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Glycosylation, Immunity, and Autoimmunity

Minireview

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Lymphocyte activation events figure prominently in initiating the adaptive immune response (Dustin and Chan, 2000). Plant lectins like phytohemagglutinin have long been used as surrogates for authentic lymphocyte activational stimuli. These carbohydrate binding proteins activate lymphocytes by cross-linking many lymphocyte cell surface glycoproteins, including some that contribute to the immunological synapse (Dustin and Chan, 2000). Although it is difficult to attach physiological significance to plant lectin–dependent activation events, they have nonetheless suggested that protein–glycan interactions may contribute to immune system function.

Glycans decorate the surfaces of all mammalian cells, and the extracellular matrix with which they interact. Their abundance and significant size (a typical N-glycan is approximately the length of one Ig domain; Rudd et al. 1999) imply roles of consequence in cell–cell and cell–matrix interactions. In general, mammalian glycans are the product of a few glycohydrolases and dozens of glycosyltransferases acting sequentially in the secretory pathway. Each glycosyltransferase uses a single nucleotide sugar substrate, and forms a specific linkage between one monosaccharide and a glycan precursor. Each cell's glycosyltransferase repertoire thus dictates which glycan structures it will express, among a much larger number of possible structures. These substantial combinatorial possibilities for oligosaccharide structure provide enormous potential for information display. The job of decoding this information is assigned in part to the large number of mammalian carbohydrate binding proteins (Varki, 1999), including the galectins, a family of lectins with specificity for galactose-containing glycans (Hughes, 1997).

Efforts to convert the information "potential" inherent in glycan structure to something more tangible have been thwarted in part because determining glycan structure is technically challenging, and is made even more difficult by heterogeneity in protein glycosylation. Mammalian cell mutants with aberrant glycan phenotypes have been important in defining glycosylation loci (Stanley and Ioffe, 1995), whereas insight into glycan function has been sought by manipulating glycosylation pathways in the animal. Recent efforts involving genetic manipulation of O- and N-glycosylation pathways in mice illuminate roles for cell surface glycans modulating extracellular molecular interactions with functional relevance to the immune system.

Distinct Immune Phenotypes in Mice with Induced Genetic Alterations in O-Glycan Synthesis

In mice, nonsialylated core 1 O-glycans (recognized by peanut agglutinin or PNA) and core 2 O-glycans (Figure 1) are expressed by $CD8^+$ T cells in a regulated manner during transitions between naive (low expression), activated (high), and memory (low) phenotypes. The physiological significance of these changes has been uncertain. Deletion of the *ST3Gal-I* sialyltransferase locus yields constitutive expression of nonsialylated core 1 O-glycans (Priatel et al., 2000). Expression of core 2 glycans also becomes constitutive, and enhanced, because a synthetic block to core 2 chain synthesis imposed by ST3Gal-I-dependent sialylation of core 1 precursors is relieved (Figure 1). Constitutive expression of these glycans by ST3Gal-I null $CD8^+$ T cells localizes primarily to CD43 and CD45, and is associated with a profound deficit in naïve $CD8^+$ T cells in the periphery and with increased apoptosis of these cells.

Nonsialylated core 1 glycans are implicated in this phenotype, since PNA induces $CD8^+$ T cell apoptosis (Priatel et al., 2000). CD43-associated core 2 glycans were also implicated since apoptosis occurs after cross-linking with 1B11, an antibody thought to be specific for CD43-bearing core 2 glycans. However, the literature now indicates that 1B11 recognizes core 2 glycans on CD45 and other glycoproteins. Moreover, CD43 deficiency does not rescue the ST3Gal-I phenotype (J. Marth, personal communication), and CD43 null mice are not reported to display $CD8^+$ T cell deficiency. Other glycoproteins thus contribute to the apoptotic phenotype.

Antibody- or PNA-induced cross-linking of O-glycans in the absence of ST3Gal-I recapitulates the apoptotic phenotype in quiescent but not in activated thymocytes (Priatel et al., 2000). This suggests that activation-dependent sensitivity to core 2 O-glycan-dependent or to nonsialylated core 1 glycan-dependent cell death, in concert with regulated expression of these glycans during the naïve to memory transition, contributes to resetting the number of $CD8^+$ T cells following an immune response. Priatel and colleagues suggest that ST3Gal-I-dependent death in vivo may be mediated by O-glycan-reactive lectins. Candidates include the galectins, since galectin-1 has been implicated in apoptosis of thymocytes via recognition of core 2 O-glycans on CD43 and CD45 (Galvan et al., 2000), and since, as will be discussed below, galectin-dependent N-glycan interactions are implicated in the control of TCR clustering and signal transduction (Demetriou et al., 2001). However, exogenous galectin-1 does not recapitulate the apoptotic phenotype observed with PNA (Priatel et al., 2000). Furthermore, T cell–specific, constitutive overexpression of a C2GlcNAcT transgene in mice yields enhanced core 2 O-glycan expression but normal numbers of peripheral $CD8^+$ T cells, while presumably leaving ST3Gal-I-modified core 1 glycans intact (Tsuboi and Fukuda, 1997). In the ST3Gal-I null mouse, apoptosis-dependent reductions in peripheral $CD8^+$ T cells are thus likely accounted for by absence of ST3Gal-I-modified core 1 glycans, in concert with or independent of enhanced core 2 glycan expression. Mechanisms to account for the ST3Gal-I null phenotype, including a role for the galectins, remain to be established.

By contrast, manipulation of terminal decoration of

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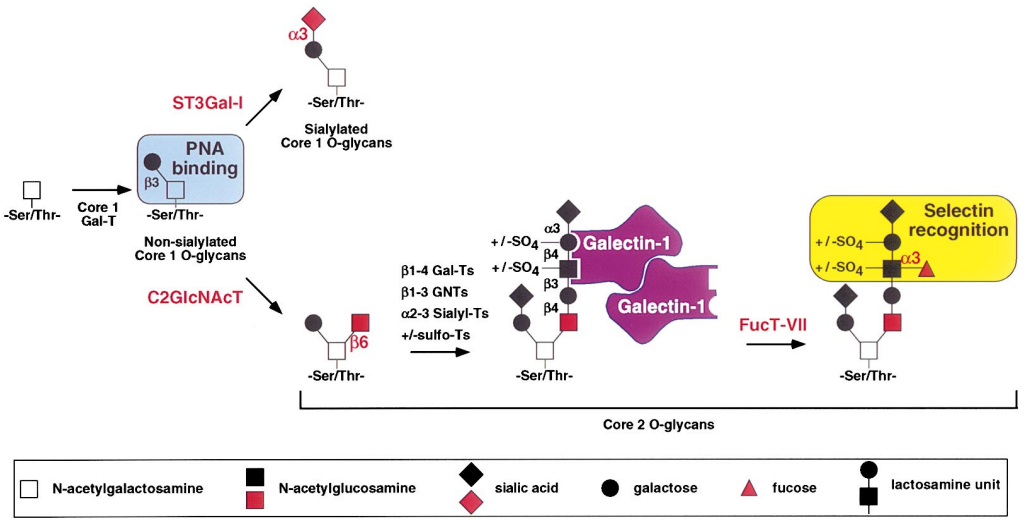

Figure 1. O-Glycans Altered in ST3Gal-I, C2GlcNAcT, and FucT-VII Null Mice

Inactivated enzymes and deleted glycan linkages are in red. ST3Gal-I accesses core 1 O-glycan substrates prior to C2GlcNAcT, and ST3Gal-I-modified core 1 O-glycans are not substrates for C2GlcNAcT; ST3Gal-I deficiency therefore leads to enhanced expression of core 2 O-glycans (Priatel et al., 2000). Galectin-1 is a homodimer whose subuits each contain a lactosamine binding domain (Hughes, 1997). Core 2 glycans may facilitate galectin-1 binding to and cross-linking of CD43 and CD45 on T cells (Galvan et al., 2000).

O-glycans by inactivation of the FucT-VII α1-3-fucosyltransferase locus leads to defects in leukocyte trafficking, but to moderate increases in peripheral T cell number (Maly et al., 1996). FucT-VII modifies sialylated and/or sulfated glycans on core 2 branches in leukocytes and high endothelial venules (HEV) (Figure 1). Its absence yields deficits in leukocyte E- and P-selectin ligand activities, and diminished selectin-dependent leukocyte recruitment. L-selectin ligand activity on HEV is also diminished, leading to faulty lymphocyte homing, which likely accounts for increases in T cell number.

Deletion of core 2 branches in mice, by inactivation of a β1-6-N-acetylglucosaminyltransferase locus (*C2GlcNAcT*) (Figure 1) is also characterized by attenuated activity of E- and P-selectin ligands on neutrophils (Ellies et al., 1998). Glycan structural studies predict that C2GlcNAcT also contributes to the activity of L-selectin ligands on HEV, yet L-selectin ligands are largely functional in the C2GlcNAcT null mice. This observation discloses that other C2GlcNAcTs (Schwientek et al., 2000) contribute to L-selectin ligand synthesis, or that alternative, C2GlcNAcT-independent pathways yield active L-selectin ligands, and reinforces the need for glycan structural data in the interpretation of glycan-deficient phenotypes. The modest T lymphocytosis in these mice is likely due to subtle decrements in lymphocyte homing, since the lymphocyte subset proportions are normal.

These studies emphasize that specific modifications on terminal O-glycans, and precise, tissue-specific control of O-glycan branching provide distinct and essential contributions to immune system function. Mechanisms to account for the role of these glycans in T cell survival and death remain to be defined.

Specific Complex-type N-Glycans in the Control of T Cell Activation and Autoimmunity

Two recent publications demonstrate that specific alterations in N-glycan structure can disable processes in the immune system that keep pathogenic self-recognition at bay. The first (Demetriou et al., 2001) involves mice with a targeted mutation in the *N-acetylglucosaminyltransferase V* (*Mgat5*) locus (Granovsky et al., 2000). Mgat5 adds N-acetylglucosamine in β1-6 linkage to the α6-linked mannose arm of tri- and tetraantennary complex N-glycans (Figure 2). Addition of this β1-6 branch, in the context of glycosyltransferases that can elongate these chains, leads to N-glycans with two or more membrane-distal polymeric lactosamine repeat units.

Most Mgat5 null mice exhibit a glomerulonephritis characteristic of autoimmune syndromes, and an exaggerated cellular immune response. Mgat5 null mice have normal numbers of immune cells, but their T cells hyperproliferate in response to anti-CD3, and have a reduced requirement for the anti-CD3 agonist approximately equal to the reduction observed with CD28 engagement. The components of the TCR complex are quantitatively normal in the absence of Mgat5, as is TCR-independent intracellular signal transduction potential. However, microscopy experiments demonstrate markedly enhanced clustering of the TCR in the membranes of Mgat5 null T cells in response to TCR agonist–coated beads. Enhanced clustering is associated with enhanced TCR internalization, microfilament reorganization, and intracellular signal transduction events.

The authors sought a mechanistic explanation for enhanced TCR clustering by considering the sites of attachment and sizes of N-glycans that modify components of the TCR complex (Rudd et al., 1999). X-ray structures of the mouse αβ TCR indicate that four or more of its seven predicted N-linked glycosylation sites are substituted with glycans. Models with prototypical N-glycans installed on this molecule, or on members of the TCR-pMHC complex, predict that these sugars are arrayed laterally, and enshroud much of the surface of the complex. Rudd et al. suggest that the lateral posi-

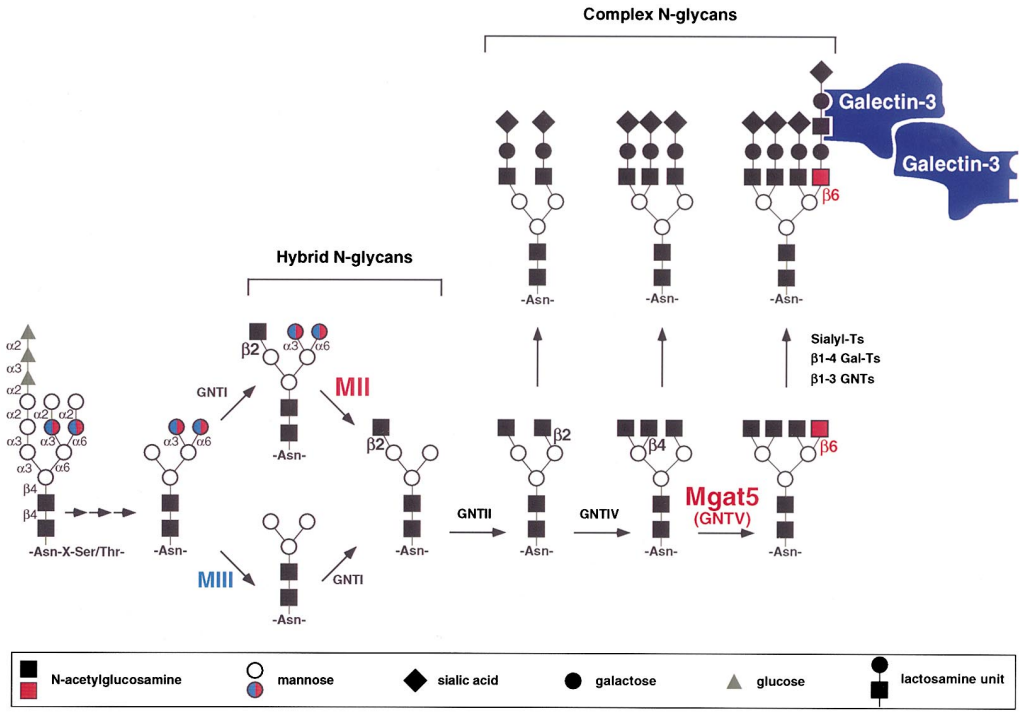

Figure 2. N-Glycans Altered in MII Null and Mgat5 Null Mice

The sequential actions of glycohydrolases and glycosyltransferases produce hybrid and complex N-glycans. Inactivated enzymes and deleted glycan linkages are in red. Galectin-3 is monomeric with a single carbohydrate recognition domain, and is depicted as a homodimer to indicate its ligand-dependent self-associating properties (Hughes, 1997).

tions predicted for TCR complex–associated N-glycans may inhibit nonspecific interactions between adjacent complexes within the plane of the membrane, and thereby diminish nonspecificity of TCR-mediated signaling.

In this context, Demetriou et al. noted that members of the galectin family of carbohydrate binding proteins (Hughes, 1997) recognize lactosamine chains that may decorate β1-6-branched N-glycans displayed by the TCR, and, in solution at least, can form multivalent complexes with their glycan ligands. These considerations suggested that one or more galectins mediate the formation of a "multivalent galectin-Mgat5 glycoprotein lattice" on the surface of a wild-type T cell. Such a lattice could actively restrain the lateral mobility of Mgat5-modified TCR complexes, raise the threshold for TCR complex clustering, and thereby help to restrict TCR complex–initiated signal transduction events to those consequent to appropriately specific TCR–pMHC interactions (Dustin and Chan, 2000). The model predicts that in Mgat5 null T cells, mobility restraints imposed by such a lattice are relieved, because deficiency of the Mgat5-dependent β1-6 branches does not allow the galectin(s) to effectively engage TCR-associated glycans. Enhanced TCR clustering and associated signal transduction events ensue.

Support for this hypothesis derives from their experiments, where lactose, a disaccharide inhibitor of galectin–lactosamine chain interactions, was observed to enhance anti-CD3-dependent TCR clustering and protein phosphorylation in wild-type T cells, while not further enhancing the already amplified clustering events in Mgat5 null T cells. Biochemical cross-linking experiments demonstrate a lactose-inhibitable association between galectin-3 and the TCR complex in wild type, but not in Mgat5 null T cells. These studies thus implicate galectin-3- and Mgat5-dependent glycans as participants in the formation of the lattice. Other galectins may also be present, however, and may interact with the TCR and other T cell glycoproteins in a lactose-inhibitable manner, and may also contribute to TCR restraint.

Considered together, these observations suggest that the autoimmune-like syndrome in Mgat5 null mice is due to a propensity for enhanced recruitment of the TCR to the peptide–MHC complex, with potential for inappropriately robust and sustained T cell activation in the absence of CD28 cosignaling, failure of anergy, and loss of self-tolerance (Demetriou et al., 2001). Many T cell glycoproteins may be decorated by Mgat5-modified N-glycans, including other components of the TCR–pMHC complex (Rudd et al., 1999). Whether these molecules participate in the postulated lattice, and how such participation may contribute to T cell physiology, are not yet explored. The large number of galectins and other lectins that may interact with N- and O-glycans, and the possibility that the T cell maintains an extensive expression repertoire of these molecules amplifies the potential for complexity. Mice with deletions of the galectin-3 and -1 loci have not been reported to experience autoimmunity, and the biology of the TCR complex in these mice has not yet been closely examined (Colnot et al. 1998; Hsu et al., 2000). Redundancy in the galectin

family, reflected in their large number and their similar glycan binding specificities (Hughes, 1997), may make such studies difficult to interpret.

A direct connection between the T cell phenotype in these mice and the autoimmune syndrome they exhibit is not established by these studies. Mgat5 deficiency also alters N-glycan structure in extra-immune compartments (Granovsky et al., 2000), which, alone, or together with aberrant T cell glycosylation, may account for autoimmunity. In this regard, it is interesting to compare the results reported by Demetriou et al. with those involving mice with a targeted deficiency in α-mannosidase II (MII), a deficiency of complex N-glycans and an autoimmune syndrome (Chui et al., 2001). MII trims two mannose residues at a key step in the conversion of high mannose to complex N-glycans (Figure 2). Initial analyses of MII null mice disclosed an absence of complex N-glycans in the erythroid lineage, and a dyserythropoietic anemia caused by mechanisms that remain undefined (Figure 2). However, these studies also identified complex N-glycans on extra-erythroid cells, including T cells, and discovered an alternate pathway for complex N-glycan formation, apparently active in many extra-erythroid tissues, and directed by a second α-mannosidase (Misago et al., 1995), now termed MIII (Figure 2).

Subsequent analyses of MII null mice find that most experience an autoimmune disease characterized by an immune complex glomerulonephritis, and by autoantibodies often found in patients with systemic lupus erythematosis (Chui et al., 2001). MII deficiency correlates with decreased expression of complex N-glycans that varies from substantial (kidney) to modest (liver). MII and MIII thus contribute in a variable and tissue-specific manner to N-glycan synthesis.

Importantly, T cells in MII null mice proliferate normally to anti-CD3 cross-linking. This result is not inconsistent with the enhanced proliferative response observed in the Mgat5 null mice, since MII null T lymphocytes continue to express complex N-glycans that are substrates for Mgat5 modification, presumably because MIII is expressed in these cells. Mechanisms accounting for autoimmunity in MII deficiency are not yet apparent. However, these observations beg the question of what mechanistic overlap, if any, accounts for the autoimmune syndromes associated with deficiencies in Mgat5 and MII. Perhaps in the MII null mice, MIII expression is occasionally extinguished in T cells, leading to an absence of complex, branched N-glycans, including those containing Mgat5-dependent β1,6-branching, hyperreactivity to TCR ligation, and an autoimmune pathogenesis similar to that observed in the Mgat5 null mice. Alternatively, the autoimmune syndromes in the two strains may be initiated and sustained through mechanisms that involve altered glycans on nonimmune tissues, and which are either independent of, or synergize with, aberrant glycosylation in the immune compartment. Lineage-specific conditional mutagenesis of the Mgat5 or MII loci, adoptive transfer approaches, and bone marrow transplantation experiments will illuminate the contributions made by the T cell, and by nonimmune cells, to autoimmunity in these mice.

Implications for Autoimmune Disease in Humans

Is there evidence that similar derangements in N-glycosylation contribute to autoimmunity in humans? There are rare carbohydrate deficiency syndromes in humans affecting proximal and largely essential steps in N-glycan synthesis, but these are associated with extremely severe, nonimmune multiorgan dysfunction early in life (Lowe and Freeze, 1999) and are not likely to be informative for autoimmune susceptibility. Humans with documented inactivating mutations in the MII or Hgat5 loci have not been reported, so their relevance to human autoimmune disease has not been studied. Linkage studies have not identified either as a clear candidate for an autoimmune susceptibility locus. Nonetheless, these loci, and their cognate N-glycans, represent important candidates for further study as contributors to the pathogenesis of systemic lupus erythematosis and other autoimmune syndromes.

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