Genetically Altered Mice with Different Sialyltransferase Deficiencies Show Tissue-specific Alterations in Sialylation and Sialic Acid 9-O-Acetylation

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Glycan chains on glycoconjugates traversing the Golgi apparatus are often terminated by sialic acid residues, which can also be 9-O-acetylated. This process involves competition between multiple Golgi enzymes. Expression levels of Golgi enzyme mRNAs do not always correlate with enzyme activity, which in turn cannot accurately predict glycan sequences found on cell surfaces. Here we examine the cell type-specific expression of terminal glycans in tissues of normal mice in comparison with animals deficient in ST6Gal-I (transfers $\alpha_2$-$6$-linked sialic acid to Gal$\beta_1$-$4$GlcNAc) or ST3Gal-I (transfers $\alpha_2$-$3$-linked sialic acid to Gal$\beta_1$-$3$GlcNAc). Tissues of ST6Gal-I null mice showed minimal binding of an $\alpha_2$-$6$-sialic acid-specific lectin, indicating that no other enzyme generates Sia$\alpha_2$-$6$Gal$\beta_1$-$4$GlcNAc and that Sia$\alpha_2$-$6$GalNAc (sialyl-Tn) is rare in mice. However, exposed Gal$\beta_1$-$4$GlcNAc termini were only moderately increased, indicating that these can be partially capped by other enzymes. Indeed, Gal$\alpha_1$-$3$Gal$\beta_1$-$4$GlcNAc and Fuc$\alpha_1$-$2$Gal$\beta_1$-$4$GlcNAc termini were enhanced in some tissues. Many tissues of ST3Gal-I null animals showed increases in Gal$\beta_1$-$3$GlcNAc termini, and some increases in poly-N-acetyllactosamines. However, overall expression of $\alpha_2$-$3$-linked sialic acid was selectively reduced only in a few instances, indicating that other ST3Gal enzymes can generate this linkage in most tissues. Highly selective losses of 9-O-acetylation of sialic acid residues were also observed, with ST6Gal-I deficiency causing loss on endothelium and ST3Gal-I deficiency giving a marked decrease on CD4$^+$ lymphocytes. These data demonstrate selective regulation of sialylation and 9-O-acetylation, point to cell types with potential physiological defects in null animals, and show in vivo evidence for competition between Golgi enzymes.

The outer surfaces of all eukaryotic cells are covered with a dense and complex array of glycosylated molecules (1). More than 200 gene products (mostly glycosyltransferases) are involved in generating the developmentally regulated and tissue-specific glycosylation characteristic of each cell type in mammals (2, 3). The biosynthesis of glycan chains takes place mostly in compartments of the endoplasmic reticulum-Golgi pathway in stepwise reactions involving specific sugar nucleotide transporters, glycosyltransferases, glycosidases, and other sugar-modifying enzymes (4–7). Expression of some of these enzymes is altered in embryogenesis (8), cancer (9, 10), injury, and inflammation (11, 12). Indeed, altered glycosylation is a universal feature of cancer cells, and certain glycan structures are well known markers for tumor progression (9, 10).

$N$- and O-glycans and glycosphingolipids are often terminated by the addition of sialic acids, a family of nine carbon carboxylated monosaccharides (13–16). Because of their terminal location and negative charge, sialic acids have the potential to inhibit many intermolecular and intercellular interactions (17). The structural diversity of sialic acids (16) can also determine or modify recognition by antibodies, by a variety of endogenous sialic acid-binding lectins, as well as by microbial agglutinins, toxins, and adhesins (18, 19). Sialic acids are involved in many other vertebrate functions, including cell-cell interactions in processes such as the trafficking of blood cells during inflammation (12), the control of neuronal plasticity (17), and the interactions of tumor cells during the metastatic process (9, 10).

Sialic acid residues can be attached to the underlying sugar chain via $\alpha_2$-$3$, $\alpha_2$-$6$, or $\alpha_2$-$8$ linkages. One or more sialyltransferases (STs)\(^3\) have been characterized and cloned for each linkage type (20–24). The $\alpha_2$-$3$ sialic acid linkages to Gal residues seem to be the most widely expressed; these are followed next in frequency by the $\alpha_2$-$6$-linkage of sialic acid to Gal or GalNAc. Some of these sialic acid residues can also be modified by the addition of O-acetyl esters, especially at the 9-position. These ester groups appear to be added by the action of a family of linkage-specific O-acetyltransferases that have yet to be cloned (16, 25–30). The addition of O-acetyl groups can substantially modify the role of sialic acids in a variety of biological processes (13–16, 31).

To help elucidate the functions of $\alpha_2$-$3$- and $\alpha_2$-$6$-linked sialic acids in an intact vertebrate animal system, the ST genes responsible for producing some of these linkages have been inactivated in the mouse. ST6Gal-I generates an $\alpha_2$-$6$-linkage of sialic acid to underlying N-acetyllactosamine (32, 33). The resulting trisaccharide structure (Sia$\alpha_2$-$6$Gal$\beta_1$-$4$GlcNAc) is

\(^3\) The abbreviations used are: ST, sialyltransferase; Sia, sialic acid; ST6Gal-I, Gal$\beta_1$-$4$GlcNAc-CMP-sialic acid ST; ST3Gal-I, Gal$\beta_1$-$3$GlcNAc-CMP-sialic acid ST; SNA, Sambucus nigra agglutinin; ECA, Erythrina cristagalli agglutinin; PNA, Arachis hypogaea peanut agglutinin; MAH, Maackia amurensis hemagglutinin; MAL, Maackia amurensis lectin; OSL-1, Griffonia simplicifolia lectin-1; TL, Lysosierpinexexcellentum tomato lectin; UEA, Ulex europaeus agglutinin; CHE-FcD, influenza C hemagglutinin esterase IgG-Fc fusion protein treated with DFP, diisopropyl fluorophosphate; HRP, horseradish peroxidase; FACS, fluorescence-activated cell sorter.

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the ligand for the Siglec-2/CD22 lectin of B-lymphocytes (34). The absence of ST6Gal-I in mice results in an immunodeficiency characterized by attenuated B lymphocyte function and failure to generate high titers of antibodies in response to T-dependent and T-independent antigens (35). Upon immunization or antigen receptor cross-linking, ST6Gal-I-deficient B cells exhibit reduced calcium fluxes and diminished phosphotyrosine accumulation on key cellular signal transducers (35).

ST3Gal-I generates an \( \alpha_2 \beta_3 - 3 \) sialic acid linkage on the Gal\( \beta_1 - 3 \)GalNAc disaccharide, primarily on O-glycan chains (36). The absence of ST3Gal-I in mice results in a marked deficiency of mature cytotoxic CD8\(^+\) T-lymphocytes, apparently via enhanced apoptosis (37, 38). Although these are the most prominent pathological consequences of these enzyme deficiencies, it remains to be seen whether there are deleterious effects in other cell types in which these enzymes are normally expressed.

Plant lectins have been powerful tools used to explore glycan structures (39, 40). Because of the specificity that each lectin has toward particular structures, even isomeric glycans with identical sugar compositions can be distinguished. Here we have used various plant lectins in flow cytometry and immunohistochemistry assays to characterize changes resulting from genetic inactivation of ST3Gal-I and ST6Gal-I genes in mice. We also used a recombinant chimeric soluble form of the influenza C hemagglutinin esterase (41) to detect changes in
uncovered clues to other potential biological consequences of organs and tissues of the normal laboratory mouse, as well as the patterns of expression of various glycan termini in the tissue-specific manner? In the course of exploring such questions, we were interested to know whether these reactions thought to take place in the late Golgi apparatus, e.g. with other STs and certain α-fucosyltransferases, and α-galactosyltransferases. It is also of interest to know whether these competing transferases always take over use of the acceptor chains or whether some are left exposed. Furthermore, do any resulting losses or changes in sialyl linkages affect sialic acid O-acetylation? Do any of the changes seen take place in a tissue-specific manner? In the course of exploring such questions we generated, for the first time, a base-line description of the patterns of expression of various glycan termini in the organs and tissues of the normal laboratory mouse, as well as uncovered clues to other potential biological consequences of ST3Gal-I and ST6Gal-I deficiency.

EXPERIMENTAL PROCEDURES

Generation of ST3Gal-I Null Mice and ST6Gal-I Null Mice—ST3Gal-I null and ST6Gal-I null mice were generated as described elsewhere (35, 42). Genomic clones of the ST6Gal-I and ST3Gal-I genes were used in constructing targeting vectors that incorporate Cre/loxP recombination sites. The selected mutations deleted critical exons necessary for protein translation and catalytic function.

Histology and Immunohistochemistry—Organs from 4–6-week-old wild-type and null animals were harvested and processed immediately for frozen or paraffin sections. Tissues to be frozen were surrounded with OCT (Optimum Cutting Temperature, Fisher Scientific) compound in freezable plastic molds, and immersed in a slurry of dry ice/2-methyl butane. The frozen blocks were stored in labeled bags at −70 °C and removed when necessary for frozen sectioning and staining. Tissues to be paraffin-embedded were fixed in 10% buffered formalin for 24 h before being transferred to 70% ethanol for the subsequent dehydration and paraffin embedding process. Day 16.5 embryos from wild-type and null animals were harvested and processed immediately for frozen sectioning and staining. For details, see Figs. 2–6 and the table in the “Supplemental Material.”

Table I

| Lectin | Binding specificity | Underlying saccharide | Controls |
|--------|---------------------|-----------------------|----------|
| SNA    | Sia2−6 required     | Gal or GalNAc         | Mild periodate abolishes (if Sia is not 9-O-acetylated) |
| PNA    | Sia blocks binding  | Galβ1–3GalNAc         | 0.1 mM lactose |
| MAH    | Sia2−3 or 3-O-sulfate| Galβ1               | Secondary reagent alone |
| ECA    | Sia blocks Fuc1−2 OK| Galβ1–4GlcNAc (weak) | Secondary reagent alone |
| GSL-1  | Galα1–3 required    | Galβ1–4GlcNAc         | 0.1 mM lactose inhibits |
| TL     | Can be varied       | (Galβ1–4GlcNAc)       | Secondary reagent alone |
| UEA    | Fuc1−2 required     | Galβ1–3GlcNAc         | Secondary reagent alone |
| MAL    | Sia2−3 or none      | Galβ1–4GlcNAc         | Secondary reagent alone |
| CHE-FcD| 9-O-acetyl Sia      | Can be varied         | CHE-Fc (untreated with DFP) |

Table II

Summary of major lectin reactivity differences observed in organs of ST3Gal-I null and ST6Gal null mice in comparison with wild-type animals

| Lectin binding | ST6Gal-I null tissues | ST3Gal-I null tissues |
|----------------|-----------------------|-----------------------|
| SNA            | Absent in all except some cartilage in embryos and some smooth muscle in adults | No change |
| PNA            | Some increase in spleen | Diffusely increased in all organs |
| MAH            | Some increase in spleen only | Decreased in spleen, thymus, and kidney and absent in adrenal |
| ECA            | Increased in spleen | No change |
| GSL-1          | Increased in spleen | No change |
| TL             | No change | Increased in spleen |
| UEA            | Small increase in spleen | No change |
| CHE-FcD        | Absent in blood vessel endothelium of all organs | Decreased binding to lymphocytes in lymph node T-cell zones |

Sialic acid 9-O-acetylation. In exploring these issues we were particularly interested in determining the outcome of these deficiencies on the potentially competitive glycosyltransferase reactions thought to take place in the late Golgi apparatus, e.g. with other STs and certain α-fucosyltransferases, and α-galactosyltransferases. It is also of interest to know whether these competing transferases always take over use of the acceptor chains or whether some are left exposed. Furthermore, do any resulting losses or changes in sialyl linkages affect sialic acid O-acetylation? Do any of the changes seen take place in a tissue-specific manner? In the course of exploring such questions we generated, for the first time, a base-line description of the patterns of expression of various glycan termini in the organs and tissues of the normal laboratory mouse, as well as uncovered clues to other potential biological consequences of ST3Gal-I and ST6Gal-I deficiency.
Glycosylation in ST-deficient Mice

RESULTS AND DISCUSSION

The glycan binding specificities of the lectins and probes used in this study (40) are outlined in Fig. 1, and the specificity of the lectins used for histochemical staining is described in Table I and in the legend to Fig. 1. The major changes seen in the ST3Gal-I or ST6Gal-I null mice are summarized in Table II. A third table (see the Supplemental Material) provides more details about the changes that were seen in specific organs of null animals.

**Altered Terminal Glycosylation in ST6Gal-I Null Mice—**No major histological abnormalities were observed in a survey of hematoxylin and eosin-stained tissues of embryonic or young adult ST6Gal-I null mice (data not shown). The major change seen was a loss of binding to the α 2–6-specific SNA lectin. As shown in Fig. 2, analysis of wild-type day 16.5 embryos showed diffusely positive staining with SNA. In contrast, almost no staining was seen in ST6Gal-I null embryos, except for some weak reaction of cartilage and smooth muscle. This staining was eliminated by prior treatment with mild periodate (not shown), indicating that it was specific. Specific staining with SNA was also seen in many organs and cell types of adult wild-type mice (see Fig. 3 for some examples and Tables II and III for a summary of the findings). In contrast, none of the organs of the adult ST6Gal-I null mice stained with SNA (except for some staining in the smooth muscle of the tracheobronchial tree). Similar results were observed using either enzyme-labeled secondary reagents or the more sensitive fluorescent reagents (Fig. 3).

Taken together, these results indicate that there may be no other enzyme that can produce the same linkage sequence (Siaα2–6Galβ1–4GlcNAc) as the ST6Gal-I enzyme. The data also indicate that Siaα2–6GalNac1-O-Ser/Thr (the sialyl-Tn epitope, which can also be recognized by SNA) (43) must be very rare in normal mouse tissues. It is possible that the latter structure accounts for the weak staining of cartilage and smooth muscle in the day 16.5 embryos and the tracheobronchial smooth muscle in adults.

The loss of ST6Gal-I activity in the null mice could potentially result in exposure of terminal Galβ1–4GlcNAc units that can be recognized by ECA. If there is no effective competition by other late Golgi enzymes, one would expect to see ECA positivity in all the places where SNA reactivity is lost. However, although there was a moderate general increase in ECA staining in the day 16.5 embryo (Fig. 2), adult animals showed increased ECA staining only within the spleen (Fig. 4) and kidneys (not shown). A moderate increase in PNA binding was also seen in the same areas (Fig. 4), likely due to the weak reactivity of PNA with Galβ1–4GlcNAc units. These data indicate that other competing glycosyltransferases must be effectively utilizing most of the Galβ1–4GlcNAc units that fail to be capped by ST6Gal-I in all the other major cell types throughout the adult body. Staining with MAH (which recognizes the product of α2–3 STs) showed no major increase over the wild-type levels (see Fig. 4). However, increased staining with GSL-I was seen in several tissues, indicating that the α1–3-galactosyltransferase generating Galβ1–3Galβ1–4GlcNAc units has a greater opportunity to act in the absence of ST6Gal-I (see Fig. 5 for an example). An additional possibility is that the Galβ1–4GlcNAc units are capped by α1–2-linked fucose residues, which do not prevent the binding of ECA (44). In keeping with this possibility, some increased staining with UEA was observed in the spleen.

**Flow Cytometry Analysis—**Single cell suspensions from thymus, spleen, or lymph nodes of 4–6-week-old mice were subjected to red blood cell lysis by ammonium chloride. Peripheral blood mononuclear cells were isolated by a density gradient using Lympholyte. Prior to staining, CHE-FcD (2 μg) was pre-complexed to phycoerythrin-conjugated goat anti-human IgG (1:100) in 100 μl of FACS buffer (1% bovine serum albumin, 0.1% sodium azide in phosphate-buffered saline) on ice for 1 h. Cell staining was performed using 1 million cells in 100 μl of FACS buffer on ice for 20 min (using anti-CD4 or anti-CD8) or 2 h (using CHE-FcD). For double and triple staining, cells were incubated first with pre-complexed CHE-FcD, washed, and then stained with the anti-CD4 and/or CD8 reagent(s). After staining, cells were washed with excess buffer and resuspended in 300 μl of fixation buffer (1% formaldehyde in phosphate-buffered saline). Stained, fixed cells were analyzed on the same day. Fluorescein isothiocyanate- or tricolor-conjugated rat anti-CD4 and anti-CD8 and phycoerythrin-conjugated goat anti-human IgG (Fc-specific) antibodies were from Caltag. Data were acquired using a BD FACScan and analyzed by the CELLQUEST program.

**Fig. 2. Lectin staining of sagittal sections of day 16.5 embryos.**

Binding of biotinylated SNA, PNA, MAH, or ECA to frozen sections of embryos from wild-type, ST6Gal-I null, and ST3Gal-I null mice was detected using HRP-streptavidin as described in "Experimental Procedures." The panels labeled "secondary" were exposed to the secondary reagent only. The asterisks indicate the sections on which major changes were seen.
Altered Terminal Glycosylation in ST3Gal-I Null Mice—A survey of embryonic and young adult tissues of ST3Gal-I null mice with hematoxylin and eosin staining showed no gross histologic differences (data not shown). There were also no consistent differences seen in staining with MAL (data not shown). However, there were very clear differences observed with PNA and MAH (see below). The ST3Gal-I null animals could lack expression of some terminal α 2–3-linked sialic acids, thereby allowing PNA to bind the exposed Galβ1–3GalNAc termini. Conversely, binding of MAH, which recognizes α2–3-linked sialic acid, could be absent or decreased in such cell types. Indeed, there was a marked increase in intensity of binding to the PNA lectin in many organs of both day 16.5 embryos (Fig. 2) and the adult animals (see Figs. 4 and 6 for examples and Tables II and III for a summary). There was particularly intensified staining in specific areas within certain organs such as the thymus, kidney, and adrenal. Although the PNA-stained thymus of wild-type mice showed a clear border delineating the positive cortex and the negative medulla, the entire organ was stained in the null animals (Fig. 6). In the kidney, there was increased binding of the PNA lectin specifically to the kidney glomeruli of the ST3Gal-I null adults (Fig. 6) and also in the day 16.5 embryos (not shown). PNA also specifically highlighted the glomeruli in the kidneys of the ST3Gal-I null adults (Fig. 6) and day 16.5 embryos (seen under higher magnification, not shown). Interestingly PNA selectively and intensely stained only the medulla but not the cortex of the adrenal gland in wild-type mice, a situation converse to that seen in the normal thymus. In ST3Gal-I null animals there was a loss of distinction between the adrenal cortex and the medulla, as both were intensely stained with PNA (Fig. 6). Noticeably, the adrenal medulla of the ST3Gal-I null animals showed an almost complete loss of staining with MAH, indicating that ST3Gal-I may be the sole enzyme responsible for capping Galβ1–3GalNAc residues throughout this endocrine organ (Fig. 6). Future studies of the aged ST3Gal-I animals may indicate whether this loss contributes to any endocrine physiological abnormalities. MAH also showed a moderate decrease in staining intensity of T cell areas in the spleen (Fig. 4), lymph nodes, and thymic medulla of the ST3Gal-I null animals (See table in Supplemental Material; no corresponding figure shown). None of the other organs showed significant changes in MAH binding (data not shown). These results were duplicated using more sensitive fluorescent secondary reagents (data not shown). No differences in staining with either SNA or ECA were observed between the wild-type and ST3Gal-I null organs (Fig. 4 and data not shown). As shown in Fig. 5, there was increased staining in the spleen of the ST3Gal-I null mice with tomato lectin. This lectin recognizes linear polylactosamine units, which are presumably extended on O-glycans in the absence of capping by ST3Gal-I.

Taken together, these data show that although the loss of
ST3Gal-I results in many exposed Galβ1–3GalNAc residues, there are other α2–3 STs active in most tissues. The other possibility is that the galactose residues are capped with 3-O-sulfate esters, which can also generate MAH binding

(45). The striking exception is the adrenal gland, where almost all of the MAH binding is eliminated, suggesting that Siaα2–3Galβ1–3GalNAc synthesized by ST3Gal-I is the major sialylated epitope in this tissue.

**Fig. 4.** Changes seen in adult spleens of ST3Gal-I and ST6Gal-I null animals using lectin histochemistry. Serial frozen sections of spleens from wild-type, ST3Gal-I, and ST6Gal-I null animals were stained with various biotinylated lectins followed by HRP-labeled streptavidin, color development, and hematoxylin counter-staining. The panels labeled "secondary" were exposed to HRP-labeled streptavidin only. **Bar = 50 μm.**
Altered Sialic Acid 9-O-Acetylation in ST6Gal-I and ST3Gal-I Null Mice—We previously presented evidence that sialic acid O-acetylation in cultured cells is regulated in a sialic acid linkage-specific fashion, apparently by the action of multiple sialic acid-specific O-acetyltransferases (46, 47). Wild-type murine CD4 positive cells derived from lymph nodes were also found to be preferentially 9-O-acetylated (26) compared with the CD8 positive population (demonstrated in Fig. 7, 90 versus 14%). ST6Gal-I null mice showed a similar pattern. Thus, despite loss of the Sia\(^{2-6}\)Gal\(^{1-4}\)GlcNAc sequence on their surfaces (35), CD4\(^+\) lymphocytes of the ST6Gal-I null mice displayed nearly wild-type levels (82%) of 9-O-acetylation compared with CD8 cells (12%). In contrast, the ST3Gal-I null mice showed a marked decrease in surface 9-O-acetylated sialic acids (n = 7). Similar results were obtained using thymic, splenic, or peripheral blood-derived lymphocytes (data not shown). Fluorescence signal intensity using phycoerythrin-conjugated FcD is shown for CD4-gated (left panels) and CD8-gated (right panels) lymphocytes, and the percentage of positive cells is indicated. Dotted lines reflect signal intensity using the esterase (nonbinding) form of the influenza C hemagglutinin esterase probe conjugated to phycoerythrin as a negative control.

![Figure 5. Changes seen in frozen sections of adult spleen using GSL-1, tomato lectin, and UEA histochemistry.](image_url)

Serial sections of spleens from wild-type, ST3Gal-I, and ST6Gal-I null mice were stained with various biotinylated lectins followed by HRP-labeled streptavidin, color development, and hematoxylin counter-staining. The panels labeled "inhibitor" were exposed to lectins in the presence of inhibitor and then HRP-labeled streptavidin only. Bar = 50 microns.

![Figure 6. Changes seen in specific organs of ST3Gal-I null mice using lectin histochemistry.](image_url)

Binding of biotinylated PNA or MAH to frozen sections of various organs from wild-type and ST3Gal-I null mice was detected with HRP-streptavidin, color development, and hematoxylin counter-staining. m, medulla; c, cortex; f, follicle; g, glomeruli. HRP-streptavidin alone gave no staining (not shown). Bar = 50 μm.

![Figure 7. Flow cytometry analysis of T cells for detection of 9-O-acetylated sialic acids (Sia).](image_url)

Lymph node-derived T-cell subsets from 4–6-week-old ST6Gal-I null and ST3Gal-I null mice and their wild-type littermates were analyzed for expression of surface 9-O-acetylated sialic acids using anti-CD4, anti-CD8 and CHE-FcD. Wild-type and ST6Gal-I null CD4 positive cells were preferentially 9-O-acetylated compared with CD8 positive cells (n = 6). In contrast, CD4 cells of the ST3Gal-I null mice showed a marked decrease in surface 9-O-acetylated sialic acids (n = 7). Similar results were obtained using thymic, splenic, or peripheral blood-derived lymphocytes (data not shown). Fluorescence signal intensity using phycoerythrin-conjugated FcD is shown for CD4-gated (left panels) and CD8-gated (right panels) lymphocytes, and the percentage of positive cells is indicated. Dotted lines reflect signal intensity using the esterase (nonbinding) form of the influenza C hemagglutinin esterase probe conjugated to phycoerythrin as a negative control.

![Figure 8. Detection of 9-O-acetylation of sialic acids in lymph node sections.](image_url)

The CHE-FcD probe was pre-complexed with phycoerythrin-conjugated goat anti-human IgG at an optimized ratio and applied to frozen, acetone-fixed, blocked sections of lymph nodes from wild-type, ST3Gal-I, or ST6Gal-I null animals for 1 h at 4 °C. Sections were then washed and mounted with Aquamount for viewing. Examples of high endothelial venules are markedhev. The punctate staining seen represents lymphocytes within the parenchyma of the nodes. Bar = 50 μm.
are carried primarily on mucin-type glycoproteins with O-linked glycans (26). Thus, the loss of the ST3Gal-I enzyme has evidently eliminated a target selective for a specific 9-O-acetyltansferase in this particular cell type.

Tissue sections stained with the CHE-FcD probe for 9-O-acetylated sialic acids showed corresponding selective changes in the two types of null animals. In the wild-type mouse, CHE-FcD stained T-lymphocyte-rich areas in the spleen, thymus, and lymph nodes. It also bound well to endothelial cells in most organs examined and to certain areas of the brain, adrenal medulla, and pancreas (summarized in Table III in the Supplemental Material, detailed data not shown). The lymph nodes of the ST6Gal-I null mice showed wild-type levels of staining of T-cell zone lymphocytes (Fig. 8). However, there was a specific loss of endothelial cell (high endothelial venule) staining by the CHE-FcD probe. This selective loss of 9-O-acetylation in vascular endothelium was also true in all other tissues studied (data not shown). Thus, the expression of the ST6Gal-I product is required for the expression of 9-O-acetylation only in endothelial cells. Conversely, in the ST3Gal-I null mice, there was a decrease in intensity of CHE-FcD staining of T-cell zone lymphocytes in lymph nodes but no decrease in endothelial staining. Fig. 8 shows an example of these findings in lymph nodes, where the staining of the T-cell-rich zone was selectively lost in the ST3Gal-I null animals and the staining of the high endothelial venules was lost in the ST6Gal-I null animals.

Conclusions and Perspectives—We hypothesized that lectin histological studies of mice with genetically altered STs would reveal important clues regarding the enzymatic regulation of terminal glycosylation in the Golgi apparatus of an intact mammalian organism. These analyses have indeed provided insight into the structural features of glycans in defined genetic and mutant backgrounds. Despite the apparent normality of the tissues upon routine histological analyses, there are multiple changes in glycans structure uncovered by the lectin analysis. Taken together, our data indicate that in vivo regulation of sialic acid linkages and sialic acid 9-O-acetylation is indeed affected by the action of ST6Gal-I and ST3Gal-I but only in a cell type-specific manner. Likewise, the level of competition for newly available acceptor sites varies according to the specific ST that is missing and the cell type in question.

Most glycosyltransferases studied to date are members of multigene families with overlapping specificities. However, the complete loss of binding of SNA in almost all cell types and tissues of the adult ST6Gal-I null mouse indicates that this is the only enzyme in the mouse that can produce the Siaα2–6Galβ1–4GlcNAc linkage. Nevertheless, the absence of this enzyme does not result in a large excess of exposed Galβ1–4GlcNAc termini in the adult animal. Instead, there was an increase in the amount of terminal Galα1–3Galβ1–4GlcNAc and Fucα1–2Galβ1–4GlcNAc units. Another explanation is that ST6Gal-I selectively sialylates only certain termini on N-glycans (48). Thus, the loss of ST6Gal-I is not necessarily expected to result in a marked increase in exposed termini. With regard to there being no obvious increase in MAH staining, we emphasize that whereas the appearance of or marked loss of lectin staining is easy to define, modest increases are not. In other words, when significant staining is already present at base line, it is difficult to quantitate moderate increases in staining.

SNA can also recognize the Siaα2–6GalNAcα1–O-Ser/Thr (Sialyl-Tn) structure that is generated by the ST6GalNAc-I and -II enzymes (49, 50). This is true whether or not the sialic acid is in the N-glycolyl form and/or is 9-O-acetylated (43). High expression of this antigen is associated with a poor prognosis in most cancers studied (51–55). The almost complete absence of SNA staining in the young adult ST6Gal-I null mouse allows us to say that the expression of the sialyl-Tn epitope is very rare and may indeed be relatively specific for the malignant state. The traces of SNA positivity in the ST6Gal-I null embryo may represent the expression of this antigen.

In contrast to the situation with ST6Gal-I, ST3Gal-I is one member of a family of ST3Gal isoenzymes, some of which are known to be able to compete with each other (20, 21, 23, 24). MAH staining could also be retained following elimination of ST3Gal-I if the terminal Gal residues were now subjected to 3-O-sulfation (45). Thus, it is not too surprising that MAH staining was substantially retained in the absence of ST3Gal-I (with a few dramatic exceptions, such as in the adrenal gland). However, in this case, compensation by other capping enzymes is quite incomplete, giving rise to markedly increased PNA staining in many tissues.

The differential expression of the mRNA for α2–3- and α2–6-sialyltransferases has been described in rat and human tissues (21, 56) and does not correlate in all instances. Furthermore, expression of mRNA does not always correlate with actual enzyme levels, which in turn, cannot always predict glycosylation. This study, the first to describe comprehensively cell type-specific expression of different linkages of terminal sialic acids in a variety of mouse tissues, forms a foundation for the histologic interpretations of future examples of sialyltransferase knock-out mice.

Finally, our findings also reveal that certain STs can regulate the expression of sialic acid 9-O-acetylation in a highly tissue-specific fashion. Thus, expression of ST6Gal-I provides selective targets for 9-O-acetylation in endothelial cells throughout the body, whereas the ST3Gal-I enzyme seems to provide targets only in CD4+ lymphocytes. The precise role of 9-O-acetylation on endothelium or on CD4+ T cells has not been defined. However, in other instances 9-O-acetylation has been known to block recognition by Siglecs or by Factor H of the complement pathway and to either block or facilitate the recognition of sialic acids by viral hemagglutinins (16).

Further studies are needed to clarify the genetic and cell biological bases of many of these regulatory events. Meanwhile, these studies have pointed to cell types and tissues in which there could be additional biological consequences of these ST deficiencies beyond those seen on B- and T-lymphocytes, e.g. the adrenal gland and kidney in ST3Gal-I null mice and several tissue types in ST6Gal-I null mice.

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