The ST6Gal I Sialytransferase Selectively Modifies N-Glycans on CD45 to Negatively Regulate Galectin-1-induced CD45 Clustering, Phosphatase Modulation, and T Cell Death

Maho Amano‡§¶, Marisa Galvan‡§¶, Jiale He‡, and Linda G. Baum‡*†

From the ‡Department of Pathology and the ¶Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, California 90095

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The role of glycosylation in these functions is specific, i.e. the different functions require specific sugars on specific glycoprotein acceptors. Regulated glycosylation of specific acceptor substrates can affect immune function by creating or masking ligands for endogenous lectins. For example, modification of cell surface oligosaccharides by the C2GnT and Fuc TVII glycosyltransferases results in specific selectin-mediated trafficking patterns for Th1 and Th2 subsets (3). Similarly, modification of CD45 by the C2GnT glycosyltransferase regulates thymocyte susceptibility to cell death induced by galectin-1 (10).

During T cell development, expression of several sialyltransferases is temporally and spatially controlled (9, 11, 12). In the human thymus, different members of the sialyltransferase family are expressed in distinct anatomic compartments, so cells in those compartments bear unique complements of sialylated oligosaccharides. For example, the SAα2,6Gal sequence, the product of the ST6Gal I sialyltransferase, is detected only on mature medullary thymocytes (12). Intriguingly, mature medullary thymocytes displaying SAα2,6Gal sequences are resistant to galectin-1-induced cell death (13, 14). Because the addition of sialic acid in the α2,6 linkage to galactose could mask terminal galactose residues required for galectin-1 binding to T cell glycoproteins (15), we asked whether expression of the ST6Gal I would control susceptibility of T cells to galectin-1-induced death.

EXPERIMENTAL PROCEDURES

Reagents and Cells—Galectin-1 was prepared as described previously (13). Murine BW5147.3 (BW5147), Phaα2,1, T200*, and human CEM and MOLT-4 cell lines were propagated as previously described (10, 16).

Lectin Flow Cytometry—Expression of cell surface oligosaccharides was detected by flow cytometry with biotinylated Phaeosolus vulgaris agglutinin (PHA)1 and Sambucus nigra agglutinin (SNA) (E-Y Labs, San Mateo, CA) (10 μg/ml) as described (12). For glycosidase inhibition, the cell lines were cultured for 72 h with 2 mM deoxymannojirimycin (DMNJ) (Oxford GlycoSystems, Inc., Rosedale, NY) or medium alone prior to lectin analysis and cell death assays.

Transfection—Rat ST6Gal I cDNA in the plasmid STγ, M-yc-pDNA 3.1 (17, 18) (gift of Dr. Karen Colley, University of Illinois, Chicago, IL) or vector alone were transfected into Phaα2,1 and T200* cells as described (9). Following selection in G418, positive Phaα2,1 clones were identified by SNA flow cytometry. Positive T200* clones were identified by RT-PCR, performed essentially according to the protocol provided in the Super Script™ One-Step RT-PCR with Platinum Taq (Invitrogen, Carlsbad, CA), using the primers 94 sense (TATGAGGCCCCTTACAC- C) and 943a antisense (GCGGAGGATGGGGAGTTGG) (18).

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** To whom correspondence should be addressed. Tel.: 310-206-5985; Fax: 310-206-0657; E-mail: lbaum@mednet.ucla.edu.
Galectin-1 Binding Assay—5 × 10⁵ cells were suspended in PBS containing the indicated amount of biotinylated galectin-1 (16) at 4 °C. After washing, the cells were incubated with streptavidin-fluorescein isothiocyanate (5 μg/ml) (Jackson Immunoresearch Laboratories, West Grove, PA) for 45 min at 4 °C. After washing, the cells were analyzed by flow cytometry.

Galectin-1 Cell Death Assays—Galectin-1 death assays were performed as described (16) with the following modifications. 10⁵ cells were incubated with 20 μM galectin-1 in 1.6 mM dithiothreitol/Dulbecco’s modified Eagle’s medium or in 1.6 mM dithiothreitol/Dulbecco’s modified Eagle’s medium alone as a control for 4–6 h at 37 °C. 0.1 mM β-lactose (final concentration) was added to dissociate galectin-1, and the cells were washed with PBS. Apoptotic cells were identified using annexin V and Staurosporine as previously described (10).

Precipitation and Western Blot Analysis—The cells lysates from 4–9 × 10⁶ cells were prepared as described (12). To precipitate SNA-binding glycoproteins, the lysates were precleared for 1 h with biotinylated bovine serum albumin (0.25 μg/250 μl cell lysate) and ImmunoPure Immobilized Streptavidin (Pierce). After centrifugation to remove insoluble material, the supernatants were incubated with SNA-biotin (1 μg/ml) for 1 h. Bound reagent was detected with horseradish peroxidase-labeled rabbit anti-goat IgG (Bio-Rad) or streptavidin-peroxidase labeled horseradish peroxidase, respectively, and visualized by ECL (Amersham Biosciences). ST6Gal I immunoblotting of whole cell lysates was performed as described in Ref. 12, with rabbit anti-rat ST6Gal I antisera (gift of Dr. K. Colley).

PNA/Gse P Digestion of CD45—Cell lysates (10⁶ cells) were separated by centrifugation to remove cell debris and probed with polyclonal rabbit anti-mouse CD45 (M-20) (Santa Cruz Biotechnology, Santa Cruz, CA). The band corresponding to CD45 was excised from the nitrocellulose, and bound antibody was stripped with Restore buffer (Pierce). After washing two times with 25 mM Tris, 150 mM NaCl, 0.05% Tween, pH 7.5 (TBS-T) followed by two washes with 50 mM sodium phosphate, pH 7.5, the membrane was incubated with 1.5 ml of 50 mM sodium phosphate containing 10,000 units of PNA/Gse F (New England BioLabs, Beverly, MA) overnight at 37 °C with rocking. The enzyme-treated membrane was washed with TBS-T and probed with SNA-biotin, as described above.

Sialyltransferase Activity Assay—The cells were lysed in 50 mM sodium cacodylate, pH 6.5, 100 mM NaCl, 1 mM MgCl₂, 1% Nonidet P-40, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. 100 μl of lysates (3 × 10⁶ cells) were incubated with 1.5 μM CMP-β-D-n-sialic acid (Calbiochem, San Diego, CA) and 500 μg of asialofetuin (Sigma) in 50 mM sodium cacodylate, pH 6.5, 1 mM MgCl₂, for 2 h at 37 °C. To stop the reaction, the mixtures were incubated for 10 min on ice. Fetuin was precipitated with anti-fetuin antibody (Accurate Chemical Co., Westbury, NY) 5 μl/150 μl lysate), and immunoprecipitates were separated by SDS-PAGE, blotted to nitrocellulose, and probed with SNA-biotin, as described above. Band intensity was determined using the MultiImage Light Cabinet, model 2.1.1 (Alpha Innotech Corp., San Leandro, CA) with ChemiImager 5500 software.

Expression of the ST6Gal I Reduces Galectin-1 Receptors CD45 or CD45, as determined by flow cytometric analysis using the relevant antibodies (data not shown).

RESULTS

N-Glycans Are Essential for Galectin-1 Death—Galectin-1 preferentially recognizes Galb1,4GlcNAc (LacNAc) sequences that can be presented on N- or O-linked glycans (15). Although prior work from our lab demonstrated that O-glycans participate in galectin-1 T cell death (9), the role of N-glycans in galectin-1 cell death is not clear. In addition, the ST6Gal I enzyme preferentially sialylates terminal galactose residues on N-glycans (12, 17); if the ST6Gal I participated in regulating galectin-1 cell death in vivo, it would likely occur through the modification of N-glycans.

To determine whether N-glycans are necessary for galectin-1 induced death, human and murine T cell lines were treated with the mannosidase I inhibitor DMNJ, to block trimming of terminal mannose residues and subsequent elongation of N-glycans with LacNAc sequences. The effectiveness of DMNJ treatment was determined by analyzing treated cells with the PHA, because inhibition of mannosidase I activity would prevent elongation of the N-glycan chain recognized by PHA (19). Cells treated with DMNJ showed a marked reduction in PHA binding compared with cells cultured in medium alone; importantly, DMNJ treatment did not affect the level of cell surface expression of galectin-1 receptors CD45 or CD45, as determined by flow cytometric analysis using the relevant antibodies (data not shown).

DMNJ-treated cells were examined for susceptibility to galectin-1-induced cell death (Fig. 1). The Pha¹⁰⁺, two T cell lines are all susceptible to galectin-1-induced cell death, whereas the BW5147 cells are resistant to galectin-1 because of the lack of core 2 O-glycans on cell surface glycoproteins (9, 10, 16). DMNJ treatment resulted in a dramatic reduction in galectin-1-induced cell death of the galectin-1-susceptible murine (Pha¹⁰⁺) and human (CEM, MOLT-4) T cell lines. Although previous studies demonstrated that the glycosylation inhibitors benzyl-α-GalNAc and swainsonine reduced T cell susceptibility to galectin-1 (9, 16), neither benzyl-α-GalNAc nor swainsonine had the dramatic inhibitory effect on cell death, whereas the BW5147 cells are resistant to galectin-1 (9, 16), neither benzyl-α-GalNAc nor swainsonine had the dramatic inhibitory effect on cell death, whereas the BW5147 cells are resistant to galectin-1.

Expression of the ST6Gal I Reduces Galectin-1 Binding to T Cells—The addition of terminal α₂,6-linked sialic acid can block galectin-1 binding to the preferred saccharide ligand LacNAc. This has been demonstrated for individual LacNAc units and for poly-LacNAc chains (15, 20–22). The ability of the terminal Saα₂,6Gal sequence to block galectin-1 binding suggested that the addition of α₂,6-linked sialic acid to T cell surface glycoproteins (12) could regulate the susceptibility of thymocytes and T cells to galectin-1.

To directly examine whether addition of α₂,6-linked sialic acid would affect susceptibility to galectin-1, we expressed the ST6Gal I in the galectin-1-susceptible murine T cell line
Expression of the ST6Gal I in isolated clones was confirmed by flow cytometry with SNA, a lectin that recognizes the SAβ2,6Gal sequence (12, 23). Both RT-PCR and immunoblot analysis with anti-ST6Gal I antibody precipitation demonstrated abundant expression of ST6Gal I mRNA and protein in SNA.9 cells (Fig. 2B) and SNA.1 cells (data not shown), whereas no reactivity was observed in control C.2 cells (Fig. 2B). ST6Gal I expression did not affect the level of expression of galectin-1 receptors CD43 or CD45 (data not shown).

We then determined whether addition of cell surface sialic acid by the ST6Gal I enzyme would reduce galectin-1 binding to T cells. As shown in Fig. 2C, galectin-1 binding to the SNA.9 cells (closed circles) was markedly reduced compared with the level of binding observed for the C.2 control cells transfected with vector alone (open circles). However, the reduced, but not absent, binding of galectin-1 to SNA.9 cells indicated that some of the potential binding sites on these cells were not modified by the ST6Gal I enzyme. For both SNA.9 and C.2 cells, galectin-1 binding was completely inhibited in the presence of 100 mM lactose (squares), demonstrating that binding was saccharide-dependent.

Expression of the ST6Gal I Reduces Susceptibility to Galectin-1—The SNA.1, SNA.9, and C.2 cells were examined for susceptibility to galectin-1-induced death. As shown in Fig. 2D, the C.2 cells transfected with vector alone were susceptible to galectin-1; ~50% of the cells underwent cell death, determined by annexin V binding and PI uptake. In contrast, the SNA.1 and SNA.9 cells demonstrated reduced susceptibility to galectin-1-induced cell death, compared with the control C.2 cells. Death assays were performed as described under “Experimental Procedures,” and the percentage of cell death was determined by binding of annexin V.

**Fig. 2.** Expression of the ST6Gal I in PhaR2.1 cells confers resistance to galectin-1-induced death. A, PhaR2.1 cells were transfected with the cDNA encoding the ST6Gal I or with vector alone. Expression of the ST6Gal I in isolated clones was confirmed by flow cytometry with SNA, a lectin that recognizes the SAβ2,6Gal sequence created by the ST6Gal I. The SNA.1 and SNA.9 clones expressing the ST6Gal I demonstrate increased SNA binding compared with the control C.2 clone transfected with vector alone. B, RT-PCR (top panel) and immunoblotting (bottom panel) demonstrate expression of the ST6Gal I mRNA and protein in SNA.9 cells but not in control C.2 cells. C, expression of the ST6Gal I reduces galectin-1 binding to the SNA.9 cells (filled symbols) compared with the control C.2 cells (open symbols). The cells were labeled with the indicated amounts of biotinylated galectin-1 in the absence (circles) or presence (squares) of 100 mM lactose, and bound galectin-1 was detected with fluorescein isothiocyanate-avidin. The cells were analyzed by flow cytometry, and the mean fluorescence channel of each sample is shown. D, the SNA.1 and SNA.9 clones demonstrate reduced susceptibility to galectin-1-induced cell death, compared with the control C.2 cells. Death assays were performed as described under “Experimental Procedures,” and the percentage of cell death was determined by binding of annexin V.
ST6Gal I Modifies CD45 and Regulates Galectin-1 T Cell Death

Fig. 3. The ST6Gal I preferentially sialylates N-glycans on CD45. A, total SNA-binding glycoproteins were precipitated from control clones transfected with vector alone (lanes C.2 and C.4) or from the SNA.1 clone expressing the ST6Gal I. Precipitated glycoproteins were probed with biotinylated SNA. The only significant difference in the profile of SNA binding glycoproteins was an increase in a band with a mass of ~200 kDa (arrow). B, the SNA reactive band is CD45. The cells were cultured in 2 mM DMNJ, as above, or in medium alone. The cell lysates were precipitated with CD45 antibody or SNA (indicated below) and probed with CD45 antibody. The band with increased SNA staining reacts with both SNA and antibody to CD45. In addition, the increased SNA binding to CD45 is abolished by pretreatment with DMNJ, which blocks synthesis of complex N-glycans. In both blots, the width of the CD45 band is diminished in DMNJ-treated cells compared with cells expressing the ST6Gal I, as a result of decreased complexity of glycosylation. C, increased SNA binding to CD45 results from sialylation of N-glycans. CD45 was detected in whole cell lysates of SNA.9 cells by immunoblotting (top panel). The CD45 bands were excised and incubated with or without PNGase F, as indicated, and reprobed with SNA-biotin. Removal of N-glycans from CD45 by PNGase F treatment reduced SNA binding.

with SNA. As shown in Fig. 3A, there was only one significant difference in the pattern of SNA-binding glycoproteins precipitated from cells expressing the ST6Gal I (SNA.1) compared with vector-transfected controls (C.2, C.4). In extracts of SNA.1 cells, there was an obvious increase in SNA binding to a band of approximate molecular mass of 200 kDa. Other SNA reactive bands of various sizes were occasionally seen in different experiments (data not shown), but these other bands were not consistently observed. In contrast, the 200-kDa band was consistently observed in ST6Gal I-expressing clones. The relative mobility of the 200-kDa band suggested that it could be CD45, a highly glycosylated protein that is known to bear SAα2,6Gal sequences on both murine and human T cells (12, 24).

To specifically determine whether the band exhibiting increased SNA binding was CD45, both SNA and CD45 antibody were used to precipitate material from vector transfected (C.4) and SNA.1 cells, and the precipitates were probed with CD45 (Fig. 3B). The 200-kDa band exhibiting increased SNA binding reacted with CD45 antibody, demonstrating that CD45 was selectively hypersialylated in the SNA.1 cells. In addition, this band migrated with the same mass as immunoprecipitated CD45. To determine whether the increased sialylation of CD45 occurred on N-glycans, the preferred glycan acceptor for the ST6Gal I, SNA.1 cells were pretreated with DMNJ prior to SNA or CD45 precipitation. DMNJ treatment reduced SNA binding to protein precipitated from SNA.1 cells to the level observed for control cells (C.4) transfected with vector alone (Fig. 3B). PNGase F treatment confirmed that, in cells overexpressing the ST6Gal I, sialic acid addition to CD45 occurred on N-glycans. Whole cell lysates of SNA.9 cells were probed with antibody to CD45. The CD45 bands were excised from the blot and incubated with or without PNGase F, and the bands were reprobed with SNA. As shown in Fig. 3C, PNGase F dramatically reduced SNA binding to CD45 from SNA.9 cells. Thus, the increased SNA binding to CD45 on cells expressing the ST6Gal I resulted from the specific addition of α2,6-linked sialic acid to N-glycans on CD45. The background level of binding of SNA to CD45 on control cells and on DMNJ-treated cells may reflect SNA recognition of SAα2,6GalNAc sequences on O-glycans on CD45 (23).

As mentioned above, the three primary receptors for galectin-1 on T cells are CD7, CD43, and CD45 (24). We specifically precipitated CD7 and CD43 from SNA.9, SNA.1, and C.2 cells and saw no difference in SNA binding to CD7 or CD43 (data not shown), indicating that the inhibitory effect on galectin-1 cell death was not due to sialylation of CD7 or CD43. We also did not detect CD7 or CD43 by immunoblotting SNA precipitates with the respective antibodies (data not shown). To further examine the acceptor substrate preference of the ST6Gal I, we expressed the ST6Gal I in the murine T200− cell line, a mutant of the BW5147 line that does not express CD45. Despite repeated attempts, we could not isolate SNA− clones from T200− cells transfected with ST6Gal I cDNA (SNA.T1), nor could we detect any increase in SNA binding to whole cell lysates of SNA.T1 cells (Fig. 4, A and B). Although RT-PCR analysis demonstrated that the ST6Gal I mRNA was present in nine
independent clones of ST6Gal I-transfected T200 cells (Fig. 4C), every clone was SNA by flow cytometry (Fig. 4A). In addition, we detected ST6Gal I protein by immunoblotting in the ST6Gal I-transfected T200 cells (Fig. 4C), although the cells were SNA. Finally, to confirm that the ST6Gal I expressed in T200 cells was enzymatically active, we used asialofetuin as an acceptor substrate to assay sialyltransferase activity. After incubation with cell lysate from either control cells (C.T1) or SNA.T1 cells, fetuin was immunoprecipitated and subjected to blotting with SNA-biotin to detect α2,6-linked sialic acid. As shown in Fig. 4C, there was a significant increase in SNA binding to fetuin incubated with SNA.T1 extract, compared with C.T1 extract. We performed densitometric analysis of the SNA reactive bands; the ratio of SNA binding to fetuin incubated with SNA.T1 cell extract compared with C.T1 cell extract was 6.3. This ratio was comparable with the ratio we observed when asialofetuin was incubated with SNA.9 cell extract compared with C.4 cell extract, 5.0 (data not shown).

These data demonstrated that equivalent ST6Gal I activity was present in the SNA.9 and SNA.T1 cells, although only the SNA.9 cells that express CD45 became SNA by flow cytometry. Thus, CD45 is the primary glycoprotein acceptor substrate for the ST6Gal I in these T cells, and in the absence of CD45, there was no detectable sialylation of other potential acceptors by the ST6Gal I in T200 cells.

**ST6Gal I Expression Inhibits CD45 Segregation on Galectin-1-treated Cells**—We have demonstrated that galectin-1 binding to T cells results in reorganization of the glycoprotein receptors CD45, CD43, and CD7 into novel membrane microdomains (24). Specifically, CD45 segregates from CD43 and CD7 and localizes to membrane blebs on dying cells. The segregation of CD45 caused by galectin-1 binding is regulated in part by expression of the C2GnT glycosyltransferase that creates branches on O-glycans bearing the LacNAc sequences recognized by galectin-1. Cells that do not express the C2GnT do not demonstrate CD45 segregation after galectin-1 and are not susceptible to galectin-1-induced cell death (10).

We examined the effects of ST6Gal I expression on CD45 segregation after galectin-1 binding (Fig. 5). On C.4 cells transfected with vector alone, galectin-1 binding resulted in the segregation of CD45 to membrane blebs on dying cells, exactly as previously described (24). In contrast, galectin-1 binding to SNA.9 cells did not result in any detectable segregation of CD45. The diffuse distribution of CD45 on the cell surface was identical for SNA.9 cells treated with either galectin-1 or buffer control. Thus, expression of the ST6Gal I inhibited galectin-1-induced CD45 segregation on the plasma membrane (Fig. 5A), as well as inhibiting galectin-1-induced cell death (Fig. 2D). A comparison of the effects of ST6Gal I expression on galectin-1-induced CD45 segregation and on galectin-1-induced cell death is shown in Fig. 5B.

**ST6Gal I Expression Abrogates Galectin-1-mediated Inhibition of PTP Activity**—Previous work has demonstrated that binding of galectin-1 to CD45 reduces the PTP activity of CD45 (25, 26). We asked whether ST6Gal I expression would modify the galectin-1-mediated effect on PTP activity. In human cell lines, the galectin-1 effect on immunoprecipitated CD45 has been examined (25, 26). However, because all of the murine CD45 antibodies that we tested would not bind CD45 in the presence of galectin-1, we measured the PTP activity of whole cell lysates. The PhaR2.1 cell line, the parental line of the SNA.1, SNA.9, and C.2 cells, demonstrated robust PTP activity (Fig. 6A). In contrast, the T200 cell line derived from the same precursor line as the PhaR2.1 cells does not express CD45 and has significantly reduced PTP activity (Fig. 6A). These results indicate that CD45 accounts for the majority of PTP activity in the PhaR2.1 cells.

To assess the effect of ST6Gal I expression on PTP activity, we examined the SNA.9 and C.2 cells at the indicated time points after galectin-1 binding. As shown in Fig. 6B, galectin-1 binding to C.2 control cells resulted in a rapid and sustained decrease in PTP activity (open circles). However, this effect was not seen when galectin-1 was added to SNA.9 cells (closed circles); the PTP activity in lysates of SNA.9 cells treated with galectin-1 did not differ appreciably from that observed for cells treated with buffer alone (100%). All of the measurable p-nitrophenol release was due to tyrosine phosphatase activity, because release was completely inhibited by the addition of bpV (phen), a tyrosine phosphatase inhibitor (10).

**DISCUSSION**

Regulated expression of glycosyltransferases affects many cell fate decisions. Altered glycosylation can directly modulate cellular responses by creating or masking ligands for endogenous lectins. For example, expression of specific glycosyltransferases creates potential selectin ligands on peripheral T cells migrating to sites of inflammation (3). Altered glycosylation can also indirectly modulate cellular responses by affecting glycoprotein conformation or by controlling intermolecular interactions. Expression of the GaIT V enzyme controls the amplitude of the T cell response to antigen (6), and sialylation of cell surface glycoproteins regulates binding of MHC class I molecules to thymocytes (4, 5).

Previous work from our group demonstrated that O-glycans...
are involved in galectin-1 induced cell death; specifically, addition of core 2 O-glycans on CD45 was required for galectin-1-mediated clustering of CD45, an initial step in the death pathway (9, 10). The present work demonstrates that N-glycans are also essential for galectin-1-induced cell death, because treatment of murine and human T cells with the mannosidase I inhibitor DMNJ, which blocks all complex N-glycosylation, virtually abolished susceptibility to galectin-1 (Fig. 1). The dramatic inhibition of cell death seen with DMNJ treatment expands our previous work (16), demonstrating that indeed, the murine PhaR2.1 cell line used in this study is highly susceptible to galectin-1, although this cell line does not express the GnT V (9). Thus, although the GnT V branch may augment galectin-1 susceptibility, other LacNAc sequences on N-glycans are sufficient for galectin-1 binding to trigger the death signal.

In the T cell lines examined in this study, the preferred acceptor substrate for the ST6Gal I was CD45. Preferred utilization of CD45 as an acceptor substrate for the ST6Gal I is supported by our finding that, despite expression of the ST6Gal I in the T200 cell line that lacks CD45, we detected no increase in SNA binding to these cells. Increased SNA binding to the other major galectin-1 receptors, CD43 or CD7, was not detected in the PhaR2.1 or the T200 cell lines. CD45 may be a preferred substrate because of accessibility of CD45 glycans to the ST6Gal I during synthesis or to recognition of peptide or conformational determinants on the CD45 backbone by the ST6Gal I enzyme.

Developmentally regulated changes in CD45 isoform expression may also control recognition by or accessibility to the ST6Gal I during glycoprotein synthesis. In human thymus, the SAa2,6Gal sequence was only detected on the CD45RA isoform on mature thymocytes (12). In murine thymus, CD45 on mature thymocytes also appears to be a preferred acceptor for the ST6Gal I, because only mature thymocytes bound CD22, a lectin that preferentially recognizes SAa2,6Gal (27). Recent work by Xu and Weiss (28) has also demonstrated preferential sialylation of high molecular weight isoforms of CD45, compared with the smallest CD45RO isoform. Few examples of this degree of preferential acceptor substrate recognition by sialyltransferases in vivo have been reported. For example, polysialyltransferase enzymes are expressed in a range of tissues, but polysialic acid is detected primarily on the neural cell adhesion molecule NCAM (29, 30). Thus, tissue specificity in both glycosyltransferase enzymes and in glycoprotein acceptor substrate expression can control cell surface glycosylation. Because galectin-1 is abundantly expressed throughout a variety of tissues, T cells will encounter galectin-1 in many organs and at many points during T cell development and peripheral activation. Thus, it is likely that the T cell response to galectin-1 will be controlled at the level of the T cell, i.e. by regulating glycosylation to control susceptibility to cell death (31).

Glycosylation of CD45 depends on a number of factors, including lymphocyte subset and stage of maturation or activation. Differential glycosylation of CD45 is controlled in part by the repertoire of glycosyltransferase enzymes expressed by the cell at each stage in T cell development (11, 12, 32–35). Regulated expression of different complements of glycosyltransferases during T cell maturation and activation implies that different glycoforms of CD45 will interact with different endogenous lectins, such as CD22, the cysteine-rich domain of the mannose receptor, or galectin-1 (10, 27, 36).

Sialylation of CD45 has recently been shown to regulate homodimerization of CD45 on the T cell surface (28). CD45 homodimerization is one mechanism to down-modulate the PTP activity of the CD45 cytoplasmic domains, an effect that would reduce T cell responsiveness to antigen. Although CD45 homodimerization has been proposed to occur spontaneously (28), we and others have shown that galectin-1 binding clusters CD45 and reduces PTP activity (Figs. 5 and 6 and Refs. 25 and 26), and the data presented here demonstrate that this effect is negatively regulated by expression of the ST6Gal I and sialylation of CD45. Galectin-1 clustering of cell surface receptors has also been demonstrated to reduce T cell responsiveness to antigen (37), suggesting that galectin-1 binding to CD45 and regulation of CD45 PTP may contribute to the observed anti-inflammatory properties of galectin-1 in a number of animal models (reviewed in Ref. 31). The addition of SAa2,6Gal sequences to CD45 may be a mechanism to finely tune immune
regulation by galectin-1 and to prevent galectin-1-induced apoptosis of specific populations, e.g. mature thymocytes.

How does ST6Gal I expression inhibit galectin-1-induced cell death? One possibility is that galectin-1 binds to LacNAc sequences on CD45 glycans; the addition of α2,6-linked sialic acid directly masks LacNAc sequences, inhibiting galectin-1 binding to and clustering of CD45 and initiation of cell death. Alternatively, the addition of sialic acid to CD45 would also impart additional negative charge. Galectin-1 may bind to other LacNAc sequences on CD45 that are not modified by sialic acid addition, but charge repulsion could prevent close packing of CD45 required to initiate cell death. On the cell surface, both direct masking of galectin-1 ligands on CD45 glycans and increased charge repulsion among CD45 molecules may contribute to inhibition of galectin-1-induced clustering, reduced PTP modulation, and resistance to death.

We are beginning to elucidate the critical roles played by specific glycosyltransferases in lymphocyte development and function. C2GnT transgenic mice demonstrated reduced T cell responses to antigen (2), whereas GnT V null mice demonstrated increased T cell responses to antigen (6). In ST3Gal I null mice, Marth and co-workers (7) found increased apoptosis of peripheral CD8 cells. This group also found profound defects in B cell function in ST6Gal I mice, although no defects in T cell development or function were reported (38). However, it is likely that complex interactions of glycosyltransferases may govern thymocyte susceptibility to galectin-1. For example, in C2GnT transgenic mice, we found increased susceptibility of immature double-positive thymocytes to galectin-1 but no increase in galectin-1 susceptibility of mature, single positive thymocytes (9). Based on our prior observation that medullary immature double-positive thymocytes to galectin-1 but no in

REFERENCES
1. Lowe, J. B. (2001) Cell 104, 809–812
2. Tsuibo, S., and Fukuda, M. (1997) EMBO J. 16, 6364–6373
3. Blander, J. M., Vieintint, I., Janeway, C. A., Jr., and Medzhitov, R. (1999) J. Immunol. 163, 3746–3752
4. Moody, A. M., Chui, D., Reche, P. A., Priatel, J.-J., Marth, J. D., and Reinherrz, E. L. (2001) Cell 107, 501–512
5. Nebert, D. M., Devine, L., Miller, J. D., Moser, J. M., Lukacher, A. E., Altman, J. D., Kavathas, P., Hoyquist, K. A., and Jameson, S. C. (2001) Immunity 15, 1051–1061
6. Demetriou, M., Granovsky, M., Quagggin, S., and Dennis, J. W. (2001) Nature 409, 733–739
7. Priatel, J.-J., Chui, D., Fonsa, N., Simmons, C. J., Richardson, K. B., Page, D. M., Fukuda, M., Varzi, N. M., and Marth, J. D. (2000) Immunity 12, 273–283
8. Kelm, S., Gerlach, J., Brossmer, R., Danzer, C. P., and Nitschke, L. (2002) J. Exp. Med. 195, 1207–1213
9. Galvan, M., Tsuibo, S., Fukuda, M., and Baum, L. G. (2000) J. Biol. Chem. 275, 16730–16737
10. Nguyen, J. T., Evans, D. P., Galvan, M., Pace, K. E., Leitenberg, D., Bui, T. N., and Baum, L. G. (2001) J. Immunol. 167, 5677–5707
11. Baum, L. G. (2002) Immunity 16, 5–8
12. Baum, L. G., Derbin, K., Perillo, N. L., Pang, M., Wu, T., and Uittenbogaart, C. (1996) J. Biol. Chem. 271, 10783–10799
13. Pfeiler, N. L., Uittenbogaart, C., Nguyen, J., and Baum, L. G. (1997) J. Exp. Med. 185, 1851–1858
14. Vespa, G. N. R., Lewis, L. A., Kozak, K. R., Moran, M., Nguyen, J. T., Baum, L. G., and Miceli, M. C. (1999) J. Immunol. 162, 799–806
15. Di Virgilio, S., Gushlak, J., Moremen, K., and Pierce, M. (1999) Glycoconjug. 9, 353–364
16. Perillo, N. L., Pace, K. E., Seilhamer, J. J., and Baum, L. G. (1995) Nature 378, 726–729
17. Weinsteins, J., de Souza-e-Silva, U., and Paulson, J. C. (1982) J. Biol. Chem. 257, 13845–13853
18. Pluck, D., Qian, R., Raus, F. M., III, and Colley, K. J. (1997) J. Biol. Chem. 272, 672–679
19. Cummings, R. D., Trowbridge, I. S., and Kornfeld, S. (1982) J. Biol. Chem. 257, 13421–13427
20. Sparrow, C. P., Leffler, H., and Barondes, S. H. (1987) J. Biol. Chem. 262, 7383–7390
21. Barondes, S. H., Cooper, D. N., Gitt, M. A., and Leffler, H. (1994) J. Biol. Chem. 269, 20807–20810
22. Merkle, R. K., and Cummings, R. D. (1988) J. Biol. Chem. 263, 16143–16149
23. Shibuya, N., Goldstein, I. J., Broekaert, W. F., Nsimba-Lubaki, M., Peeters, B., and Peumans, W. J. (1987) J. Biol. Chem. 262, 1596–1601
24. Pace, K. E., Lee, C., Stewart, P. L., and Baum, L. G. (1999) J. Immunol. 163, 3801–3811
25. Walske, H., Schulz, U., Neels, P., and Brock, J. (1999) Immunol. Lett. 67, 193–202
26. Fouillit, M., Joubert-Caron, R., Poirier, F., Bourin, P., Monestori, E., Leve-Strauss, M., Raphael, M., Bladier, D., and Caron, M. (2000) Glycoconjug. 10, 413–419
27. Sigro, D., and Stamenkovic, I. (1994) Scand. J. Immunol. 39, 433–438
28. Xu, Z., and Weiss, A. (2002) Nat. Immunol. 3, 764–771
29. Franceschini, I., Angata, K., Ong, E., Hong, A., Doherty, P., and Fukuda, M. (2001) Glycoconjug. 11, 211–219
30. Close, B. E., Tao, K., and Colley, K. J. (2000) J. Biol. Chem. 275, 4484–4491
31. Bashinovich, G. A., Baum, L. G., Tinari, N., Paganielli, R., Natoli, C., Liu, F.-T., and Isacchelli, S. (2002) Trends Immunol. 23, 313–320
32. Gillespie, W., Paulson, J. C., Pang, M., Kelm, S., and Baum, L. G. (1993) J. Biol. Chem. 268, 3801–3804
33. Kaufmann, M., Blier, C., Takashima, S., Schwartz-Albies, R., Tsuji, S., and Percher, H. (1999) Int. Immunol. 11, 731–738
34. Ohta, T., Kitamura, K., Maizel, A. L., and Takeda, A. (1994) Biochem. Biophys. Res. Commun. 200, 1283–1289
35. Whiteheart, S. W., McLeish, J. C., and Hart, G. W. (1990) Cell. Immunol. 125, 337–353
36. Martinez-Pomares, L., Crocker, P. R., Da Silva, R., Holmes, N., Colomanas, C., Rodd, P., Dweck, R., and Gordon, S. (1999) J. Biol. Chem. 274, 35211–35218
37. Chung, C. D., Patel, V. P., Moran, M., Lewis, L. A., and Miceli, M. C. (2000) J. Immunol. 165, 3722–3729
38. Hennet, T., Chui, D., Hiraoka, N., Simmons, C. J., Richardson, K. B., Page, D. M., Fukuda, M., Varzi, N. M., and Marth, J. D. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4504–4509
39. Comelli, E. M., Amado, M., Head, S. R., and Paulson, J. C. (2002) Glycoconjug. 12, 650