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# Genetic defects in *N*-glycosylation and cellular diversity in mammals

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and Michael Demetriou†

Glycoproteins in mammalian cells are modified with complex-type asparagine-linked glycans of variable chain lengths and composition. Observations of mice carrying mutations in glycosyltransferase genes imply that *N*-glycan structures regulate T-cell receptor clustering and hence sensitivity to agonists. We argue that the heterogeneity inherent in *N*-glycosylation contributes to cellular diversity and, thereby, to adaptability in the immune system.

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## Abbreviations

<b>APC</b>	antigen-presenting cell
<b>CDG</b>	congenital disorder of glycosylation
<b>CRD</b>	carbohydrate-recognition domain
<b>peptide–MHC</b>	antigenic peptide bound to major histocompatibility complex
<b>PyMT</b>	polyomavirus middle T oncogene
<b>TCR</b>	T-cell receptor

## Introduction

*N*- and *O*-linked glycans are found on both cell-surface and secreted proteins, many of which control proliferation and cell fate decisions in animals. Tissue-specific expression of glycosyltransferases is a significant factor controlling the glycan profiles observed in differentiated cells [1]. In addition, many glycosyltransferases compete for acceptor intermediates, causing bifurcations of the pathways and additional structural complexity [2]. Heterogeneity at specific Asn-X-Ser/Thr sites on individual glycoproteins is very common and diversifies the molecular population into glycoforms. The protein environment of each Asn-X-Ser/Thr influences access by processing enzymes and inefficiencies in this process result in only a fraction of *N*-glycans receiving certain substitutions. As a result, each Asn-X-Ser/Thr site of a glycoprotein is commonly populated by a set of biosynthetically related *N*-glycan structures.

The specific activity with respect to a particular functional property can vary between individual glycoforms and thus the potency of a glycoprotein is actually the weighted average of the specific activities of the glycoform population. For example, the peptide hormones lutropin and erythropoietin are produced with structurally diverse glycans, and the discrete glycoforms differ in their affinity for hepatic lectins, thereby determining serum half-lives and potency

*in vivo* [3,4]. By balancing the proportions of glycoforms with different serum half-life, the potency and kinetics of a cytokine response can be modified or adapted to extrinsic conditions. In a similar manner, variation in receptor glycosylation has the potential to modify their physical associations in the membrane and thereby the ligand occupancy thresholds for signal transduction. The terminal portions of mature *N*-glycans are generally not held close to the glycoprotein on which they reside. This leaves them free to bind to multivalent lectins at the cell surface. Various receptor glycoforms would have differing affinities for cell-surface lectins. The interaction of receptors with such lectins could potentially serve as an adjustable means of linking various signaling systems and hence tuning the cellular responses to various stimuli, as previously suggested by Feizi and Childs [5]. Receptor systems that form highly cooperative macromolecular clusters in response to agonists, such as T-cell receptors (TCRs) and cell adhesion receptors, appear to be sensitive to glycoform variation. Herein we discuss phenotypes associated with mutations in enzymes of the glycosylation pathways and their implications concerning the molecular functions of cell-surface *N*-glycans.

## Congenital disorders of glycosylation

The first stage of protein *N*-glycosylation, conserved from yeast to man, is the synthesis of Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub>-PP-dolichol and transfer of the oligosaccharide to proteins in the ER. Five congenital disorders of glycosylation (CDGs) are known, each with a characteristic enzymatic defect either in the biosynthesis of Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub>-PP-dolichol or in its transfer to asparagine. These are known as type I CDGs. Infants with these defects share clinical features, including developmental delay, multiple organ abnormalities and severe neurologic dysfunction [6\*,7]. These deficiencies result from the partial failure to glycosylate proteins with oligosaccharides at the usual Asn-X-Ser/Thr sites. A complete deficit of *N*-glycosylation is expected to be incompatible with life, as the inhibition of oligosaccharyltransferase by tunicamycin is toxic to yeast and mammalian cells. Glc<sub>1</sub>Man<sub>7-9</sub>GlcNAc<sub>2</sub> on newly synthesized glycoproteins binds calnexin, calreticulin and ER  $\alpha$ -glucosyltransferase. The last acts as a sensor of glycoprotein conformations and, combined with the action of  $\alpha$ -glucosidase II, a deglycosylation/reglycosylation cycle continues and retains the glycoprotein until proper folding occurs, thus fulfilling a function analogous to that of chaperones [8\*,9].

CDG type I patients display a remarkable variability in clinical phenotypes, even, for example, between patients that have the same mutant alleles of the phosphomannomutase gene *PMM2* [6\*]. The genetic background of each

patient can presumably influence the expressivity of a phenotype in a complex manner. The chaperone function of Glc<sub>1</sub>Man<sub>7-9</sub>GlcNAc<sub>2</sub> may vary depending on polymorphism in the underlying polypeptides and this could influence the clinical phenotype. ‘Silent’ polymorphisms are common in the population, such as the underglycosylated state present in CDG patients, and may be innocuous until revealed by a ‘molecular stress’. This is analogous to the inactivation of the chaperone Hsp90 in *Drosophila*, which revealed the accumulated genetic variation between strain backgrounds as morphological mutations [10]. In an evolutionary context, environmental stresses that compromise the buffering capacity of protein chaperones, including those that rely on *N*-glycans, may reveal a reservoir of genetic diversity for rapid adaptation [10].

### Complex-type *N*-glycans in embryogenesis and immune regulation

The position and specificity of key enzymes in the *N*-glycan biosynthesis pathway are depicted in Figure 1a. The GlcNAc-TI gene *Mgat1* is ubiquitously expressed and required in the biosynthesis of all hybrid and complex-type *N*-glycans (Figure 1a). *Mgat1*<sup>-/-</sup> mouse embryos die at around embryonic day E9.5 with failures of neural tube formation, vascularization and determination of left/right body plan asymmetry [11,12]. Maternal sources of the *Mgat1* enzyme and its *N*-glycan products are present in pre-E6 day embryos, and may support development until E9.5, after which complex-type glycans are largely depleted [13]. In chimeric embryos, *Mgat1*<sup>-/-</sup> cells contribute to various tissues, with the exception of lung bronchial epithelium [14]. Consistent with this observation, the *Mgat1*<sup>-/-</sup> embryos display a severe failure to organize bronchial epithelium. As *Mgat1* mutant Chinese hamster ovary cells grow normally in culture, the *in vivo* data on the failure of *Mgat1*<sup>-/-</sup> embryos support the notion that complex-type *N*-glycans mediate cell–cell interactions in the animal. However, when growth conditions are limiting, as in low-serum cultures, mutant tumor cells with defects in either GlcNAc-TV (*Mgat5*) or the Golgi UDP-Gal transporter were observed to grow at reduced rates and displayed an increased dependency on autocrine growth factors [15].

These studies suggested that complex-type *N*-glycans on certain cell-surface receptors affect their sensitivity to growth factors and/or adhesion signals. Indeed, some glycosyltransferase mutant mice may appear normal at birth. Phenotypes can be revealed, however, by stress and aging, as well as by expressing the mutations in different inbred strains of mice. In this regard, there was a relatively low incidence of mammary tumor growth and metastases induced by the polyomavirus middle T oncogene (PyMT) in *Mgat5*<sup>-/-</sup> mice on the 129/sv x FVB background. The PyMT protein is an intracellular docking protein that transforms cells by activating the Ras signaling pathways, as well as phosphatidylinositol 3-kinase and protein

kinase B (PKB/Akt). The activation of these latter enzymes is the downstream effect of focal adhesion signaling, which appears to be impaired in *Mgat5*<sup>-/-</sup> tumor cells and embryonic fibroblasts. Glycans modified by *Mgat5* are present on integrin  $\alpha 5\beta 1$  and their depletion may stabilize substratum attachment and result in an impairment of PyMT signaling and tumor growth in *Mgat5*<sup>-/-</sup> mice [16\*\*]. A cancer phenotype has also been revealed in *Mgat3*<sup>-/-</sup> mice by treating them with a carcinogen. Diethylnitrosamine-induced hepatocarcinomas progress more slowly in *Mgat3*<sup>-/-</sup> mice compared with wild-type littermates. Further analysis of the mice revealed that a paracrine growth factor dependent upon *Mgat3* promotes tumor growth in the mice [17\*].

In the absence of GlcNAc-TII (*Mgat2*), complex-type *N*-glycans are replaced by hybrid-type glycans (Figure 1a). Mis-sense mutations have been identified in *Mgat2* that reduce enzyme activity by >95% in two CDG type IIa patients [18]. This is a rare autosomal recessive disorder characterized by multisystemic involvement and severe impairment of the nervous system. *Mgat2*<sup>-/-</sup> mice are runted and die at variable times after birth with multiple organ defects (D Chui, JD Marth, personal communication), further evidence that complex-type *N*-glycans are required for normal embryogenesis.

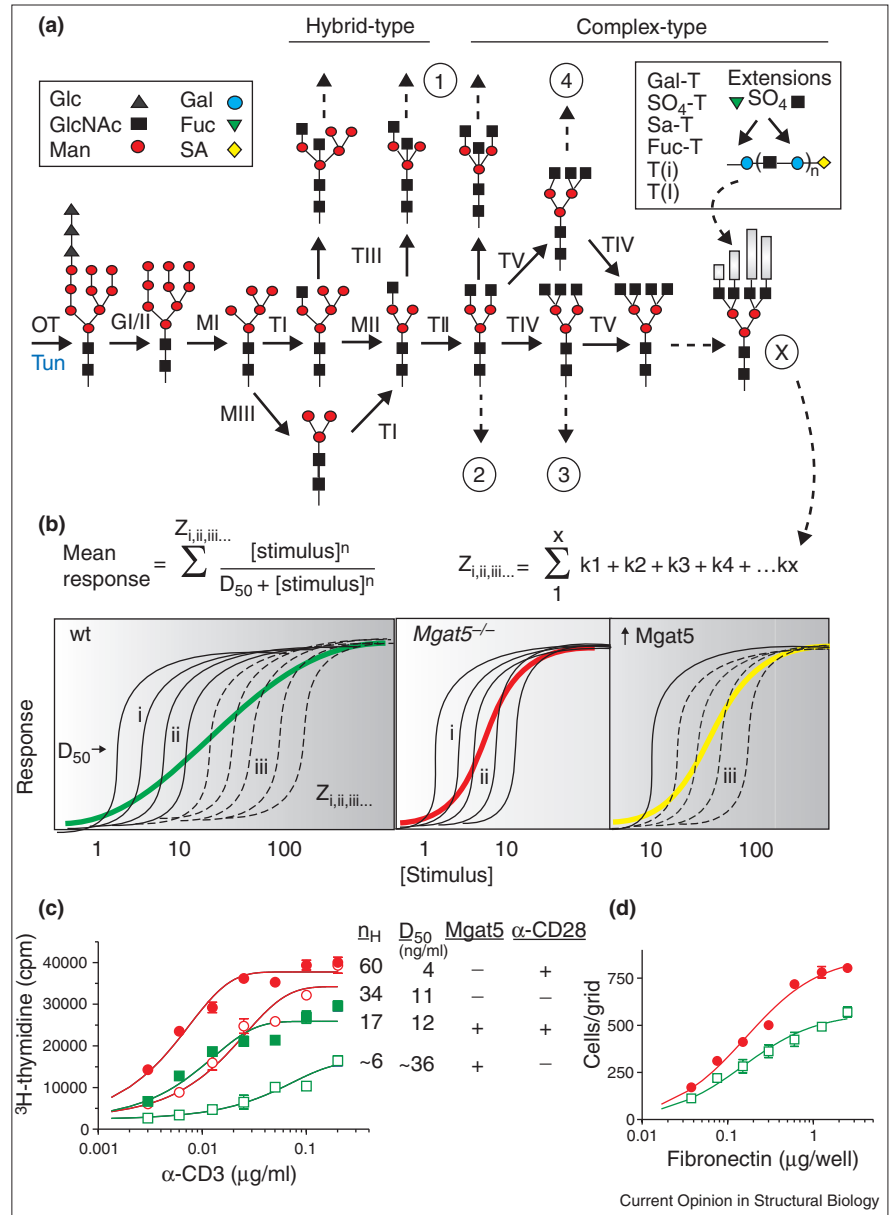
Mutations disrupting fewer glycan structures generally result in viable animals with tissue-restricted or conditional phenotypes. These include mutations that affect either late steps in the processing pathway or enzymes with functional redundancies. Immune phenotypes have been detected in a number of viable glycosyltransferase mutant mice, indicating a particular sensitivity of immune cells to changes in glycosylation. Sialyltransferase ST6Gal modifies *N*-linked glycans with  $\alpha 2$ -6-linked sialic acid; mice lacking this enzyme display impaired B-cell proliferation [19]. Mice deficient in ST3Gal-1, a sialyltransferase that substitutes core 1 (Gal $\beta$ 1-3GalNAc) *O*-glycans, also display the subtle phenotype of CD8<sup>+</sup> T-cell depletion by apoptosis, but retention of CD8<sup>+</sup> memory T cells [20]. The E-, P- and L-selectins and their ligands control leukocyte traffic. Mutations in several glycosyltransferases that produce selectin ligands disrupt trafficking, but mutant phenotypes differ qualitatively, presumably because of the details of glycan structure and their tissue distribution. These include Fuc-TIV, Fuc-TVII [21], the *O*-linked core 2 GlcNAc-T(L) branching enzyme [22] and a  $\beta$ 3GlcNAc-T that extends core 1 *O*-glycans with 6-sulfo sialyl-LeX [23]. Mice deficient in the *N*-linked processing enzymes  $\alpha$ -mannosidase II [24] or *Mgat5* both develop autoimmune kidney disease with age [25\*\*].

### *Mgat5*-modified glycans regulate TCR sensitivity to antigen

*Mgat5*<sup>-/-</sup> mice lack detectable GlcNAc $\beta$ 1,6Man $\alpha$ 1,6-branched *N*-glycan products and are born healthy from CD1 outbred and 129/sv inbred mouse strains [16\*\*].

**Figure 1**

Glycosylation, signaling thresholds and cell population diversity. **(a)** Scheme of the *N*-glycan biosynthesis pathway. Oligosaccharyltransferase, OT;  $\alpha$ -glucosidases, Gl and GII;  $\beta$ -*N*-acetylglucosaminyltransferases, T1, TII, TIII, TIV, TV, T(i);  $\alpha$ 1,2-mannosidases, MI;  $\alpha$ 1,3/6-mannosidases, MII and MIII;  $\beta$ 1,4-galactosyltransferases, Gal-T;  $\alpha$ -fucosyltransferases, Fuc-T;  $\alpha$ -sialyltransferases, Sa-T; sulfotransferase, SO<sub>4</sub>-T. Gene names for T1 to TV are *Mgat1* to *Mgat5*, respectively. Tun, tunicamycin. The circled numbers 1 to x represent biosynthetically related subsets of glycans labeled here only to illustrate the model shown below. **(b)** Hypothetical model to represent variability or plasticity within the T-cell population. For each T cell, entry into S phase is a switch-like event that initiates activation and several rounds of cell division. Each black line represents individual cells or groups of cells that share a similar *N*-glycan structural profile. The weighted contributions of glycoforms ( $k_1$ – $k_x$ ) determine the cell phenotype ( $Z_i$ ), the response to agonist. The mean response to agonists is the sum of responses for the population over all  $Z_i$ . The dashed lines depict the influence of *Mgat5* glycans. The mean response has a  $D_{50}$  and Hill slope as depicted by the colored line for the wild-type (wt), *Mgat5* deficiency (*Mgat5*<sup>-/-</sup>) and *Mgat5* overexpression ( $\uparrow$  *Mgat5*). The last panel may also apply to ST6Gal-deficient cells. Note the variance in the cell population is largest for the wild-type and reduced in the mutants. **(c)** Purified T cells were stimulated at low density for 48 h with increasing concentrations of soluble anti-TCR antibody in the presence or absence of anti-CD28 antibody (data from [25•]). The code for genotypes and stimulation in each curve are indicated in the table on the right, along with the ligand concentration providing a 50% response ( $D_{50}$ ) and the Hill coefficient ( $n_H$ ). **(d)** Adhesion of *Mgat5*<sup>-/-</sup> (solid) and wild-type (open) leukocytes to increasing concentrations of fibronectin. *Mgat5*-modified glycans on the TCR complex and adhesion receptors impair TCR clustering and formation of focal adhesions, respectively, in both cases reducing the cooperativity ( $n_H$ ) and increasing the ligand threshold ( $D_{50}$ ).

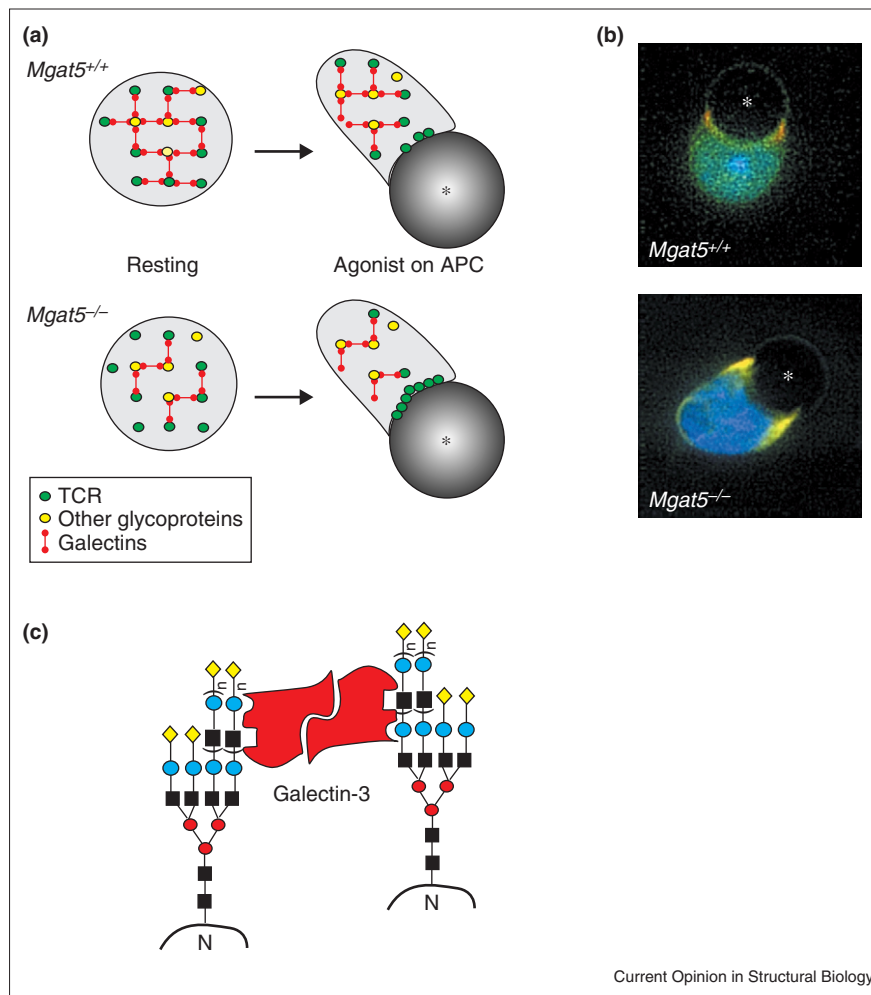


However, small litter sizes and higher perinatal mortality have been observed with the *Mgat5* mutation on a PLJ mouse background, a hyperimmune mouse strain (J Pawling, M Demetriou *et al.*, unpublished data). *Mgat5*<sup>-/-</sup> mice displayed an age-dependent autoimmune disease characterized by glomerulonephritis [25•]. T-cell-dependent immune reactions were exaggerated in *Mgat5*<sup>-/-</sup> mice and T cells in culture were hypersensitive to TCR agonists. Delayed type hypersensitivity was more severe and susceptibility to experimental autoimmune encephalomyelitis, a model for human multiple sclerosis, was greater in mutant

mice. In contrast, *Mgat5*<sup>-/-</sup> B cells responded normally to a variety of stimuli, indicating that the defect is restricted to selected cell types.

TCRs are recruited into 'immune synapses' by peptide-MHC complexes on antigen-presenting cells (APCs). A threshold number of receptors are required in the immune synapse to sustain intracellular signaling and trigger entry into S phase [26]. This rate-limiting event of sustained TCR clustering was enhanced in *Mgat5*<sup>-/-</sup> cells (Figure 2). TCR-dependent tyrosine phosphorylation,

Figure 2



Restriction of receptor clustering by galectins. (a) A model depicting restricted mobility of TCR by interaction with a galectin–glycoprotein network. The lattice has more binding interactions and therefore greater avidity in *Mgat5*<sup>+/+</sup> cells. The ball marked by an asterisk represents an APC or an anti-TCR antibody-coated bead. (b) Merged images of *Mgat5*<sup>-/-</sup> and *Mgat5*<sup>+/+</sup> cells, showing the TCR (green) and actin microfilament (red) distribution in T cells stimulated by anti-CD3 $\epsilon$  coated beads (marked with an asterisk) (taken from [25\*\*]). (c) Schematic diagram of a galectin-3 homodimer, represented as a red dumbbell in (a), binding to N-glycans and cross-linking two glycoproteins.

actin microfilament reorganization and Ca<sup>2+</sup> mobilization were enhanced in *Mgat5*<sup>-/-</sup> T cells, but intracellular signaling downstream of TCR induced by phorbol ester was unchanged. These results indicate that T-cell hypersensitivity in *Mgat5*<sup>-/-</sup> cells is due to change at the cell surface [25\*\*]. In quiescent T cells, *Mgat5* gene expression is low and rate limiting in the production of cell-surface N-glycans with  $\beta$ 1,6GlcNAc branches. *Mgat5* gene transcription, enzyme activity and glycan products increase following activation [25\*\*]. *Mgat5* gene expression is positively regulated by Ras–Raf–Ets activation downstream of TCR. Therefore, *Mgat5* glycans are regulated during T-cell maturation and are also determinants of TCR sensitivity to agonists.

Some glycoproteins recycle through the endocytic pathway and back to the cell surface, but this does not appear to involve remodeling of N-glycan branching [27]. As such, changes in N-glycan branching at the cell surface occur through *de novo* synthesis of glycoproteins. The timescale of this process is hours, whereas TCR-dependent tyrosine phosphorylation, Ca<sup>2+</sup> mobilization and actin rearrangement

occur in minutes after agonist-induced receptor clustering. Therefore, *Mgat5*-dependent regulation of TCR sensitivity to antigen is a long timescale, slow form of negative feedback, governed by the steady-state activity of signaling pathways that are downstream of TCR. This type of delayed negative feedback has been observed in other pathways and provides a mechanism to adjust or tune receptor sensitivity to match ambient conditions [28]. T cells in different tissue environments may alter *Mgat5*-modified glycan levels, thereby adapting the TCR sensitivity and response threshold.

### Lectin–glycan interactions modulate lymphocyte receptor signaling

The TCR  $\alpha$  and  $\beta$  chains together have seven N-glycans and CD3  $\gamma$  and  $\delta$  each have one chain. Glycosylation is necessary for the assembly of these peptides into a mature TCR complex. The N-glycans protrude  $\sim$ 30 Å from the protein surface and a fraction of these glycans appear, by their positive leucoagglutinin L-PHA reactivity, to be  $\beta$ 1,6GlcNAc-branched complex-type structures [29], which are preferentially substituted with poly N-acetylglucosamine

[30]. Electron density for the chitobiose core (GlcNAc<sub>2</sub>) of *N*-glycans is often observed in high-resolution X-ray structures [31••]. However, density for distal *N*-glycan sequences is generally not visible in X-ray structures, consistent with their mobility and lack of order in the crystal. Biantennary complex-type *N*-glycans have been modeled onto TCR complex. Based on their size and positions, it has been proposed that *N*-glycans may limit nonspecific receptor aggregation and thereby also spurious T-cell activation [31••]. The glycans may also align the geometry for TCR binding towards peptide–MHC complexes on the APC [31••].

We recently reported that glycoproteins of the TCR complex bind to galectins at the cell surface, which impedes TCR clustering in response to agonist [25••]. The galectin family is conserved in metazoans and features either one or two carbohydrate-recognition domains (CRDs) per molecule. Galectin-1 and galectin-3 have one CRD and form homodimers with CRDs spaced ~50 Å apart and oriented in opposing directions [32], a feature ideally suited for cross-linking glycoproteins with multiple glycans [33] (Figure 2). Treating wild-type T cells with lactose or lactosamine to dissociate the galectins from their endogenous ligands enhanced TCR clustering and tyrosine phosphorylation in response to agonist, in essence creating a phenocopy of *Mgat5*<sup>-/-</sup> [25••]. Furthermore, cell-surface galectin-3 was found physically associated with TCR complex, an interaction enhanced by the expression of *Mgat5* glycans. Monomeric affinities of galectins for lactosamine and lactose are in the 10<sup>-3</sup> M range [34], which is comparable to peptide–MHC-induced oligomerization of TCR measured in solution and lower than TCR affinity for peptide–MHC to form an immune synapse [26]. Poly *N*-acetyllactosamine, a slightly higher affinity ligand (10<sup>-4</sup> M) for galectin-3 [34], is preferentially added to *Mgat5*-modified glycans [30] (Figure 1a). Therefore, *Mgat5* glycans on the TCR complex and other glycoproteins appear to form a multivalent lattice held together by galectins, which slow the migration of the TCR into clusters at the immune synapse (Figure 2a,c).

There are at least ten mammalian galectins with overlapping expression patterns. Mice deficient in galectin-1 or galectin-3 are healthy, but have not yet been examined for T cell or stress-related phenotypes [35]. Galectin-3 and the TCR complex are present in the T-cell lattice and galectin-1 has been reported to bind to glycoproteins designated CD2, CD3, CD4, CD7, CD43 and CD45 on T cells [36], but other components of the lattice remain to be defined. Exogenous galectin-3 and galectin-1 modulate T-cell activation *in vitro* [36], antagonize TCR signaling [37•] and, when injected into mice, galectin-1 can suppress autoimmune disease [38]. The expression of galectin-3 at the cell surface of cancer cells is associated with an increase in tumor growth, invasion and metastasis [39•], possibly as a result of enhanced turnover of integrin–substratum contacts, which would give rise to an increase in focal adhesions.

Siglecs are sialic-acid-binding lectins implicated in lymphoid and myeloid cell functions. CD22 (siglec-2) binds ST6Gal products (SAα2,6Galβ) *in cis* on the B-cell surface. Unlike galectins, CD22 is a transmembrane protein with a phosphorylated cytosolic domain, and recruits Grb2, Shc, SHP1 and SHIP, causing reduced B-cell receptor signaling [40]. B cells of CD22-deficient mice are hypersensitive to antigen stimulation. However, ST6Gal-deficient mice display impaired B-cell proliferation, attenuated antibody production and, although cell-surface CD22 is present, it is not bound to ligand [41]. This suggests that the recruitment of CD22 into B-cell receptor signaling complexes is inhibited by SAα2,6Galβ. Therefore, SAα2,6Galβ appears to be a negative regulator of a negative regulator (i.e. CD22) and loss of SAα2,6Galβ allows CD22 to dampen the B-cell response. Such a possible scenario is depicted by the model in Figure 1b (represented by the right panel). ST6Gal activity [42] and CD22 occupancy with sialic acid are regulated with B-cell activation [43]. Similar to *Mgat5* regulation in T cells, this suggests that the signaling threshold for B-cell receptors may be regulated by differential sialylation, which controls the availability of CD22.

### Structural diversity of glycans increases functional diversity

Individual cells typically show small variances of many molecular parameters that, together, confer a Gaussian spectrum of responsiveness in the cell population. The protein glycosylation machinery appears to be designed to increase molecular heterogeneity and, presumably in some instances, functional diversity within cell populations as well. Although different receptor glycoforms may vary only slightly in their affinities for lectins and signal transduction, exponential amplification of lymphocyte clones can convert small differences into large systemic events [44]. T-cell clones undergo multiple rounds of cell division once triggered by peptide–MHC binding above a threshold affinity. Once this stochastic event occurs, strong positive feedback by cytokines creates a highly cooperative and sustained expansion of cells. Many other interactions between cells influence the balance of Th1/Th2 helper T cells, development of memory T cells and cessation of the response. The immune system appears to be particularly sensitive to small functional differences at the cellular level.

Sustained clustering of ~8000 TCRs results in an increase in the concentration of protein kinases and of docking proteins on the inner surface of the membrane; this results in the triggering of T-cell activation [45]. CD28 is a co-receptor of the TCR. Binding of CD28 to CD80 on APCs enhances the recruitment of intracellular signaling molecules, thereby reducing by fivefold the number of TCRs required for activation [46,47]. The *Mgat5* deficiency sensitizes this system still further and lowers the threshold for the TCR response to agonists; this is independent of the ligation of CD28 [25••] (Figure 1c). In addition, the apparent Hill coefficient ( $n_H$ ) was increased in *Mgat5*-deficient cells and this represents the synchrony of the responding cell population in this

experiment. Although each T cell responds individually in a switch-like manner, the inherent heterogeneity between cells for *N*-glycans and other factors such as CD28 levels tends to reduce the synchrony or apparent cooperativity of the responding cell population (Figure 1c) [48]. The *Mgat5* mutation eliminates a subset of *N*-glycans, reducing both molecular heterogeneity in the remaining *N*-glycans and presumably the heterogeneity of the glycoprotein–galectin lattice (Figure 1b). *Mgat5* glycans are present on  $\alpha 1\beta 5$  integrin and, in a similar manner, *Mgat5*<sup>-/-</sup> cells show increased cooperativity for adhesion [16\*\*], whereas overexpression of *Mgat5* has the opposite effect on the adhesion to a substratum of fibronectin [49] (Figure 1d).

Coffey [50] has argued convincingly that complex biological systems, such as the immune system, might be modeled using nonlinear dynamics and chaos theory, beginning with simple rules of interaction governing the behavior of cells in the system. The calculated outcomes over time appear unpredictable, but they are confined to a bounded set and are highly dependent on basal conditions and particularly the genetic background. Coffey further notes that living systems tend to evolve towards the boundary of order and chaos where the system has maximum fitness. Loss of diversity in the lymphocyte population is thought to have an influence on the probability of acquiring infections and autoimmune diseases, as suggested by many gene knockout experiments in mice. For example, CD28-deficient mice are more resistant to autoimmune disease, but more sensitive to certain pathogens. Resistance to infections and development of cancer versus the maintenance of self-tolerance are, in some respects, opposing objectives of the immune system and are balanced for overall fitness. *Mgat5*-deficient mice are prone to developing autoimmune diseases, but they show a relatively low incidence of breast cancer progression in a transgenic oncogene model of cancer [16\*\*].

## Conclusions

Structurally diverse glycans are present on many receptors that may also be regulated by interactions with lectins, as discussed here for TCR, integrins and CD22. It would appear that the incremental effects of glycoform variation on highly cooperative events, such as TCR clustering at the molecular level and lymphocyte activation at the cell population level, can set thresholds for systemic events such as susceptibility to autoimmune disease. Although further evidence is needed to support our hypothesis, it is possible that the structural diversity of glycans may be an important feature of adaptability and fitness designed to regulate receptor thresholds and variances of cellular responses to extrinsic stimuli.

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## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Paulson JC, Colley KJ: **Glycosyltransferases: structure, localization, and control of cell type-specific glycosylation.** *J Biol Chem* 1989, **264**:17615-17618.
  2. Schachter H: **Biosynthetic controls that determine the branching and microheterogeneity of protein-bound oligosaccharides.** *Biochem Cell Biol* 1986, **64**:163-181.
  3. Fiete D, Srivastava V, Hindsgaul O, Baenziger JU: **A hepatic reticuloendothelial cell receptor specific for SO<sub>4</sub>-4GalNAc $\beta$ 1,4GlcNAc $\beta$ 1,Man $\alpha$  that mediates rapid clearance of lutropin.** *Cell* 1991, **67**:1103-1110.
  4. Takeuchi M, Kobata A: **Structures and functional roles of the sugar chains of human erythropoietins.** *Glycobiology* 1991, **1**:337-346.
  5. Feizi T, Childs RA: **Carbohydrates as antigenic determinants of glycoproteins.** *Biochem J* 1987, **245**:1-11.
  6. Carchon H, Van Schaftingen E, Matthijs G, Jaeken J: **Carbohydrate-deficient glycoprotein syndrome type IA (phosphomannomutase-deficiency).** *Biochim Biophys Acta* 1999, **1455**:155-165.  
This paper reviews the type I CDG syndromes, with a focus on the clinical symptoms and the mis-sense mutations in the *PMM2* gene. This issue of the journal has several other reviews on CDG syndromes that cover all aspects of the problem.
  7. Freeze HH, Aebi M: **Molecular basis of carbohydrate-deficient glycoprotein syndromes type I with normal phosphomannomutase activity.** *Biochim Biophys Acta* 1999, **1455**:167-178.
  8. Helenius A, Aebi M: **Intracellular functions of N-linked glycans.** *Science* 2001, **291**:2364-2369.  
This brief review covers the role of *N*-glycans in protein folding and their intracellular transport and targeting functions. This is part of a special feature by *Science* magazine on glycobiology and the accompanying articles are also very good.
  9. Parodi AJ: **Protein glucosylation and its role in protein folding.** *Annu Rev Biochem* 2000, **69**:69-93.
  10. Rutherford SL, Lindquist S: **Hsp90 as a capacitor for morphological evolution.** *Nature* 1998, **396**:336-342.
  11. Metzler M, Gertz A, Sarkar M, Schachter H, Schrader JW, Marth JD: **Complex asparagine-linked oligosaccharides are required for morphogenic events during post-implantation development.** *EMBO J* 1994, **13**:2056-2065.
  12. Ioffe E, Stanley P: **Mice lacking N-acetylglucosaminyltransferase I activity die at mid-gestation, revealing an essential role for complex or hybrid N-linked carbohydrates.** *Proc Natl Acad Sci USA* 1994, **91**:728-732.
  13. Ioffe E, Liu Y, Stanley P: **Complex N-glycans in *Mgat1* null preimplantation embryos arise from maternal *Mgat1* RNA.** *Glycobiology* 1997, **7**:913-919.
  14. Ioffe E, Liu Y, Stanley P: **Essential role for complex N-glycans in forming an organized layer of bronchial epithelium.** *Proc Natl Acad Sci USA* 1996, **93**:11041-11046.
  15. VanderElst I, Dennis JW: **N-linked oligosaccharide processing and autocrine-stimulation of tumor cell proliferation.** *Exp Cell Res* 1991, **192**:612-613.
  16. Granovsky M, Fata J, Pawling J, Muller WJ, Khokha R, Dennis JW: **Suppression of tumor growth and metastasis in *Mgat5*-deficient mice.** *Nat Med* 2000, **6**:306-312.  
*Mgat5*<sup>-/-</sup> mice were crossed with transgenic mice expressing the oncogene polyoma virus. PyMT-induced tumor growth and metastasis were markedly suppressed in *Mgat5*<sup>-/-</sup> mice. *Mgat5* promoted focal adhesion turnover, which appears to amplify PyMT-dependent activation of PI3 kinase/PKB, a key event in PyMT oncogenesis.
  17. Yang X, Bhaumik M, Bhattacharyya R, Gong S, Rogler CE, Stanley P: **New evidence for an extra-hepatic role of N-acetylglucosaminyltransferase III in the progression of diethylnitrosamine-induced liver tumors in mice.** *Cancer Res* 2000, **6**:3313-3319.  
Two independent *Mgat3*<sup>-/-</sup> mouse strains suppress chemical-induced liver cancer. As *Mgat3* is not expressed in the liver, expression was introduced by a MUP/*Mgat3* transgene, an experiment further suggesting that the positive effects of *Mgat3* on tumor growth were due to extrahepatic glycoprotein.

18. Tan J, Dunn J, Jaeken J, Schachter H: **Mutations in the MGAT2 gene controlling complex N-glycan synthesis cause carbohydrate-deficient glycoprotein syndrome type II, an autosomal recessive disease with defective brain development.** *Am J Hum Genet* 1996, **59**:810-817.
19. Hennet T, Chui D, Paulson JC, Marth JD: **Immune regulation by the ST6Gal sialyltransferase.** *Proc Natl Acad Sci USA* 1998, **95**:4504-4509.
20. Priatel JJ, Chui D, Hiraoka N, Simmons CJ, Richardson KB, Page DM, Fukuda M, Varki NM, Marth JD: **The ST3Gal-I sialyltransferase controls CD8+ T lymphocyte homeostasis by modulating O-glycan biosynthesis.** *Immunity* 2000, **12**:273-283.
21. Maly P, Thall AD, Petryniak B, Rogers CE, Smith PL, Marks RM, Kelly RJ, Gersten KM, Cheng G, Saunders TL *et al.*: **The  $\alpha(1,3)$ fucosyltransferase fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis.** *Cell* 1996, **86**:643-653.
22. Snapp KR, Heitzig CE, Ellies LG, Marth JD, Kansas GS: **Differential requirements for the O-linked branching enzyme core 2  $\beta(1-6)$ -N-glucosaminyltransferase in biosynthesis of ligands for E-selectin and P-selectin.** *Blood* 2001, **97**:3806-3811.
23. Yeh J, Hiraoka N, Petryniak B, Nakayama J, Ellies LG, Rabuka D, Hinds Gaul O, Marth JD, Lowe JB, Fukuda M: **Novel sulfated lymphocyte homing receptors and their control by a core 1 extension  $\beta(1,3)$ -N-acetylglucosaminyltransferase.** *Cell* 2001, **105**:957-969.
24. Chui D, Sellakumar G, Green R, Sutton-Smith M, McQuistan T, Marek K, Morris H, Dell A, Marth J: **Genetic remodeling of protein glycosylation *in vivo* induces autoimmune disease.** *Proc Natl Acad Sci USA* 2001, **98**:1142-1147.
25. Demetriou M, Granovsky M, Quaggin S, Dennis JW: **Negative regulation of T-cell activation and autoimmunity by Mgat5 N-glycosylation.** *Nature* 2001, **409**:733-739.  
 The autoimmune and T-cell hypersensitivity phenotypes in *Mgat5*<sup>-/-</sup> mice are documented. Furthermore, *Mgat5*<sup>-/-</sup> T cells show enhanced TCR clustering, receptor internalization and intracellular signaling. The presence of a glycoprotein-galectin lattice on the cells was demonstrated by competition with sugars and by chemical cross-linking of TCR and galectin-3. The results suggest a general mechanism for regulation of receptor clustering through differential glycosylation and interaction with cell-surface lectins.
26. Reich Z, Boniface JJ, Lyons DS, Borochoy N, Wachtel EJ, Davis MM: **Ligand-specific oligomerization of T-cell receptor molecules.** *Nature* 1997, **387**:617-620.
27. Helgemo SL, Hart GW: **Recycling cell surface glycoproteins undergo limited oligosaccharide reprocessing in LEC1 mutant Chinese hamster ovary cells.** *Glycobiology* 1998, **8**:1173-1182.
28. Alon U, Surette MG, Barkai N, Leibler S: **Robustness in bacterial chemotaxis.** *Nature* 1999, **397**:168-171.
29. Hubbard SC, Kranz DM, Longmore GD, Sitkovsky MV, Eisen HN: **Glycosylation of the T-cell antigen-specific receptor and its potential role in lectin-mediated cytotoxicity.** *Proc Natl Acad Sci USA* 1986, **83**:1852-1856.
30. Cummings RD, Kornfeld S: **The distribution of repeating Gal  $\beta(1-4)$ GlcNAc  $\beta(1-3)$  sequences in asparagine-linked oligosaccharides of the mouse lymphoma cell line BW5147 and PHAR 2.1.** *J Biol Chem* 1984, **259**:6253-6260.
31. Rudd PM, Wormald MR, Stanfield RL, Huang M, Mattsson N, Speir JA, DiGennaro JA, Fetrow JS, Dwek RA, Wilson IA: **Roles for glycosylation of cell surface receptors involved in cellular immune recognition.** *J Mol Biol* 1999, **293**:351-366.  
 Molecular models for CD3 subunits were made by aligning nonredundant sets of known protein structures using a threading algorithm. Glycosylation was added to examine the spatial arrangement, thereby suggesting possible consequences in receptor clustering and agonist recognition.
32. Lobsanov YD, Gitt MA, Leffler H, Barondes SH, Rini JM: **X-ray crystal structure of the human dimeric S-Lac lectin, L-14-II, in complex with lactose at 2.9-Å resolution.** *J Biol Chem* 1993, **268**:27034-27038.
33. Mandal DK, Brewer CF: **Cross-linking activity of the 14-kilodalton beta-galactoside-specific vertebrate lectin with asialofetuin: comparison with several galactose-specific plant lectins.** *Biochemistry* 1993, **31**:8465-8472.
34. Sato S, Hughes RC: **Binding specificity of a baby hamster kidney lectin for H type I and II chains, polylectosamine glycans, and appropriately glycosylated forms of laminin and fibronectin.** *J Biol Chem* 1992, **267**:6983-6990.
35. Colnot C, Fowles D, Ripoche MA, Bouchaert I, Poirier F: **Embryonic implantation in galectin 1/galectin 3 double mutant mice.** *Dev Dyn* 1998, **211**:306-313.
36. Pace KE, Lee C, Stewart PL, Baum LG: **Restricted receptor segregation into membrane microdomains occurs on human T cells during apoptosis induced by galectin-1.** *J Immunol* 1999, **163**:3801-3811.
37. Chung CD, Patel VP, Moran M, Lewis LA, Carrie Miceli M: **Galectin-1 induces partial TCR zeta-chain phosphorylation and antagonizes processive TCR signal transduction.** *J Immunol* 2000, **165**:3722-3729.  
 The addition of exogenous galectin-1 to T cells antagonized TCR clustering into signaling GM1-enriched 'rafts', inhibiting full activation but permissive for partial TCR signaling (IFN $\gamma$  production, CD69 and apoptosis). A GFP-Lck reporter gene was expressed in a T-cell line and used to visualize the TCR signaling complexes at the membrane. The authors conclude that galectins act as counter-receptors that limit TCR recruitment.
38. Offner H, Celnik B, Bringman TS, Casentini-Borocz D, Nedwin GE, Vandenbark AA: **Recombinant human beta-galactoside binding lectin suppresses clinical and histological signs of experimental autoimmune encephalomyelitis.** *J Neuroimmunol* 1990, **28**:177-184.
39. Gong HC, Honjo Y, Nangia-Makker P, Hogan V, Mazurak N, Bresalier RS, Raz A: **The NH<sub>2</sub> terminus of galectin-3 governs cellular compartmentalization and functions in cancer cells.** *Cancer Res* 1999, **59**:6239-6245.  
 A form of galectin-3 truncated at the N terminus does not localize to the cell surface, but the CRD is functional. Cells expressing the wild-type are more malignant than the truncated form, suggesting cell-surface expression is important.
40. Poe JC, Fujimoto M, Jansen PJ, Miller AS, Tedder TF: **CD22 forms a quaternary complex with SHIP, Grb2, and Shc. A pathway for regulation of B lymphocyte antigen receptor-induced calcium flux.** *J Biol Chem* 2000, **275**:17420-17427.
41. Hennet T, Chui D, Paulson JC, Marth JD: **Immune regulation by the ST6Gal sialyltransferase.** *Proc Natl Acad Sci USA* 1998, **95**:4504-4509.
42. Wuensch SA, Huang RY, Ewing J, Liang X, Lau JT: **Murine B cell differentiation is accompanied by programmed expression of multiple novel  $\beta$ -galactoside  $\alpha(2,6)$ -sialyltransferase mRNA forms.** *Glycobiology* 2000, **10**:67-75.
43. Razi N, Varki A: **Masking and unmasking of the sialic acid-binding lectin activity of CD22 (siglec-2) on B lymphocytes.** *Proc Natl Acad Sci USA* 1998, **95**:7469-7474.
44. Germain RN: **The art of the probable: system control in the adaptive immune system.** *Science* 2001, **293**:240-245.
45. Viola A, Lanzavecchia A: **T cell activation determined by T cell receptor number and tunable thresholds.** *Science* 1996, **273**:104-106.
46. Viola A, Schroeder S, Sakakibara Y, Lanzavecchia A: **T lymphocyte costimulation mediated by reorganization of membrane microdomains.** *Science* 1999, **283**:680-682.
47. Wulfiging C, Davis MM: **A receptor/cytoskeletal movement triggered by costimulation during T cell activation.** *Science* 1998, **282**:2266-2269.
48. Ferrell JE Jr, Machleder EM: **The biochemical basis of an all-or-none cell fate switch in *Xenopus* oocytes.** *Science* 1998, **280**:895-898.
49. Demetriou M, Nabi IR, Coppolino M, Dedhar S, Dennis JW: **Reduced contact-inhibition and substratum adhesion in epithelial cells expressing GlcNAc-transferase V.** *J Cell Biol* 1995, **130**:383-392.
50. Coffey DS: **Self-organization, complexity and chaos: the new biology for medicine.** *Nat Med* 1998, **4**:882-885.