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Mice deficient in the *St3gal3* gene product α 2,3 sialyltransferase (ST3Gal-III) exhibit enhanced allergic eosinophilic airway inflammation

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Abstract

Background—Sialic acid-binding immunoglobulin-like lectin (Siglec)-F is a proapoptotic receptor on mouse eosinophils, but little is known about its natural tissue ligand.

Objective—We previously reported that the *St3gal3* gene product α 2,3 sialyltransferase (ST3Gal-III) is required for constitutive Siglec-F lung ligand synthesis. We therefore hypothesized that attenuation of ST3Gal-III will decrease Siglec-F ligand levels and enhance allergic eosinophilic airway inflammation.

Methods—C57BL/6 wild-type mice and *St3gal3* heterozygous or homozygous deficient (*St3gal3*^{+/-} and *St3gal3*^{-/-}) mice were used. Eosinophilic airway inflammation was induced through sensitization to ovalbumin (OVA) and repeated airway OVA challenge. Siglec-F human IgG₁ fusion protein (Siglec-F-Fc) was used to detect Siglec-F ligands. Lung tissue and bronchoalveolar lavage fluid (BALF) were analyzed for inflammation, as well as various cytokines and chemokines. Serum was analyzed for allergen-specific immunoglobulin levels.

Results—Western blotting with Siglec-F-Fc detected approximately 500-kDa and approximately 200-kDa candidate Siglec-F ligands that were less abundant in *St3gal3*^{+/-} lung extracts and nearly absent in *St3gal3*^{-/-} lung extracts. After OVA sensitization and challenge, Siglec-F ligands were increased in wild-type mouse lungs but less so in *St3gal3* mutants, whereas peribronchial and BALF eosinophil numbers were greater in the mutants, with the following rank order: *St3gal3*^{-/-} *St3gal3*^{+/-} > wild-type mice. Levels of various cytokines and chemokines in BALF were not

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significantly different among these 3 types of mice, although OVA-specific serum IgG₁ levels were increased in *St3gal3*^{-/-} mice.

Conclusions—After OVA sensitization and challenge, *St3gal3*^{+/-} and *St3gal3*^{-/-} mice have more intense allergic eosinophilic airway inflammation and less sialylated Siglec-F ligands in their airways. One possible explanation for these findings is that levels of sialylated airway ligands for Siglec-F might be diminished in mice with attenuated levels of ST3Gal-III, resulting in a reduction in a natural proapoptotic pathway for controlling airway eosinophilia.

Keywords

Eosinophils; asthma; Siglec-F; 6'-sulfated sialyl Lewis X; 6'-sulfated sialyl N-acetyl-D-lactosamine; apoptosis; glycan ligands; lung; *St3gal3*

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are a family of single-pass, transmembrane, cell-surface receptors found predominantly on leukocytes.¹⁻³ Among them, Siglec-F in mice and Siglec-8 in human subjects are functional paralogs inhibitory receptors expressed by eosinophils.⁴ Because engagement of Siglec-F or Siglec-8 induces eosinophil apoptosis,⁵⁻¹⁰ targeting these molecules might help to fill an unmet need in the treatment of various eosinophilic diseases.¹¹⁻¹³

Previous studies have reported that both Siglec-F and Siglec-8 uniquely and preferentially recognize the same sialoside ligand 6'-sulfo sialyl Lewis X (6'-su-sLe^x; NeuAcα2-3[6-SO₃]Galβ1-4 [Fucα1-3]GlcNAc).^{14,15} For Siglec-F, constitutive and cytokine-inducible lung epithelial ligands have been detected.^{9,16-18} These ligands contain α2,3-linked terminal sialic acid residues and in mouse lung require the *St3gal3* gene product α2,3 sialyltransferase (ST3Gal-III) for their constitutive synthesis.^{17,18} We therefore hypothesized that attenuation of ST3Gal-III will decrease Siglec-F airway ligand levels and selectively enhance eosinophilic airway inflammation. Using *St3gal3* heterozygous and homozygous deficient (*St3gal3*^{+/-} and *St3gal3*^{-/-}) mice, we found that they exhibit a more intense allergic eosinophilic airway inflammatory reaction and produce less sialylated high molecular weight Siglec-F ligands in their airways.

Methods

Glycan array analysis of Siglec-F and Siglec-8 ligand binding

The sugar-binding specificities of Siglec-F and Siglec-8 were reanalyzed on the printed glycan microarray, which was developed by the Consortium for Functional Glycomics. Previously reported analyses were carried out with an earlier array format, the microtiter plate array (Version 2.0), which only had 184 biotinylated glycosides and controls immobilized in streptavidin-coated micro titer plates.^{14,15} Version 4.1 of the printed glycan microarray was composed of 465 defined, amino-functionalized glycan structures printed as replicates of 6 on an NHS-activated microscope slide. Siglec-F human IgG₁ Fc chimera (Siglec-F-Fc; R&D Systems, Minneapolis, Minn) or Siglec-8 human IgG₁ Fc chimera (Siglec-8-Fc)¹⁹ were applied in 70 μL of Tris-buffered saline at several different concentrations to the microarray under a cover slip and incubated at room temperature in a humidified chamber. After 1 hour, the cover slip was carefully removed, and the slides were washed in buffer to remove excess protein. The protein-glycan complexes were detected with an Alexa Fluor 488-conjugated goat anti-human IgG (Invitrogen, Carlsbad, Calif) by using the same procedure. After removing the cover slip, the slides were washed, air dried, and scanned with a PerkinElmer ProScanArray fluorescence scanner (PerkinElmer, Waltham, Mass) set at an excitation of 485 nm and an emission of 535 nm, and the data were processed with Imogene Software (BioDiscovery, El Segundo, Calif). After removing

the high and low measurements of each set of replicates, the averages and SDs of the remaining 4 values were used to determine the average relative fluorescence units. Data are reported as a histogram of relative fluorescence units versus glycan structure.

Animals, ovalbumin sensitization, and airway challenge

Mice used in these experiments were 8 to 10 weeks old and included C57BL/6 mice (wild-type [WT]) and *St3gal3* heterozygous deficient (*St3gal3*^{+/-}) and homozygous deficient (*St3gal3*^{-/-}) mice. Breeding sets of *St3gal3*^{-/-} mice were kindly provided by Dr Jamey Marth (University of California, San Diego, Calif), as previously described, but because of breeding limitations of these mutant mice, all *St3gal3*^{-/-} mice used in these studies came from the approximately 25% of *St3gal3*^{+/-} × *St3gal3*^{+/-} mice off-spring that were homozygous null.^{17,20} Mice were intraperitoneally sensitized with 500 µg of ovalbumin (OVA; Sigma-Aldrich, St Louis, Mo) in 1 mg of alum on days 1 and 8 and then challenged intranasally with 200 µg of OVA on days 17, 19, and 21 to induce allergic airway inflammation. All mice were killed 24 hours after final challenge. Control mice were injected and challenged with PBS. All procedures performed on mice were in accordance with the National Institutes of Health's guidelines for humane treatment and approved by the Johns Hopkins University Institutional Animal Use and Care Committees.

Siglec-F ligand expression

Expression levels of Siglec-F binding protein in lung-derived samples were determined by means of Western blotting with a 4% to 15% polyacrylamide gel (Bio-Rad Laboratories, Hercules, Calif). Protein extracted from lung tissue homogenates was generated as previously described.¹⁷ After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories) and incubated overnight with 4% BSA (Sigma-Aldrich). The membranes were incubated with Siglec-F-Fc (0.5 mg/mL, 1 hour, room temperature; R&D Systems). After washing, the membranes were incubated with horseradish peroxidase-linked polyclonal sheep anti-human IgG polyclonal antibody (45 minutes, room temperature; GE Healthcare, Piscataway, NJ). Bands were visualized with the ECL Western blotting detection system (GE Healthcare UK Ltd, Buckinghamshire, United Kingdom). Some of these samples were pretreated with sialidase (*Clostridium perfringens*, 1.6 mU/mL, 2 hours, 37°C; Sigma-Aldrich) to confirm sialidase sensitivity.

Tissue distribution of potential Siglec-F ligands was studied by means of immunohistochemistry, as described previously.¹⁷ Briefly, the 10-µm cryostat sections of lung and tracheal tissues were incubated with Siglec-F-Fc (1 µg/mL, 1 hour, 37°C) and detected by using an alkaline phosphatase imaging method. Isotype-matched humanized IgG₁ mAb (omalizumab, 1 µg/mL, 1 hour, 37°C; Genentech, South San Francisco, Calif) was used as a negative control. In some experiments samples were pretreated with sialidase (10 mU/mL, 24 hours, 37°C; Sigma-Aldrich).

Bronchoalveolar lavage and peripheral blood cell counting

Mouse lungs were lavaged by using 5 repeated instillations of 0.6 mL of saline each through the tracheal cannula. The first 1.2 mL of bronchoalveolar lavage fluid (BALF) was centrifuged, and the supernatant was used for the measurements of various cytokines and chemokines. Cells were counted with a hemocytometer. Differential cell counts in BALF and blood sample smears were performed under standard light microscopy after Diff-Quik staining.

Peribronchial eosinophil enumeration

Major basic protein (MBP) immunohistochemistry was performed on 5- μ m paraffin sections, as previously described.⁹ Rat anti-mouse MBP mAb was obtained from the laboratory of Dr James J. Lee (Mayo Clinic, Scottsdale, Ariz). Numbers of MBP⁺ cells in the peribronchial area were counted by a blinded observer to quantitate the severity of eosinophilic airway inflammation, as previously described.¹⁶ The number of cells staining positive was counted in 10 randomly selected peribronchial regions to a depth of 30 μ m beneath the epithelium. Results are expressed as the number of cells staining positive for MBP per 150- to 200- μ m-sized internal diameter bronchiole.

Measurement of immunoglobulin levels

Serum total IgE (1:100 dilution; detection range, 0.156-20 μ g/mL) and OVA-specific IgE (1.56-200 ng/mL), IgG₁ (1:20,000 dilution, 31.3-4000 μ g/mL), and IgG_{2a} (1:100 dilution, 0.156-20 μ g/mL) levels were determined by using commercial ELISA kits, per the manufacturer's instructions (R&D Systems).

Measurement of cytokines and chemokines

IL-3 (0.2-21,632 pg/mL), IL-4 (2.1-9,372 pg/mL), IL-5 (0.3-13,315 pg/mL), IL-10 (1.0-12,066 pg/mL), IL-17A (0.8-43,337 pg/mL), GM-CSF (5.6-3,401 pg/mL), IFN- γ (1.2-30,164 pg/mL), and TNF- α (1.4-59,626 pg/mL) concentrations in BALF were determined with a Bio-Plex kit (Bio-Rad Laboratories), according to the manufacturer's instructions. IL-13 (1.5-500 pg/mL), IL-33 (14.3-2000 pg/mL), eotaxin (CCL11; 3-1000 pg/mL), and eotaxin-2 (CCL24; 1:4 dilution, 0.0156-12 ng/mL) concentrations were determined by using commercial ELISA kits, according to the manufacturer's instructions (R&D Systems).

Statistical analysis

Data are shown as means $\pm$ SEMs or individual dot plots with medians. Statistical significance between groups was evaluated by means of ANOVA and the Tukey multiple comparison test. *P* values of less than .05 were considered statistically significant.

Results

Siglec-F recognizes 6'-su-sLe^x and a closely related nonfucosylated structure, 6'-sulfated sialyl N-acetyl-D-lactosamine

Previous studies have shown that both Siglec-8 and Siglec-F uniquely and specifically recognize the same glycan structure (6'-su-sLe^x), but only 184 glycans were available as part of this original screening process.^{14,15} Because of the availability of an expanded panel of glycans subsequently made available through the Consortium for Functional Glycomics (Version 4.1, <http://www.functionalglycomics.org>), repeat screening was initiated. By using Siglec-F-Fc and Siglec-8-Fc as probes, 465 carbohydrate-based structures were tested for specific Siglec-F and Siglec-8 binding. As expected, both Siglec-F-Fc and Siglec-8-Fc once again had unique affinity for 6'-su-sLe^x, but both also recognized its nonfucosylated form, 6'-sulfated sialyl N-acetyl-D-lactosamine (6'-su-sLacNAc; not present on the glycan panel Version 2.0 when previously tested) both of which contain an α 2,3-linked terminal sialic acid on a galactose that also carries a 6-sulfate group (Fig 1). Twenty other ligands with terminal NeuAc α 2,3 linked to Gal did not bind either Siglec, including closely related glycans, such as 6-su-sLe^x and sLe^x.^{14, 15} These results support the hypothesis that unique α 2,3-linked sialic acid-containing airway glycans recognize Siglec-8 and Siglec-F and that sialyltransferases capable of creating terminal α 2,3-linked sialic acid residues will be required for natural Siglec-F and Siglec-8 ligand synthesis.¹⁸

Attenuation of ST3Gal-III decreases levels of sialic acid containing Siglec-F ligands in mouse airways

Western blotting of whole-lung extracts was performed to explore possible endogenous lung ligands for Siglec-F. By using Siglec-F-Fc as a probe, approximately 500-kDa and approximately 200-kDa sialidase-sensitive bands were detected in resting whole-lung extracts (Fig 2, A). These bands were less abundant in *St3gal3*^{+/-} lung extracts and almost absent in *St3gal3*^{-/-} lung extracts under nonsensitized and challenged condition. Levels of the approximately 200-kDa candidate ligands increased after OVA sensitization and challenge in WT mouse whole-lung extracts (Fig 2, B) and remained reduced or absent in OVA-sensitized and OVA-challenged *St3gal3*^{+/-} and *St3gal3*^{-/-} lung extracts. However, levels of the approximately 500-kDa candidate ligands were increased after OVA sensitization and challenge in WT mouse lung extracts, as well as in *St3gal3*^{+/-} and *St3gal3*^{-/-} lung extracts (Fig 2, B). In summary, data in Fig 2, A, show (1) the presence of at least 2 sialidase-sensitive ligands from resting mouse lung, one approximately 200 kDa and another broader band at approximately 500 kDa; (2) reduction but not elimination of the approximately 200-kDa material in the *St3gal3*^{+/-} and *St3gal3*^{-/-} mice; and (3) reduction of the approximately 500-kDa material in the *St3gal3*^{+/-} mice and its elimination in the *St3gal3*^{-/-} mice. Data in Fig 2, B, show (1) an increase in the approximately 500-kDa Siglec-F ligand in whole-lung extracts after OVA sensitization and challenge compared with PBS control, even in the *St3gal3*^{+/-} and *St3gal3*^{-/-} mice (and based on Fig 2, C, data; this material might be coming from airway glandular sources) and (2) an increase in the approximately 200-kDa Siglec-F ligand in whole-lung extracts after OVA sensitization and challenge compared with PBS control in the WT mice but less so in the *St3gal3*^{+/-} and *St3gal3*^{-/-} mice.

Immunohistochemistry was performed on tracheal and bronchial tissue sections using Siglec-F-Fc to determine the localization of endogenous Siglec-F tissue ligands in the airway and effects of ST3Gal-III attenuation (Fig 2, C). As previous studies reported,^{9,16,17} expression levels of Siglec-F ligands were constitutive (Fig 2, panel a) and increased in airway epithelium in OVA-challenged WT mice compared with that of PBS-challenged mice (Fig 2, C; compare panels a vs d). In airway epithelial cells after OVA challenge, these inducible ligands were less abundant in *St3gal3*^{+/-} mice (Fig 2, C; compare panels d vs e) and absent in *St3gal3*^{-/-} mice (Fig 2, C; compare panels d and f). In addition, ligands were studied and detected for the first time in tracheal submucosal glands, and there were no obvious differences in Siglec-F ligand expression before or after OVA challenge between WT mice (Fig 2, C; panels g and j), *St3gal3*^{+/-} mice (Fig 2, C; panels h and k), and *St3gal3*^{-/-} mice (Fig 2, C; panels i and l). Further analysis of these tracheal submucosal ligands for Siglec-F revealed that they were completely sialidase sensitive (see Fig E1 in this article's Online Repository at www.jacionline.org). These results indicate that there are at least 2 sources of sialidase-sensitive lung ligands for Siglec-F: one constitutively produced by airway epithelium that manifest ST3gal-III-dependent increases under allergic inflammatory conditions and a second one constitutively produced in tracheal submucosal glands in which production is ST3gal-III independent and is also increased under allergic inflammatory conditions.

St3gal3^{-/-} mice show a selective enhancement in eosinophilic airway inflammation in the OVA-induced allergic lung inflammation model

OVA sensitization and repeated intranasal challenge was performed on WT, *St3gal3*^{+/-} and *St3gal3*^{-/-} mice to test the hypothesis that diminished ST3Gal-III-dependent Siglec-F ligand production will lead to enhancement of lung eosinophilia. As shown in Fig 3, A and B, all 3 types of mice had lung inflammation after OVA sensitization and challenge, with increases in BALF total leukocyte and eosinophil counts significantly greater in *St3gal3*^{-/-} mice than

in the other mice (Fig 3, A, panels a and e), as well as a higher percentage of eosinophils in BALF (see Fig E2, A, in this article's Online Repository at www.jacionline.org). However, when lung sections labeled with anti-MBP mAb (Fig 3, B) were quantified (Fig 3, C), a similar statistically significant increase in tissue eosinophil counts was seen for *St3gal3*^{+/-} and *St3gal3*^{-/-} mice compared with WT mice after OVA sensitization and challenge. There was a nonstatistically significant increase in blood eosinophilia in the *St3gal3*^{-/-} mice that was unaffected by OVA sensitization and challenge (see Fig E2, B). These data demonstrate a preferential increase in peribronchial and luminal eosinophil counts for *St3gal3*^{+/-} and *St3gal3*^{-/-} mice compared with WT mice after OVA sensitization and challenge.

***St3gal3*^{-/-} mice show increases in OVA-specific IgG₁ levels in the OVA-induced allergic lung inflammation model**

Given the enhanced eosinophilic inflammation mentioned above, it was important to determine whether the *St3gal3*^{+/-} and *St3gal3*^{-/-} mice displayed any other evidence of enhanced allergic responses. Serum total IgE levels were significantly increased in *St3gal3*^{-/-} mice after OVA sensitization and challenge compared with those seen in PBS control animals (Fig 4, A). Although there was a higher mean level of total IgE in the *St3gal3*^{-/-} mice, this change was not statistically significant compared with WT or *St3gal3*^{+/-} OVA mice and was due to 1 high outlier in this group of mice (not shown). All 3 types of OVA-sensitized and challenged mice showed increases in serum OVA-specific IgE, IgG₁, and IgG_{2a} levels compared with PBS control animals (Fig 4, B-D). When levels were directly compared from OVA-sensitized and challenged WT, *St3gal3*^{+/-}, and *St3gal3*^{-/-} mice, a significant increase was only seen in OVA-specific IgG₁ levels (Fig 4, C).

Levels of various cytokines and chemokines in the BALF of *St3gal3*^{-/-} mice are not different than those in WT mice in the OVA-induced allergic lung inflammation model

BALF was tested for a variety of potentially relevant mediators to test the hypothesis that the exaggerated lung eosinophilia in the *St3gal3*^{+/-} and *St3gal3*^{-/-} mice was due to an altered allergic cytokine and chemokine response. As shown in Fig E3 in this article's Online Repository at www.jacionline.org, IL-3, IL-4, IL-10, IL-13, IL-17A, IL-33, eotaxin, eotaxin-2, GM-CSF, IFN- γ , and TNF- α levels were not statistically different between OVA-sensitized and challenged WT, *St3gal3*^{+/-}, and *St3gal3*^{-/-} mouse BALF. Unexpectedly, IL-5 levels were higher in BALF of WT-OVA mice than OVA-challenged *St3gal3*^{-/-} mice, even though eosinophil levels in BALF were higher in OVA-challenged *St3gal3*^{-/-} mice. Overall, there were only 2 situations in which there was a significant increase in cytokine levels between the BALF of OVA- versus PBS-treated mice: (1) IL-5 levels were significantly increased in WT-OVA-challenged mice compared with WT-PBS control animals and (2) IL-13 levels were significantly increased in *St3gal3*^{+/-} and *St3gal3*^{-/-} OVA-challenged mice compared with WT-PBS control animals, even though IL-13 levels were not different between WT, *St3gal3*^{+/-}, and *St3gal3*^{-/-} OVA-challenged mice. Taken together, these data suggest that the increased lung eosinophilia is not readily explained by an increase in levels of any of the 10 mediators measured 24 hours after the last intranasal OVA challenge and that the increased eosinophilia seen in *St3gal3*^{+/-} and *St3gal3*^{-/-} OVA-challenged mice occurs along with an unexpected blunting of IL-5 production compared with that seen in WT mice.

Discussion

This article reports several novel findings. First, the data not only confirm the binding specificity of 6'-su-sLe^x for both Siglec-8 and Siglec-F^{14,15} but also demonstrate that the fucose residue in 6'-su-sLe^x is not required to maintain this ligand-binding specificity (Fig 1). This is important because both Siglecs still recognize the same unique glycans,

suggesting that it is relevant to use mouse models involving Siglec-F for the development of glycan-based therapeutics targeting Siglec-8. Second, the results confirm and extend the findings of Guo et al¹⁷ by supporting the critical role of *St3gal3* in generating sialidase-sensitive mouse glycan ligands for Siglec-F on the lung epithelium. Interestingly, newly described submucosal ligands do not appear to require *St3gal3* to generate ligands (Fig 2). By using Western blotting of whole-lung extracts, 2 candidate ligands of approximately 500 kDa and approximately 200 kDa have been identified, with complete attenuation of the approximately 500-kDa ligand seen in *St3gal3*^{-/-} unchallenged mouse lungs and reduced, but not absent, levels of the approximately 200-kDa ligands in *St3gal3*^{-/-} unchallenged mouse lungs (Fig 2, A). After challenge, increases in levels of the approximately 500-kDa ligand are seen regardless of *St3gal3* attenuation, whereas levels of the approximately 200-kDa ligands remain reduced in *St3gal3*-attenuated mice. Although the exact nature of these 2 candidate ligands is still under investigation,¹⁸ it appears likely that the approximately 500-kDa ligand might be primarily glandular in origin.

With respect to the biological significance of Siglec-F ligands, this article is the first to demonstrate that a specific enzyme responsible for decorating glycans with sialic acid, namely ST3Gal-III, can influence lung eosinophilic inflammation. Increased lung eosinophilia in the *St3gal3*^{+/-} and *St3gal3*^{-/-} mice was selective among infiltrating leukocytes in both BALF and peribronchial tissues (Fig 3). Although the increase in BALF eosinophil counts was greatest in the *St3gal3*^{-/-} mice, eosinophil counts were equally and significantly increased in peribronchial tissues in both the *St3gal3*^{+/-} and *St3gal3*^{-/-} mice (Fig 3, C). Even though Siglec-F is also expressed by alveolar macrophages,²¹ attenuation of *St3gal3* had no effect on their numbers, which is consistent with a lack of biological effect seen through Siglec-F targeting on these cells compared with eosinophils.²² The selective lung eosinophilia was not associated with any detectable alteration in levels of a panel of potentially relevant BALF cytokines or chemokines (see Fig E3). One possible explanation is that Siglec-F regulation of lung eosinophilia is upstream of these proinflammatory cytokines in allergen-induced responses. The dissociation between the numbers of eosinophils (increased) and the levels of BALF IL-5 (decreased) in *St3gal3*^{-/-} mice is intriguing and might result from the lack of eosinophil apoptosis in *St3gal3*^{-/-} mice. Many cytokines contain amino acids that would permit N- or O-linked glycosylation, but little is known as to whether alterations in sialylation of cytokines or chemokines affect their biology or half-life. Although an increase in OVA-specific IgG₁ levels was seen, there were no other changes in serologic markers of sensitization observed in the *St3gal3*^{-/-} mice after sensitization and challenge (Fig 4). The reason for the increase in OVA-specific IgG₁ levels is unknown but could be due to changes in the function or longevity of B cells or plasma cells as a result of an alteration of the glycans that are produced by the ST3Gal-III enzyme. Taken together, these data are consistent with previous reports that mice deficient in Siglec-F have selectively exaggerated eosinophilic inflammation^{9,16} and that targeting Siglec-F with antibodies selectively reduces eosinophilic inflammation.^{10,23-25}

There are several potential shortcomings of the current work. Analysis of airways hyperreactivity was not performed, in part because it was not directly relevant to the hypotheses being tested and in part because prior studies involving alteration of Siglec-F did not show any significant effects on airways hyperreactivity.^{12,26} Despite the highly selective nature of the eosinophilic inflammation observed in *St3gal3*-attenuated mice, further work is needed to unequivocally prove that this is due to exclusively reduced amounts of sialylated airway glycan ligands acting through Siglec-F to induce eosinophil apoptosis. Indeed, it is possible that deletion of *St3gal3* might have an effect on these mice in other ways. Future experiments are needed, for example, to determine whether the *St3gal3* gene deletion has an effect directly on eosinophils *per se* and whether reconstitution of the ST3Gal-III enzyme into the airway abrogates the lung eosinophilia to conclude that

eosinophilic inflammation is more intense because and only because of elimination of Siglec-F ligands in the lung. Whether these ligands contain 6'-su-sLe^x, 6'-su-sLacNAc, or both remains to be determined. Furthermore, the relative roles for the approximately 500-kDa versus the approximately 200-kDa ligands and the relative roles of gland-derived versus epithelium-derived ligands in mediating Siglec-F-dependent apoptosis are not yet known. Efforts are underway to isolate enough of each of these candidate ligands for glycomic and proteomic characterization. Nevertheless, the fact that *St3gal3*^{+/-} and *St3gal3*^{-/-} mice have more intense allergic eosinophilic airway inflammation and less sialylated Siglec-F ligands in their airways strongly suggest that reducing levels of airway glycan ligands for Siglec-F diminishes a natural proapoptotic pathway for controlling airway eosinophilia and that disorders associated with reductions in the function of ST3Gal-III in the lung might manifest exaggerated eosinophilic airway inflammation. These data also support the notion that specific Siglecs and their sialylated glycan ligands are important for controlling lung eosinophilia and could potentially be exploited for therapeutic benefit.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BALF	Bronchoalveolar lavage fluid
MBP	Major basic protein
OVA	Ovalbumin
Siglec	Sialic acid-binding immunoglobulin-like lectin
Siglec-8-Fc	Siglec-8 human IgG ₁ Fc chimera

Siglec-F-Fc	Siglec-F human IgG ₁ Fc chimera
ST3Gal-III	<i>St3gal3</i> gene product α 2,3 sialyltransferase
6'-su-sLacNAc	6'-Sulfated sialyl N-acetyl-D-lactosamine
6'-su-sLe^x	6'-Sulfated sialyl Lewis X
WT	Wild-type

Key messages

- This is the first report of sialic acid biosynthesis affecting eosinophilic inflammation in that mice deficient in a single sialyltransferase that adds sialic acid to defined substrates, such as glycoproteins, have exaggerated allergic pulmonary eosinophilic responses.
- This enhanced lung eosinophilia might be due to a change in the sialic acid content of airway glycoproteins, including those that would normally be present and have a proapoptotic effect on these infiltrating eosinophils through their cell-surface Siglec-F.
- These data support the notion that specific Siglecs and their sialylated glycan ligands are important for controlling lung eosinophilia and could be exploited for therapeutic benefit.

Fig 1.

A, Glycan array screening with Version 4.2 of the Consortium for Functional Glycomics glycan array (<http://www.functionalglycomics.org>) reveals 2 shared ligands for Siglec-F-Fc (1 μ g/mL) and Siglec-8-Fc (200 μ g/mL) among 465 glycans tested, namely 6'-su-sLe^x and 6'-su-sLacNAc. Values represent means $\pm$ SDs of replicates from a single assay. Structures are displayed with nomenclature used in the *Essentials of Glycobiology* textbook, second edition (<http://www.ncbi.nlm.nih.gov/books/NBK1908/>). *Diamonds*, N-acetylneuraminic acid (sialic acid); *circles*, galactose; *squares*, N-acetylglucosamine; *triangles*, fucose. **B**, Chemical structures of the 2 shared ligands, 6'-su-sLe^x and 6'-su-sLacNAc, that differ only with respect to the presence or absence, respectively, of the fucose residue.

Fig 2. $St3gal3^{+/-}$ and $St3gal3^{-/-}$ mice demonstrate decreased Siglec-F ligand expression in the lung and airway. **A**, Constitutive ligand characterization in WT, $St3gal3^{+/-}$, and $St3gal3^{-/-}$ mouse whole-lung parenchymal extracts with or without sialidase pretreatment probed by means of Western blotting with Siglec-F-Fc and for β -actin as a positive control. For each lane, 150 μ g of lung extract protein was loaded. Data are representative of 3 experiments with similar results. **B**, Western blotting of mouse whole-lung parenchymal extracts in control and OVA-sensitized and challenged mice probed with Siglec-F-Fc or β -actin. Data are representative of 3 experiments with similar results. **C**, Representative detection and localization of Siglec-F ligand by using Siglec-F-Fc in immunohistochemistry from lung tissues of normal WT, $St3gal3^{+/-}$, and $St3gal3^{-/-}$ mice in tracheal epithelium (panels a-f) and submucosal tracheal glands (panels g-l). All samples were obtained 24 hours after final challenge with PBS or OVA. Data are representative of 3 experiments with similar results.

Fig 3.

Attenuation of *St3gal3* is associated with enhanced OVA-induced allergic eosinophilic airway inflammation. **A**, BALF cytology of each indicated cell type using samples obtained 24 hours after PBS or OVA final challenge from WT, *St3gal3*^{+/-}, and *St3gal3*^{-/-} mice (n = 8-12). **B**, Representative lung tissue sections from WT (panels a and b), *St3gal3*^{+/-} (panels c and d), and *St3gal3*^{-/-} (panels e and f) mice obtained 24 hours after PBS (panels a, c, and e) or OVA (panels b, d, and f) final challenge analyzed by using immunohistochemistry after being labeled with anti-MBP mAb. Representative photomicrographs are provided for each group (magnification $\times 200$). **C**, Peribronchial eosinophils (MBP⁺ cells) in lung tissue sections (like those in Fig 3, B) were enumerated. Values represent means $\pm$ SDs (n = 5-7). For Fig 3, A and C, horizontal bars indicate direct statistical comparisons between OVA-challenged WT, *St3gal3*^{+/-}, or *St3gal3*^{-/-} mice. NS, Not significant.

Fig 4.

Effect of attenuation of *St3gal3* on OVA-induced serologic markers associated with allergic sensitization. Total IgE (A), OVA-specific IgE (B), OVA-specific IgG₁ (C), and OVA-specific IgG_{2a} (D) levels in serum obtained 24 hours after PBS or OVA final challenge (n = 9-13). **P* < .05, ***P* < .01, and ****P* < .001 relative to the WT-PBS control mice.

Horizontal bars indicate direct statistical comparisons between OVA-challenged WT, *St3gal3*^{+/-}, or *St3gal3*^{-/-} mice. NS, Not significant.