycocalyx, a ~100 nM wide macromolecular structure consisting of glycans attached to proteins and lipids. The abundance, size, and complexity of glycan structures suggest a significant role for information encoding distinct from the genome. The majority of cell surface receptors and transporters are co-translationally modified with Asparagine (N)-linked glycans in the endoplasmic reticulum, with further modifications in the Golgi apparatus following sequential but incomplete action of a series of glycohydrolases and glycosyltransferases (18,19) (Fig. 1). In this manner, the same protein is modified into multiple glycoforms that differ in binding avidity for the galectin family of soluble glycan binding proteins (20). Galectins, a 15-member family in mammals that possess two or more glycan binding sites as monomers and/or multimers, are ubiquitously expressed at the cell surface and extracellular matrix and interact with multi-valent glycan ligands to form ‘lattices’ (1,20,21). At the cell surface, N-glycans appear to be the major ligand for galectins (22,23), a thermodynamically favorable interaction (24) that regulates glycoprotein distribution and concentration in the plasma membrane (1,3,5,10,25–27). Galectins have been classified into three structural groups (28): proto-type galectins which possess a single carbohydrate recognition domain (CRD) and frequently dimerize (galectin-1, -2, -5, 7, -10, -11, -13, -14 and -15), tandem repeat galectins with two CRDs connected by a short linker region that may also dimerize (galectin-4, -6, -8, -9 and -12) (13) and chimeric galectins with a single CRD connected to an N-terminal region that allows formation of pentamers in the presence of multivalent ligand (galectin-3) (21). The minimal binding structure for galectins is N-acetyllactosamine (Galactose β1,4 N-acetylglucosamine = Galβ1,4GlcNAc) (29), with binding avidity for individual glycoproteins increasing in proportion to the number of N-acetyllactosamine units per N-glycan and the number of N-glycans per protein(3). Although this implies significant binding redundancy among galectins, addition of fucose and/or terminal α2,6-sialic acid differentially alters binding of specific galectins, consistent with differences in biological function (11,12,29,30). Moreover, structural differences in CRD orientation and valency may contribute to selective binding of glycoprotein partners based on protein specific differences in N-glycan topology (11,20,31). Galectins are also expressed in the cytoplasm, where they may have effects that are distinct from interactions with cell surface glycoproteins (32), complicating interpretation of phenotypes in mice with targeted

deficiency of galectin. In contrast, alterations in Golgi production of galectin ligands (i.e. N-acetyllactosamine) is expected to alter all cell surface glycoprotein – galectin interactions.

Herein, we define the galectin – glycoprotein lattice as a macro-molecular structure that encompasses all galectins and glycoproteins interacting at the cell surface. In theory, any transmembrane glycoprotein appropriately modified in the endoplasmic reticulum (ER)/ Golgi may interact with one or more galectins at the cell surface and thereby be regulated by the galectin – glycoprotein lattice. Experimentally defining all endogenous galectin – glycoprotein interactions at the cell surface in space and time is a daunting task that will require years of investigation by multiple groups. However, when combined with gene encoded differences in N-glycan number, computational modeling of the Golgi is highly predictive of cell surface glycoprotein regulation by the galectin -glycoprotein lattice (3). The ability to computationally predict regulation of individual glycoproteins based on Golgi activity and N-glycan number provides a powerful tool to probe the function of the galectin – glycoprotein lattice.

Convergent work by multiple groups suggests the galectin-glycoprotein lattice controls glycoprotein activity at the cell surface by two primary molecular mechanisms: 1) regulation of membrane localization/lateral mobility/clustering and 2) inhibition of endocytosis (1,3,5,8,[10],25,26,33–35) (Table 1). Inside the cell, cytoplasmic domains of transmembrane glycoproteins bind adaptor proteins that interact with the cytoskeleton (eg. actin microfilaments, the spectrin scaffold) and/or the endocytic machinery. However, at the cell surface the extracellular domain of the same glycoprotein may also bind galectins, an interaction that opposes cytoskeletal-mediated movement in the plane of the membrane and/ or surface loss to endocytosis (1,3,5,25,26). In this manner, the galectin-glycoprotein lattice couples the molecular organization on the outer leaflet of the plasma membrane with organization on the inner leaflet to control lateral mobility and endocytosis of surface glycoproteins.

Specificity of individual glycoproteins for the galectin-glycoprotein lattice depends on the structural diversity of N-glycans produced in the Golgi as well as the number of N-glycans per glycoprotein (i.e. occupied N-X-S/T sites) (3). The number of N-glycans per glycoprotein is genetically encoded, with growth promoting receptors such as the TCR and receptor tyrosine kinases frequently having a high density of N-glycans per protein molecule (i.e. five or more N-glycans). In contrast, growth inhibitory receptors, such as Transforming Growth Factor-β receptors I and II (TβR) and CTLA-4, possess few N-glycan sites (i.e. four or less N-glycans). Thus, when Golgi activity is low (i.e. limited N-acetyllactosamine production), positive regulators of cell growth are predicted to predominate the cell surface resulting in a growth promoting state. However, with increasing Golgi production of N-acetylalctosamine, negative regulators of cell growth are incorporated into the galectin-glycoprotein lattice, enhancing surface residency with switch-like kinetics to inhibit growth. In this manner, Golgi activity has the potential to globally set growth characteristics of cells, controlling transitions from growth to arrest/differentiation (3). This paradigm has been demonstrated for receptor tyrosine kinases/TβR in epithelial cells and TCR/CTLA-4 in T cells (3). N-acetyllactosamine content in N-glycans is conditional on the expression and activity of Golgi enzymes as well as metabolic supply of sugar-nucleotide substrates by the hexosamine pathway (3,6). Thus, the galectin-glycoprotein lattice appears to incorporate both genetic and metabolic signals to control cellular function, cell fate decisions and disease states. Below, we first detail data indicating that the galectin-glycoprotein lattice utilizes these mechanisms to control basal, activation and arrest/differentiation signaling in T cells, then describe regulation of N-glycan production by metabolism and the Golgi and finally, its impact on T-cell function and autoimmunity.

Ligand independent basal TCR signaling and the galectin-glycoprotein lattice

The peripheral naive T-cell repertoire is governed by complex homeostatic mechanisms (36–38). Basal growth signaling by TCR and Rous sarcoma oncogene (src) tyrosine kinases [lymphocyte protein tyrosine kinase (lck) and/or Fyn proto-oncogene (Fyn)] is necessary to sustain naive T cells and ensure their survival in the absence of pathogenic stimuli (39–41). Initial reports suggested that TCR survival signals require interaction with self-peptide-MHC (42,43), however subsequent studies using adoptive transfer approaches indicated little role for self-peptide-MHC (44,45). These studies suggest a mechanism for spontaneous ligand-independent signaling by TCR. Indeed, work by our lab indicates that the galectin–glycoprotein lattice negatively regulates basal growth signaling via TCR and Lck in the absence of TCR ligand (5), providing a molecular mechanism for ligand-independent signaling by TCR and the potential to control long term survival of naive T cells. The association of TCR with the galectin-glycoprotein lattice inhibits Lck activation by blocking spontaneous TCR oligomerization and subsequently, a conformational change that recruits CD4, and in turn, Filamentous (F)-actin mediated transfer of TCR/CD4-Lck complexes to GM1-enriched microdomains (GEM) (Fig. 2). The galectin-glycoprotein lattice also regulates the tyrosine phosphatase CD45, maintaining CD45 in GEMs and suppressing Lck activation by opposing F-actin mediated exclusion from GEMs (Fig. 2).

Regulation of spontaneous TCR clustering and signaling

Differential partitioning of plasma membrane proteins into functional microdomains have a crucial role in signaling pathways. The earliest event in agonist-induced TCR signaling appears to be a conformational change in CD3Σ that recruits the adaptor protein non-catalytic region of tyrosine kinase adaptor protein 1 (Nck) to the TCR complex following TCR dimerization, an event independent of tyrosine phosphorylation (46,47). Nck binds the Wiskott Aldrich Syndrome protein (WASp) and Src homology 2 domain containing leukocyte protein of 76kDa (SLP-76) (48), recruiting F-actin to TCR (48–50), transferring TCR to GEMs (51) and activating the Src family protein tyrosine kinase Lck (52,53). Autophosphorylation of Lck at activating Tyr^{394} enhances kinase activity and is required for initiating the TCR signaling cascade. Approximately 75–95% of cytoplasmic Lck is associated with CD4 (or CD8), a co-receptor also recruited to the TCR complex by peptide-MHC.

Remarkably, we have found that all of the aforementioned molecular events are recapitulated in the absence of TCR ligand by disrupting the galectin-glycoprotein lattice (5) (Fig. 2). The human TCRα/β-CD3 complex has 12 N-glycan sites (54,55) and has been demonstrated to bind galectin-1 and galectin-3 (1, 10). Using galectin affinity chromatography and T-cell lysates, Baum and colleagues observed interaction of galectin-1 and -3 with multiple glycoproteins including the TCR complex (10,11). Co-immunoprecipitation studies done in our lab demonstrated association of endogenous galectin-3 with the TCR complex in an N-glycan dependent manner, an experiment that requires chemical cross-linking of the cell surface to stabilize the low-affinity binding of galectin-3 to TCR (1). Indeed, excessive *in vitro* washing of *ex vivo* T cells markedly reduces the level of cell surface galectin-1 and 3 observed by fluorescence-activated cell sorting (FACS) analysis (A. Grigorian and M. Demetriou, unpublished data). Addition of exogenous galectin-1 and 3 results in the re-distribution of multiple T-cell glycoproteins into different microdomains and T-cell death *in vitro* (10,11), an assay that may disrupt endogenous galectin-glycoprotein interactions. Interpretation of phenotypes in mice with gene targeted deficiency in individual galectins are complicated by potential redundancies between galectins and effects secondary to loss of expression in the cytoplasm. However,

disrupting the interaction of endogenous galectins with cell surface glycoproteins can be achieved by: 1) reducing N-acetyllactosamine production in the Golgi, either by gene-targeted deficiency of the Golgi enzymes *Mgat5* or β1,3GnT3 and treating cells with the Mannosidase II inhibitor swainsonine (SW) (see Fig. 1); 2) short-term co-incubation of cells with soluble N-acetyllactosamine (or lactose) to compete with galectin binding; and 3) genetic changes that reduce the number of N-glycans attached to individual glyoproteins. Although these approaches do not specify relevant galectins, they all result in the same phenotype in T cells, namely enhanced TCR mobility in the plane of the membrane and hyper-active TCR signaling(1,5,7,35). In resting T cells, disruption of the galectin-glycoprotein lattice (via Mgat5 deficiency, lactose or swainsonine treatment) recruits CD4, Nck, SLP-76, WASp, and F-actin to TCR in the absence of TCR ligand (5). This recapitulates the phenotype observed by anti-CD3 induced TCR clustering(5;46;47), suggesting the galectin-glycoprotein lattice actively prevents spontaneous TCR clustering in the absence of ligand. Recruitment of F-actin to TCR following ligand-induced clustering transfers TCR to GEMs. Similarly, co-localization studies utilizing Cholera Toxin B (CTB) patching techniques in live cells as well as sucrose density separation of Triton X-100 resistant membrane fractions both demonstrated that disrupting the galectin-glycoprotein lattice (via Mgat5 deficiency, lactose or swainsonine treatment) localized TCR and CD4-Lck to GEMs. This transfer was mediated by F-actin, as blocking F-actin reorganization with Latrunculin-A prevented the transfer. Recruitment of CD4, Nck, SLP-76, WASp, and F-actin to TCR and partition of the complex to GEMs did not require Lck activity, as Lck deficiency and/or co-incubation with PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine), a potent and selective inhibitor of the Src-family tyrosine kinases, had no effect. Rather, F-actin transfer of TCR-CD4-Lck complexes to GEMs following galectin-glycoprotein lattice disruption (via Mgat5 deficiency, lactose or swainsonine treatment) led to hyper-phosphorylation of Lck at activating Tyr^{394} and to a lesser extent, zeta-chain (TCR) associated protein kinase (Zap-70) and linker for activation of T cells (Lat). Latrunculin-A blocked transfer of TCR-CD4-Lck complexes to GEMs and prevented Lck activation. Furthermore, deficiency of TCR or CD4 prevented Lck transfer to GEMs and Lck activation. Together, these data indicate that the galectin-glycoprotein lattice regulates basal signaling through Lck by actively preventing spontaneous TCR clustering, subsequent recruitment of CD4-Lck, Nck, SLP-76, and WASp to TCR and F-actin mediated transfer to GEMs. This mechanism also appears active in $CD8^+$ T cells, as disruption of the galectin-glycoprotein lattice with lactose induces CD8 – TCR interactions and enhances responsiveness to tumor antigens (8).

Membrane localization of CD45 and basal TCR signaling

Src tyrosine kinases are the primary substrate for CD45, a receptor protein tyrosine phosphatase that dephosphorylates both inhibitory Tyr^{505} and activating $Tyr^{394}$of Lck (56,57). CD45 is highly expressed at the cell surface of all nucleated hematopoietic cells, comprising an estimated 10% of the lymphocyte cell surface. A small proportion of CD45 localizes to GEMs, where it inactivates Lck by dephosphorylating Tyr^{394} and suppresses TCR signaling (58–62). Partition of CD45 to GEMs is mediated by its extracellular domain (60), which is highly glycosylated with N-glycans containing N-acetyllactosamine (63), the ligand for galectins. Baum and colleagues (10,11) have found that under conditions that induce T-cell death *in vitro*, addition of exogenous galectin-1 clusters CD45 while the cell surface distribution of CD45 remains uniform following addition of exogenous galectin-3. We have found that disrupting the galectin-glycoprotein lattice via Mgat5 deficiency, lactose and/or swainsonine treatment reduces co-immunoprecipitation of galectin-3 with CD45 and leads to F-actin mediated exclusion of CD45 from GEMs in resting T cells and activation of Lck (5) (Fig. 2). This was independent of Lck activity and TCR-CD4-Lck movement as Lck deficiency, co-incubation with PP2, and deficiency of TCR and CD4 had no effect. In

contrast, co-incubation with Latrunculin A increased CD45 localization to GEMs and reversed CD45 exclusion from GEMs following galectin-glycoprotein lattice disruption, indicating F-actin opposes the galectin-glycoprotein lattice to maintain CD45 outside of GEMs (Fig. 2). The cytoplasmic domain of CD45 binds ankyrin, tethering CD45 to the actin cytoskeleton via interaction with the spectrin-ankyrin scaffold (64). Galectin-glycoprotein lattice-mediated partition of CD45 to GEMs functions to inactivate Lck as hyper-phosphorylation of Tyr^{394} following galectin-glycoprotein lattice disruption is prevented by CD45 deficiency. Moreover, increased localization of CD45 to GEMs following F-actin depolymerization with Latrunculin-A is associated with reduced phosphorylation of Tyr^{394} in the absence but not presence of a selective CD45 phosphatase inhibitor.

Together, these data indicate that the galectin-glycoprotein lattice suppresses basal TCR signaling in resting T cells by concurrently maintaining CD45 within GEMs and preventing partition of TCR/CD4-Lck to GEMs. The galectin-glycoprotein lattice blocks spontaneous TCR clustering in the absence of ligand, preventing recruitment of CD4, Nck, SLP-76, WASp to TCR, F-actin transfer to GEMs and Lck activation. Partitioning of CD45 to GEMs by the galectin-glycoprotein lattice inactivates Lck, suppressing basal TCR signaling. Thus, changes in galectin-glycoprotein lattice strength, which may be achieved either through changes in N-glycosylation or galectin levels, appears able to titrate basal TCR signaling irrespective of peptide-MHC.

T-cell activation thresholds and the galectin-glycoprotein lattice

The initiation of a T-cell response to foreign antigen entails TCR recognition of foreign peptide bound to MHC displayed on the surface of antigen presenting cells (APCs) and the formation of a specialized contact site, the immunological synapse (IS) (65,66). T-cell activation and subsequent proliferation requires clustering of a threshold number of TCRs by peptide-MHC at the IS(67), a number reduced by binding of the co-stimulatory receptor CD28 to CD80/CD86 up-regulated on activated APCs (68). Ligand-specific oligomerization of TCRs and the stability of these clusters are crucial for signaling and sustained T-cell activation(69). Following activation, the T cell undergoes multiple rounds of cell division followed by growth arrest and differentiation into effector cells.

The galectin-glycoprotein lattice negatively regulates T-cell activation thresholds by inhibiting ligand-dependent TCR clustering while concurrently promoting CD45 at the immune synapse (1,5,35,70) (Fig. 3). The galectin-glycoprotein lattice appears to pre-set activation thresholds at the early immune synapse in part by regulating membrane microdomain structure in the resting state. GEMs cluster at the immune synapse (71), while the galectin-glycoprotein lattice excludes TCR/CD-Lck and promotes retention of CD45 in GEMs in resting T cells. These considerations predict that disruption of the galectin-glycoprotein lattice will promote TCR and inhibit CD45 clustering at the early immune synapse. Indeed, using immunofluorescence microscopy of T cells stimulated with anti-CD3 antibody coated microbeads we find that disruption of the galectin-glycoprotein lattice by Mgat5 deficiency, SW or lactose treatment increases TCR clustering, actin microfilaments and/or Lck-Tyr^{394} phosphorylation levels while decreasing CD45 concentration at the cell-microbead contact site(1,5). Disruption of the galectin-glycoprotein lattice by these approaches also results in enhanced agonist-induced TCR endocytosis, phosphorylation of CD3ζ, Zap70, LAT, and/or Akt as well as increased mobilization of intracellular Ca^{2+}. Similarly, Miceli and colleagues demonstrated that addition of galectin-1 inhibited agonist induced lck clustering at the immune synapse, TCR signaling and T-cell activation (70). Togayachi *et al.* (7) demonstrated that gene targeted deficiency of poly-nacetyllactosamine, the high affinity ligand for galectins, also results in hyperactive TCR signaling and enhanced T-cell proliferation. Remarkably, clustering of GEMs at the cell-microbead contact sites

appears to be unaffected by Mgat5 deficiency, consistent with alterations in TCR and CD45 partitioning to GEMs occurring prior to encounter with TCR agonist (5). A negative regulatory role for CD45 retention by the galectin-glycoprotein lattice at the early immune synapse is demonstrated by forced expression of CD45 at the immune synapse of lattice disrupted T cells (5). This reverses hyper-phosphorylation of Lck-Tyr^{394}, Zap70, and LAT and is consistent with previous suggestions for a negative regulatory role of CD45 in GEMs and the immune synapse (61,62,72). These effects on the TCR signaling cascade are not due to intrinsic changes in intracellular signaling potential as T-cell proliferation induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) and the Ca^{2+} ionophore ionomycin stimulation is unaffected by galectin-glycoprotein lattice disruption (1). Based on these studies, we conclude the galectin-glycoprotein lattice regulates TCR and CD45 membrane mobility and localization to titrate T-cell activation thresholds and proliferation in a dose-dependent manner (1,4,6,7,35). The magnitude of the effect is similar to that produced by CD28 co-stimulatory signal, as enhancement of proliferation induced by the Mgat5 deficiency is approximately equivalent to that of CD28 co-receptor engagement (1).

TCR binds both galectin-1 and galectin-3 as well as peptide-MHC, the latter necessary for inducing TCR clustering at the IS. Importantly, the affinity of TCR for activating peptide-MHC complexes is similar to that of galectin affinity for N-acetyllactosamine in N-glycans (~10^{-5}) (29). Recent studies done by our lab and others in the field suggest that peptide-MHC needs to overcome galectin - TCR interactions to induce clustering and T-cell activation (Fig. 3). The competition between galectin and peptide-MHC for TCR appears to depend on their relative binding affinities. Indeed, experiments removing defined N-glycosylation sites in the TCR constant domain as well as deficiency in Mgat5 both reduce avidity of TCR for galectins and enhances the effective avidity for peptide-MHC (35) and anti-CD3 antibody (1), respectively. Thus, changes in the strength of the galectin-glycoprotein lattice have the potential to convert non-activating or antagonistic peptide-MHC complexes into agonists. Furthermore, increasing galectin avidity for the N-glycans attached to TCR may have the opposite effect and effectively reduce the pool of peptide-MHC complexes that can activate the T cell. Self-peptide MHC complexes are normally unable to activate T cells, however the above considerations raise the possibility that reductions in N-glycosylation that weaken the galectin-glycoprotein lattice may convert these into activating complexes, thereby promoting loss of self-tolerance. Consistent with this hypothesis, genetic deficiencies in various Golgi enzymes required for galectin ligand production promote autoimmunity (1,4). For example, Mgat5-deficient mice display various immune defects including spontaneous autoimmune-mediated glomerulonephritis, enhanced susceptibility to delayed-type hypersensitivity and experimental autoimmune encephalomyelitis (EAE), the latter a model for Multiple Sclerosis (MS) (1).

Regulation of T-gell growth arrest and differentiation

The T-cell immune response is shaped by an intricate interplay between stimulatory and inhibitory signals. Receptors that function as inhibitory regulators of the T-cell response are critical regulators of T-cell tolerance and autoimmune pathogenesis. CTLA-4 (CD152) is an inhibitory receptor induced to the cell surface 4–5 days following T-cell activation to promote growth arrest (73). CTLA-4 and CD28 are sequence-related receptors that compete for CD80/CD86 co-stimulatory ligand on APCs to negatively and positively regulate T-cell proliferation, respectively. CD28 is constitutively expressed at the T-cell surface in resting and activated cells. In contrast, CTLA-4 is predominantly found in endosomes/lysosomes due to constitutive endocytosis (half-life of ~2 hours) mediated by AP-2, with a small fraction induced to the cell surface 4–5 days after T-cell activation. The affinity of CTLA-4 for CD80/CD86 exceeds that of CD28 and small amounts of surface CTLA-4 lead to growth arrest. Mice deficient in CTLA-4 display widespread loss of immune tolerance, developing a

lymphoproliferative disorder with inflammatory destruction of multiple organs leading to death by ~4 weeks of age(74).

Human CTLA-4 possesses two N-glycans and our model for the role of N-glycan number in regulating glycoprotein incorporation in the galectin-glycoprotein lattice predicts that CTLA-4 surface retention should be highly sensitive to changes in N-acetyllactosamine production by the Golgi. Indeed, we find that Mgat5 deficiency, SW and lactose treatment reduce surface levels of CTLA-4 in activated T cells (3) (Fig. 4). The Ala17 polymorphism of human CTLA-4 reduces the number of N-glycans attached to CTLA-4 by ~50% (i.e. from two to one) and is associated with reduced CTLA-4 surface expression and T-cell hyperactivity (75,76). Mgat5 and CTLA-4 gene expression increases in proportion to TCR signal strength (1,2,73). Mgat5$^{-/-}$ T cells have hyperactive TCR signaling and indeed, intracellular levels of CTLA-4 are significantly increased compared to activated control cells. Thus, despite increased CTLA-4 protein production from enhanced TCR signaling, surface levels of CTLA-4 are reduced in Mgat5-deficient T cells due to disruption of the galectin-glycoprotein lattice. However, with strong co-stimulation (CD28 ligation), cell surface levels of CTLA-4 were similar in activated Mgat5$^{-/-}$ and Mgat5$^{+/+}$ T cells. This may arise from even greater increases in CTLA-4 transcription/translation and/or upregulation of N-acetyllactosamine content in CTLA-4 by other Golgi enzymes such as Mgat4. Studies using CFSE (5,6-carboxyfluorescein diacetate succinimidyl ester) to track individual cell divisions confirmed that lattice disruption decreases both the proportion of cells expressing cell surface CTLA-4 as well as associated surface levels at all cell divisions, except at very high levels of stimulation. CD28 has five N-glycan attachment sites and surface levels are not significantly altered with Mgat5 deficiency. CTLA-4 surface expression in resting CD4^{+} forkhead box p3 (Foxp3)$^{+}$ regulatory T cells (Tregs) is not altered by Mgat5 deficiency, a cell type that has low membrane turnover and constitutively expresses surface CTLA-4. These findings indicate that the galectin-glycoprotein lattice regulates CTLA-4 surface levels in activated effector T cells, where membrane turnover is high, but not resting Treg cells where membrane turnover is minimal (Fig. 4). TCR signaling up-regulates Mgat5 gene expression and N-acetyllactosamine content in N-glycans 3–5 days following activation of naive T cells (3), thereby promoting CTLA-4 retention at the cell surface via incorporation into the galectin-glycoprotein lattice and T-cell growth arrest.

Following growth arrest, activated CD4^{+} T cells differentiate into effector T cells such as T helper 1 (T_H1), T helper 2 (T_H2), T helper 17 (T_H17), and/or induced Treg cells. TCR signal strength (77,78) couples with cytokines produced by the activated innate immune system to regulate differentiation, with IFN-γ and IL-12 promoting T_H1, Transforming Growth Factor-β (TGF-β) + IL-6 (79) or TGFβ + IL-21 (80,81) enhancing T_H17, IL-4 triggering T_H2 differentiation and TGF-β inducing Tregs. T_H1 and T_H17 cells are pro-inflammatory and independently enhance autoimmunity (82), secreting IFN-γ and IL-17, respectively. T_H2 cells secrete IL-4, IL-5, IL-10, and IL-13 and defend against extracellular pathogens, provide help to B cells (humoral immunity) and are anti-inflammatory. Tregs potently limit effector T-cell proliferation to inhibit autoimmunity. The relative balance of these different cell types influences susceptibility to autoimmunity, allergic responses and tumor immune surveillance. Bottomly and colleagues (77,78) demonstrated that increasing TCR signal strength promotes differentiation of naive T cells into T_H1 over T_H2 cells. Consistent with these results, we found that hyper-active TCR signaling in Mgat5-deficient splenocytes and naive CD4^{+} T cells is associated with increased IFN-γ and decreased IL-4 production compared to wild-type control cells (2). Similarly, swainsonine treatment of T cells from humans and mice also increased IFN-γ production induced by TCR stimulation. *In vivo*, both unmanipulated and autoimmune afflicted Mgat5$^{-/-}$ mice display increased frequency of Tim3^{+}CD4^{+} T cells (4), where Tim3 serves as a marker of both T_H1 and T_H17 cells (83). The direct effects of Mgat5 deficiency on T_H17 differentiation have not been investigated;

however Rabinovich and colleagues (84) demonstrated that galectin-1 deficiency suppresses both T_H1 and T_H17 responses. This suppression was associated with increased sensitivity of T_H1 and T_H17 T cells to galectin-1 mediated apoptosis; however galectin-1 also suppresses TCR signal strength (70) and based on the work of Bottomly and colleagues (77,78), this is expected to reduce T_H1 and promote T_H2 differentiation. By co-culturing wild-type and Gal-$1^{-/-}$ T_H1 and T_H2 T cells, Miceli and colleagues (14) recently reported that T_H2 derived galectin-1 promotes T_H1 death while T_H1 derived galectin-1 promoted T_H2 cytokine production (14). Enhanced T_H1 responses have been observed in Gal-$3^{-/-}$ mice in asthma and parasite infection models by Liu and colleagues and Roque-Barreira and colleagues, respectively (85,86). Kuchroo and colleagues (15) reported that exogenous galectin-9 suppresses T_H1 responses, at least in part by binding Tim-3 and promoting apoptosis. Together these data indicate that the galectin-glycoprotein lattice negatively regulates T_H1 and T_H17 differentiation and promotes T_H2 differentiation both by suppressing TCR signaling and promoting T_H1 and T_H17 cell death.

Foxp3^+ Tregs are a subclass of $CD4^+$ T cell that develop in the thymus or are induced in the periphery by TGFβ, express CD25, proliferate poorly in response to TCR stimulation and are able to suppress effector T cell responses and organ-specific autoimmunity (87). Paradoxically, the *in vivo* frequency of Foxp3^+CD25$^+$CD4$^+$ T cells are increased in both healthy and autoimmune afflicted Mgat$5^{-/-}$ mice (4). The N-glycan structures produced by Mgat5 are ~2 times higher in Tregs (Foxp3^+CD4$^+$) relative to naive T cells (Foxp3^-CD4$^+$) (88). Similarly, levels of galectin-1, -3, and -10 are significantly increased in mouse and/or human Tregs (89–91). These data indicate that the strength of the galectin-glycoprotein lattice is significantly increased in Treg cells, a feature that may contribute to their hypo-proliferative phenotype. Consistent with this interpretation, down-regulation of galectin-10 promotes proliferation of human Treg cells (91). The role of the galectin-glycoprotein lattice in regulating Treg function requires further investigation. However, blocking galectin-1 with neutralizing antibody (90) and suppressing galectin-10 expression (91) both reduce the ability of Tregs to suppress naive T-cell activation. Mgat5 deficiency promotes autoimmunity despite increased Treg frequency(4), consistent with defective Treg function and hyper-active effector T-cell responses both contributing to disease pathogenesis.

A number of other cell surface receptors contribute to growth arrest signaling and differentiation in activated T cells. Programmed-death-1 receptor (PD-1) and B- and T-lymphocyte attenuator (BTLA) are induced in activated T cells, promote growth arrest and inhibit autoimmunity (92,93). The PD-1 receptor has also been shown to be highly upregulated in exhausted $CD8^+$ T cells during chronic viral infection, promoting viral persistence (94). Blocking TGFβ receptor I and II (TGFβRI/II) signaling *in vivo* induces spontaneous activation and differentiation of naive T cells into T_H1/T_H2 effector cells and inflammatory infiltration of multiple organs (95), paralleling CTLA-4 deficiency (74). Inducible co-stimulator (ICOS) is induced in activated T cells and regulates T_H1/T_H2 differentiation with minimal affects on growth. BTLA, TGFβRI/II, and ICOS have three and PD-1 has four N-X-S/T sites, suggesting surface retention is also likely to be sensitive to changes in the galectin-glycoprotein lattice. Consistent with these conclusions, Dennis' group (25) has found that retention of TGFβRI/II in epithelial cells is positively regulated by the galectin-glycoprotein lattice. Based on these considerations, it is likely that the galectin-glycoprotein lattice regulates surface distribution and retention of multiple receptors in parallel to promote growth arrest and differentiation in activated T cells.

Genetic and metabolic regulation of the galectin-glycoprotein lattice

The galectin-glycoprotein lattice requires availability of both galectins and their N-acetyllactosamine ligands. The 15 mammalian galectins are widely distributed, but

expression differences have been observed among various T cell sub-types (12,14,89). *In vivo*, galectins secreted into the extracellular matrix by one cell type can be incorporated into the galectin-glycoprotein lattice of a second cell, complicating interpretation of cell-specific galectin production. In contrast, regulation of cell surface N-glycans is intrinsic to the cell examined and the evidence indicates regulation at both the genetic and metabolic level (1,3,6,20).

N-acetyllactosamine content within individual glycoproteins depends on the number of attached N-glycans (i.e. N=occupied N-X-S/T sites, X≠P), a distinct feature of each glycoprotein sequence, and the degree of branching per N-glycan as determined by the Golgi. The number of N-X-S/T sites varies widely among glycoproteins, with growth promoting receptors (eg. TCR, receptor tyrosine kinases) predominantly harboring large numbers of N-X-S/T sites (i.e. N>5) while growth inhibitory receptors (eg. CTLA-4, PD-1, TGFβRI/II) largely displaying few N-glycans (N≤4) (3). The large difference in galectin avidity for high and low N-glycan modified receptors allows Golgi mediated changes in N-glycan branching to differentially control surface retention and endocytosis rates of these receptors and therefore transitions between growth and arrest signaling (3).

N-glycan branching is mediated by the Golgi N-acetylglucosaminyltransferases Mgat1, 2, 4, and 5, which catalyze the addition of N-acetylglucosamine (GlcNAc) from UDP-GlcNAc to N-glycan precursors transiting the medial Golgi, but with declining efficiency (Fig. 1). These are then substituted with galactose by β1,4 galactosyltransferases to generate mono-, bi-, tri- and tetra-antennary N-acetyllactosamine (Galβ1,4GlcANc) branched N-glycans. N-acetyllactosamine is the minimal unit for galectin binding, but further modifications can alter binding avidity. β1,6GlcNAc branched N-glycans produced by Mgat5 are preferentially extended with additional N-acetyllactosamine units by β1,3GlcNAc transferase (iGnT) to form poly-N-acetyllactosamine (96,97), the highest-avidity ligand for the galectins (29,30). Capping of N-acetyllactosamine units with terminal *Φιγ*.2,6 linked sialic acid by the ST6Gal1 transferase inhibits galectin-1 but not galectin-3 binding, whereas galectin-2 binding is reduced by either α2,3 or α2,6 linked sialic acid (12,29,98,99). Capping N-acetyllactosamine with α1,3Gal, rather than sialic acid, may enhance galectin binding (29). Addition of fucose to N-acetyllactosamine also appears to influence galectin avidity (29,30). Importantly, T-cell activation and differentiation are associated with alterations in these structures. Relative to the resting state, N-glycan branching is increased ~4–8 fold in activated T cells, consistent with enhanced Mgat5 gene and protein expression following TCR signaling (1,2). T-cell activation is also associated with a marked increase in terminal α1,3 galactose and a concurrent decrease in terminal α2,6 sialic acid (100). Combined with increased branching, these changes are expected to markedly strengthen the galectin-glycoprotein lattice, thereby promoting CTLA-4 surface retention and T-cell growth arrest (3). Relative to T_H1 and T_H17 cells, T_H2 T cells express significantly more ST6Gal1 (i.e. α2,6 sialic acid), reducing galectin-1 binding avidity and associated apoptosis (12,14). Very importantly, comparing resting T cells from various inbred mouse strains we found significant differences in N-glycan branching and Golgi enzyme activity, indicating that the Golgi branching pathway is highly polymorphic and contributes to genetic differences in T-cell activity and autoimmune susceptibility among mouse strains (4).

Multiple lines of evidence suggest that the activities of the Golgi branching enzymes are dependent upon metabolic production of their donor substrate UDP-GlcNAc by the hexosamine pathway (3,6,101) (Fig. 1). *De novo* UDP-GlcNAc biosynthesis requires key metabolites of carbohydrate (glucose), amino acid (glutamine, NH_4), lipid (Acetyl-CoA) and nucleotide (UTP) metabolism. For these reasons, the hexosamine pathway is considered a critical sensor of nutrient availability in cells. Using Jurkat T cells and/or resting naive mouse T cells, we found that simple addition of one or more of these metabolites to culture

media enhanced N-glycan branching (6). N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) are salvaged into the hexosamine pathway following breakdown of glycans in lysosomes and secretion into the cytoplasm. The Taniguchi, Dennis, and Demetriou groups (3,6,101) have observed that supplementing various cell types leads with GlcNAc enhances N-glycan branching While both GlcNAc and GlcN can be utilized for UDP-GlcNAc biosynthesis, GlcN may also be shunted back into glycolysis for energy production. Indeed, we observed that supplementing mouse T cells with increasing concentrations of GlcN first enhances then reduces N-glycan branching, whereas GlcNAc supplementation shows only a progressive increase to higher levels (6). Combining two hexosamine pathwaymetabolites (eg. GlcNAc + uridine) is more effective at increasing N-glycan branching than doubling the dose of a single metabolite, suggesting that each metabolite is limiting for UDP-GlcNAc production (6). Based on phenotypes observed with Mgat5 defeciency, hexosamine pathway supplementation mediated increases in N-glycan branching is expected to suppress T-cell activity by strengthening the galectin-glycoprotein lattice. Indeed, we find that GlcNAc supplementation inhibits TCR signaling, induction of the T-cell activation marker CD69, T-cell proliferation, T_H1 differentiation, and CTLA-4 endocytosis(6). Importantly, the effects of GlcNAc supplementation on T-cell function are abrogated by co-incubating cells with SW or deoxymannojirimycin (DMN), Golgi inhibitors which block N-glycan branching (Fig. 1). TCR signaling significantly enhances endogenous production of UDP-GlcNAc, thereby strengthening the galectin-glycoprotein lattice and promoting CTLA-4 surface retention (3). Thus, metabolic regulation of N-glycan branching appears to be an important conditional regulator of T-cell activity and differentiation.

Implications in autoimmunity

The immune system has evolved to provide host defense while maintaining self tolerance. Dysregulation at any stage of T-cell growth may lead to aberrant cell function and initiation of autoimmunity. The galectin-glycoprotein lattice negatively regulates basal, activation and arrest signaling of naive T cells, promotes T_H2 while inhibiting T_H1/T_H17 responses and possibly enhances Treg suppressor function (1–3,5,12,14,15,90,91). Thus, weakening the galectin-glycoprotein lattice is expected to drive multiple phenotypes known to independently regulate risk of autoimmunity. Consistent with this, mice deficient in Mgat5 develop spontaneous autoimmune kidney disease and display increased sensitivity to EAE (1). Others have demonstrated that injection of galectin-1 or galectin-9 into mice suppresses EAE (15,102) while genetic deficiency of galectin-1 increases EAE susceptibility (12). Inbred mouse strains are characterized by differences in susceptibility to EAE and remarkably, we observed that strains susceptible to EAE [PL/J, SJL, and Non-Obese Diabetic (NOD) strains] display significantly reduced N-glycan branching in T cells compared to strains resistant to EAE (129/Sv, Balb/c, and B10.S) (4). The PL/J strain displayed the lowest levels of N-glycan branching in this group, with mass spectroscopy and enzyme assays demonstrating natural deficiencies in multiple N-glycosylation pathway enzymes (i.e. Mgat1, Mgat2, and Mgat5). Moreover, T cells from PL/J mice have a hyperactive phenotype with increased TCR signaling and proliferation relative to 129/Sv T cells. Unlike T cells from 129/Sv mice, co-incubating PL/J T cells with SW does not further enhance proliferation, a result consistent with the pre-existing defects in N-glycan branching in the PL/J strain. PL/J mice develop a spontaneous late-onset clinical disease manifested by inflammatory demyelination and neurodegeneration, a disease markedly enhanced by $Mgat5^{+/-}$ and $Mgat5^{-/-}$ genotypes in a gene dose-dependent manner. This disease phenocopies progressive MS to a much better degree than typical EAE models and is the first spontaneous MS model that arises from loss of gene function. Unpublished data from our lab indicates that gene targeted deficiency of galectin-3 slightly enhances T-cell proliferation (M. Demetriou, unpublished data) while Lukic and colleagues (103) found that galetin-3 deficiency marginally reduces EAE severity without altering disease incidence,

suggesting redundancy between galectins in the T cell galectin-glycoprotein lattice. Lukic and colleagues also observed a T_H2 bias produced by Gal-$3^{-/-}$ dendritic cells, however this finding conflicts with enhanced T_H1 responses observed by others in Gal-$3^{-/-}$ mice (85,86).

Hexosamine pathway supplementation increases N-glycan branching in T cells and is therefore expected to suppress autoimmune pathogenesis. Indeed, we find that GlcNAc supplementation of encephalitogenic T cells *in vitro* reduced incidence and severity of EAE following adoptive transfer of the cells into naive recipient mice (6). This was associated with reduced IFN-γ but increased IL-6 production. In addition, oral GlcNAc supplementation enhances N-glycan branching in T cells *in vivo* and inhibits spontaneous autoimmune diabetes in NOD mice. Others have found that GlcN suppresses T cell activation and T_H1 differentiation *in vitro* at doses that promote N-glycan branching *in vitro* (6), and when given to mice, inhibits graft rejection and EAE (104,105). Remarkably, Murch and colleagues (106) found that oral GlcNAc therapy inhibited clinical disease in 8 of 12 children with treatment-resistant inflammatory bowel disease. Assuming a weight of 40–50 kg for these children, which was not reported in the study, the dose used was ~60–150 mg/kg/day, the same dose range that maximally raises T cell N-glycan branching and inhibits autoimmune diabetes in mice (6). Autoimmunity is a complex trait disease with both genetic and environmental components. The production of N-acetyllactosamine in N-glycans is sensitive to genetic regulation, the nutrient environment and metabolism, factors that combine to control T cell activity, differentiation, and autoimmunity. With further understanding of the hexosamine pathway and its regulation of the immune system, metabolic therapy utilizing oral supplementation with GlcNAc may prove to be a potential intervention in human autoimmunity.

Conclusion

The inherent complexity of N-glycosylation provides a wide spectrum of avidities for galectins at the cell surface, and combines with the number of N-glycans per glycoprotein to titrate galectin binding. In T cells, the galectin-glycoprotein lattice integrates three temporally distinct facets of T cell growth, namely basal, activation and arrest/differentiation signaling. TCR, CD45, and CTLA-4 surface concentration and membrane localization are controlled by the galectin-glycoprotein lattice, thereby negatively regulating T-cell growth throughout the growth cycle. Galectin-glycoprotein lattice strength may be altered by changes in galectin availability, Golgi enzyme expression/activity and Golgi supply of N-glycan substrates. The latter is under metabolic control by the hexosamine pathway, which in turn is influenced by the cell's nutrient environment and TCR signaling. Thus, it appears that cellular metabolism may directly regulate growth and differentiation mechanisms of the cell by altering the galectin-glycoprotein lattice (Fig. 5). Subsequent differences in receptor distribution at the cell surface may then alter cell signaling to the nucleus to modify cellular growth and function. This unique paradigm has critical implications for complex trait diseases such as autoimmunity, where genetic and metabolic inputs to the galectin-glycoprotein lattice combine to influence T cell activity and autoimmune susceptibility. As the galectin-glycoprotein lattice also plays important roles in cancer, tissue renewal, osteoporosis and non-autoimmune (Type 2) diabetes (25, 33, 107, 108), genetic and/or metabolic dysregulation of the galectin-glycoprotein lattice may play an important role in many of the chronic diseases afflicting western civilization.

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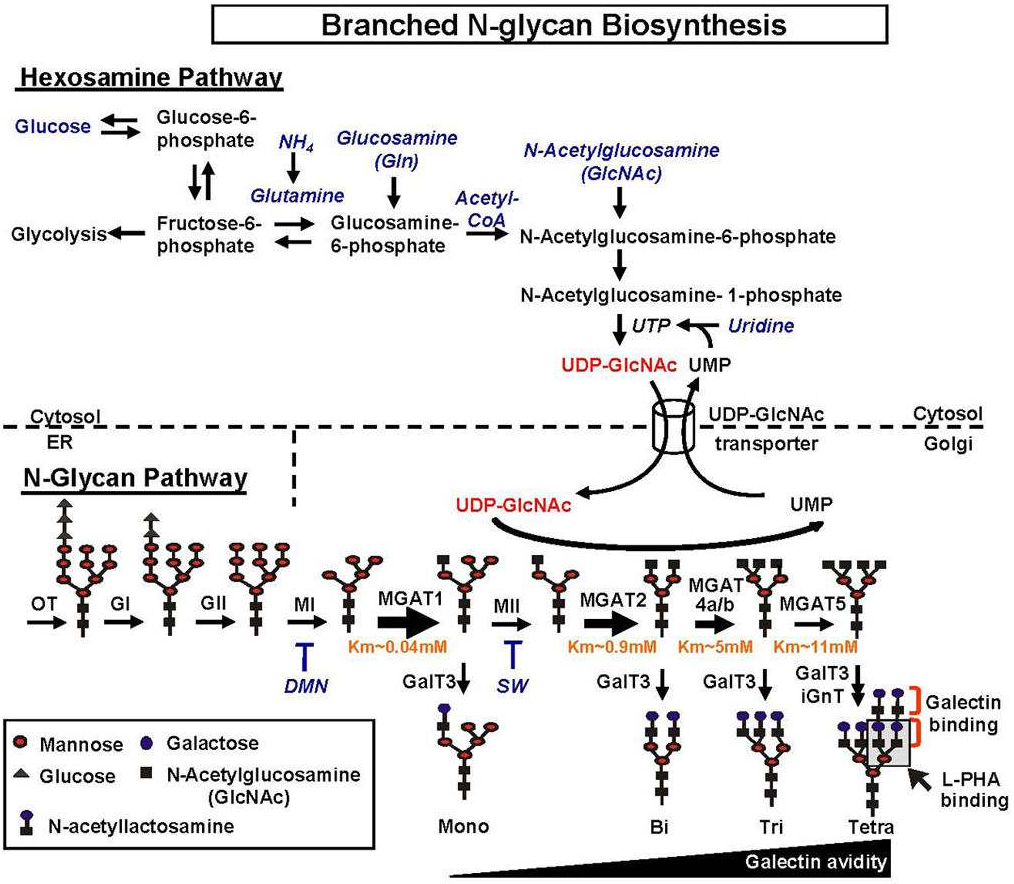

Fig. 1. Regulation of GlcNAc-branched N-glycan biosynthesis by the Hexosamine and N-glycan pathways

UDP-GlcNAc is required by the N-acetylglucosaminyltransferases Mgat1, 2, 4 & 5 and iGnT. Size of the arrows for Mgat1, 2, 4, and 5 depicts relative affinity for UDP-GlcNAc, with K_m below arrows. Cytosolic UDP-GlcNAc enters the Golgi via anti-porter exchange with Golgi UMP, a reaction product of the N-acetylglucosaminyltransferases. Galectins bind N-acetyllactosamine, with avidity increasing in proportion to the number N-acetyllactosamine units (i.e. branching). β1,6GlcNAc-branching by Mgat5 promotes poly-N-acetyllactosamine production, further enhancing avidity for galectins. DMN, deoxymannojirimycin; SW, swainsonine; GI, glucosidase I; GII, glucosidase II; MI, mannosidase I; MIIx, mannosidase IIx; GalT3, galactosyltransferase3. Modified from Grigorian et al. J. Biol. Chem. 2007;**282**:20027–20035.

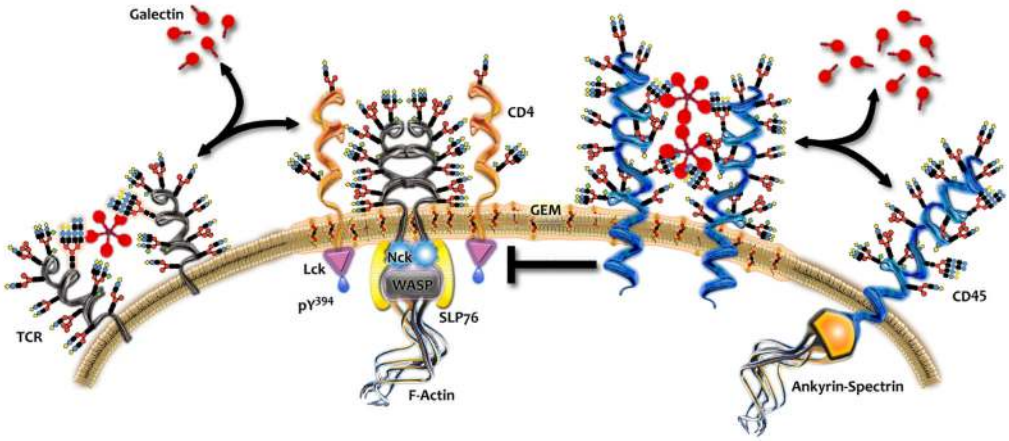

Fig. 2. Regulation of basal TCR signaling by the galectin-glycoprotein lattice

Galectin binding to N-glycans attached to the TCR complex (which is d**epicted as a single ribbon)** inhibits basal signaling via Lck by actively blocking spontaneous TCR oligomerization and subsequent recruitment of CD4-Lck, Nck, WASp, and SLP-76 to TCR, F-actin reorganization and transfer of the complex to GM1 enriched membrane microdomains (GEMs) *(LEFT)*. Concurrently, galectin binding to N-glycans attached to the tyrosine phosphatase CD45 counteracts F-actin, maintaining CD45 in GEMs, dephosphorylating Tyr^{394} and inactivating Lck *(RIGHT*). CD45 is tethered to F-actin via the ankyrin-spectrin scaffold.

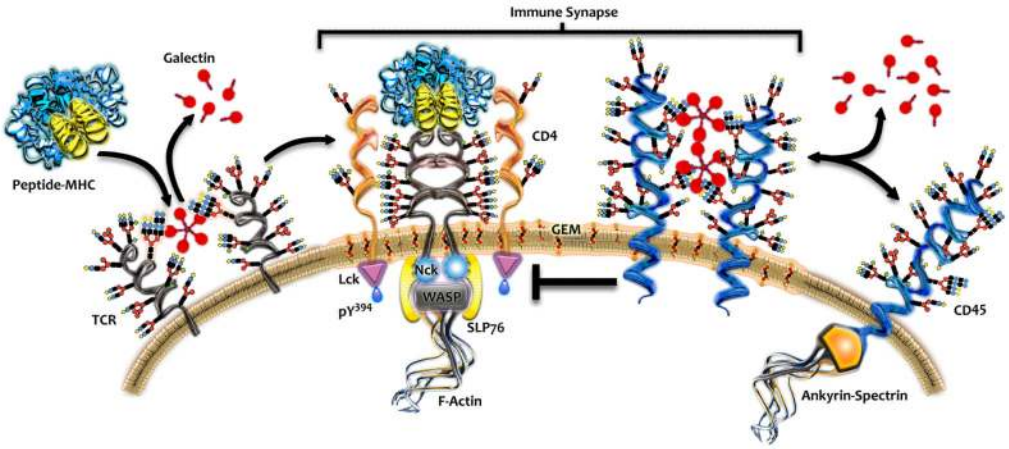

Fig. 3. Regulation of T cell activation thresholds by the galectin-glycoprotein lattice

Galectin binding to N-glycans attached to the TCR complex (which is d**epicted as a single ribbon)** restricts clustering of TCR at the immune synapse in response to agonist *(LEFT).* Peptide-MHC overcomes galectin-TCR interactions to induce clustering, F-actin transfer to the immune synapse and signaling. Concurrently, galectin targets CD45 to the immune synapse, suppressing Lck activation and TCR signaling. Differential partitioning of TCR/CD4-Lck versus CD45 in the early immune synapse is in part predetermined by galectin-glycoprotein lattice control of GEM structure prior to encounter with TCR ligand.

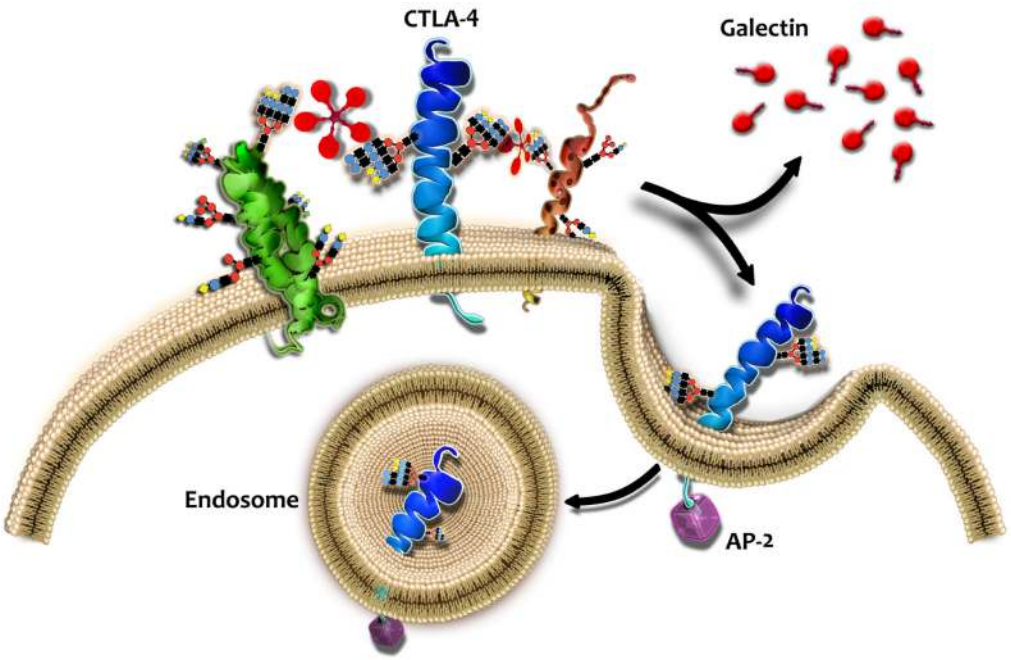

Fig. 4. Regulation of CTLA-4 mediated growth arrest by the galectin-glycoprotein lattice

Galectin binding to N-glycans attached to CTLA-4 enhances surface retention by opposing endocytic loss, resulting in sustained and increased arrest signaling. CTLA-4 has only two N-glycan sites and undergoes rapid degradation due to high constitutive endocytosis rates via AP-2-mediated targeting into clathrin-coated pits. TCR signaling increases metabolic flux to N-glycan branching, promoting incorporation of CTLA-4 into the galectin-glycoprotein lattice to sustain surface retention and augment growth arrest.

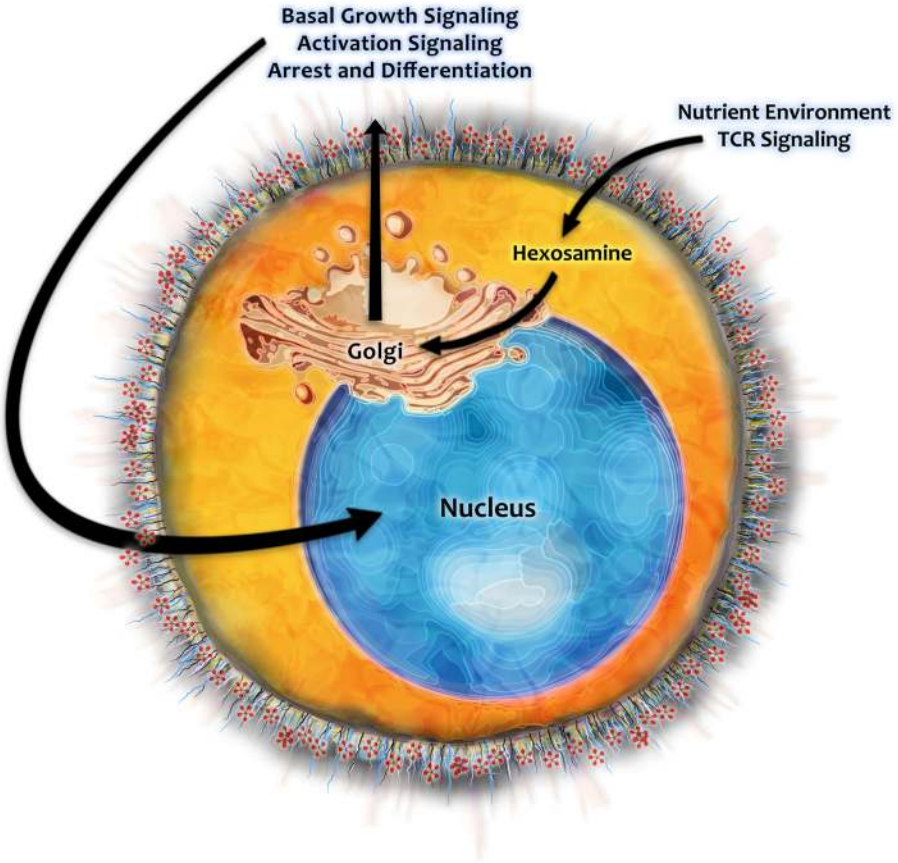

Fig. 5. Metabolism controls cell growth by regulating the galectin-glycoprotein lattice

The nutrient environment and/or growth signaling by TCR influences UDP-GlcNAc production by the hexosamine pathway, which in turn regulates N-glycan branching in the Golgi to control galectin-glycoprotein lattice strength. Subsequent changes in cell surface receptor membrane localization and concentration results in differential responses to external factors (eg. Growth factors, peptide-MHC) that signal to the nucleus.

Table 1

Regulation of cell surface glycoproteins by the galectin – glycoprotein lattice

Glycoprotein	Galectin	Function	References
a) Regulation of membrane localization/lateral mobility/clustering			
TCR complex	Gal-1, -3, others?	Exclusion from GM1 microdomains and immune synapse, inhibition of clustering and signaling	1,5,7,10,11,35,70
CD4, CD8	Gal-3, others?	Exclusion from TCR complex	5,8
CD45	Gal-1, -3, others?	Inclusion in GM1 microdomains and immune synapse, inhibition of TCR signaling	5,10,11
EGFR	Gal-3, others?	Exclusion from Caveolin-1 microdomains	26
b) Negative regulation of endocytosis to enhance surface residency			
CTLA-4	?	T cell growth arrest	3,75,76
TβR	Gal-3, others?	Epithelial/mesenchymal cell growth arrest	3,25
EGFR, PDGF, IGFR, FGFR	Gal-3, others?	Promotes cytokine signaling, epithelial-mesenchymal transition, cell motility, tumor growth and metastasis	3,25
GLUT-4	?	Glucose transporter, clearance of systemic glucose by muscle and adipocytes	3
GLUT-2	Gal-9, others?	Glucose transporter, Glucose sensing in pancreatic β-cells	33
TRPV-5	Gal-1, others?	Renal Ca^{+2} Channel	35
c) Regulation of T cell apoptosis*			
CD3, CD7, CD43, CD45	Gal-1	Apoptosis (thymocytes, T_H1, T_H17)	9,10,11,12,14
CD7, CD29, CD45, CD71	Gal-3	T cell Apoptosis	11,16
Tim-3	Gal-9	T_H1 apoptosis	15

*not an exhaustive list of galectin mediated apoptosis