JLB

Article

Altered eosinophil profile in mice with ST6Gal-1 deficiency: an additional role for ST6Gal-1 generated by the P1 promoter in regulating allergic inflammation

Mehrab Nasirikenari,* *E. V. Chandrasekaran*,† *Khushi L. Matta*,† *Brahm H. Segal*,‡,§ *Paul N. Bogner*,|| *Amit A. Lugade*,§ *Yasmin Thanavala*,§ *James J. Lee*,¶ *and Joseph T. Y. Lau*,*,1

Departments of *Molecular and Cellular Biology, †Cancer Biology, ‡Medicine, ||Pathology, and §Immunology, Roswell Park Cancer Institute, Buffalo, New York, USA; and ¶Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, Arizona, USA

RECEIVED NOVEMBER 18, 2008; REVISED OCTOBER 23, 2009; ACCEPTED OCTOBER 26, 2009. DOI: 10.1189/jlb.1108704

ABSTRACT

Cumulative evidence indicates that the sialyltransferase ST6Gal-1 and the sialyl-glycans, which it constructs, are functionally pleiotropic. Expression of the ST6Gal-1 gene is mediated by six distinct promoter/regulatory regions, and we hypothesized that these promoters may be used differentially to produce ST6Gal-1 for different biologic purposes. To examine this hypothesis, we compared a mouse with a complete deficiency in ST6Gal-1 (*Siat1* null) with another mouse that we have created previously with a disruption only in the P1 promoter (*Siat1*ΔP1). We noted previously greater neutrophilic inflammation associated with ST6Gal-1 deficiency. Here, we report that ST6Gal-1-deficient mice also have significantly elevated eosinophilic responses. Upon i.p. thioglycollate elicitation, eosinophils accounted for over 20% of the total peritoneal inflammatory cell pool in ST6Gal-1-deficient animals, which was threefold greater than in corresponding wild-type animals. A principal feature of allergic respiratory inflammation is pulmonary eosinophilia, we evaluated the role of ST6Gal-1 in allergic lung inflammation. Using OVA and ABPA experimental models of allergic airways, we showed that ST6Gal-1 deficiency led to greater airway inflammation characterized by excessive airway eosinophilia. The severity of airway inflammation was similar between *Siat1*ΔP1 and *Siat1* null mice, indicating a role for P1-generated ST6Gal-1 in regulating eosinophilic inflammation. Colony-forming assays suggested greater IL-5-dependent eosinophil progenitor numbers in the marrow of ST6Gal-1-deficient animals. Moreover, allergen provocation of wild-type mice led to a significant reduction in P1-mediated ST6Gal-1 mRNA and accompanied decline in circulatory ST6Gal-1 levels. Taken together, the data implicate ST6Gal-1 as a participant in regulating not only Th1 but also Th2 responses, and ST6Gal-1 deficiency can lead to the development of more severe allergic inflammation with excessive eosinophil production. *J. Leukoc. Biol.* **87: 457–466; 2010.**

Introduction

Sialic acid-containing glycans contribute to diverse aspects of the immune and inflammation repertoire and in the maintenance of blood homeostasis. Construction of the sialic acid linkages is mediated by a family of 18 distinct sialyltransferases. Experimental inactivation of sialyltransferases showed that ST3Gal-IV mediates leukocyte arrest during inflammation [1], and ST3Gal-I is a dominant modifier contributing to variability in von Willebrand disease [2, 3] and in regulating $CD8^+$ T lymphocyte homeostasis [4]. The contribution of the sialyltransferase ST6Gal-1 in humoral immunity is well-established. The interaction of its cognate α2,6-sialyl catalytic product with the B cell complex accessory molecule CD22 (Siglec-2) contributes to B cell activation, differentiation, and maturation [5–8]. A role for the α2,6-sialyl ligands constructed by ST6Gal-1 in the homing of leukocytes to the bone marrow has also been suggested [7]. Sialylation of the integrin β1 subunit by ST6Gal-1 has also been implicated in macrophage and tumor cell adhesiveness [9]. Recent studies from our laboratory have revealed an unexpected role of hepatically produced ST6Gal-1 in regulating inflammation, circulating neutrophil homeostasis, and replenishing granulocyte numbers [10]. Together, these advances have underscored the idea that ST6Gal-1 and the sialyl-glycan structures that it constructs have multiple biologic roles that differ depending on the cells that express the sialyltransferase.

The notion of ST6Gal-1 pleiotropy was prompted by early observations that transcription of the ST6Gal-1 gene originates from

Abbreviations: ABPA=allergic bronchopulmonary aspergillosis, BAL=bronchoalveolar lavage, BALF=BAL fluid, CFU-eo=CFU-eosinophil, CMP-NeuNAc=cytidine monophosphate-N-acetylneuraminic acid, Ct=comparative threshold, DC=dendritic cell, i.n.=intranasal, NIH=National Institutes of Health, PI=propidium iodide

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

0741-5400/10/0087-457 © Society for Leukocyte Biology

six physically distinct promoter/initiation sites, resulting in dramatically varied levels of ST6Gal-1 in different tissues [11, 12]. For example, four distinct promoters are sequentially recruited for ST6Gal-1 expression during B cell activation and differentiation [12]. In contrast, specifically ablating the P1 promoter while leaving the remaining promoters intact [13] resulted in a normal humoral response but in an exaggerated acute neutrophilic response coupled with an expanded capacity for granulopoiesis and greater sensitivity to G-CSF [10]. Furthermore, the severity of the inflammation in the P1-ablated mouse (*Siat1*ΔP1) was equivalent to that displayed by the totally ST6Gal-1-deficient mouse (*Siat1* null). This observation indicated that the pool of ST6Gal-1 relevant to the regulation of granulopoiesis and recruitment of granulocytes in acute inflammation was generated from P1-mediated transcription of the ST6Gal-1 gene.

Asthma is a disease of chronic inflammation of the airway marked by episodic acute exacerbations leading to airway obstruction and reversible variable airflow limitations. The principle features of allergic respiratory inflammation associated with asthma are pulmonary eosinophilia, airway hyper-responsiveness, excessive airway mucus production, elevated serum IgE, and in chronic disease settings, airway remodeling marked by collagen deposition and increases in airway smooth muscle mass. The onset and progression of asthma are mediated by Th2 inflammatory responses orchestrated principally by the production of cytokines such as IL-4, IL-5, IL-9, and IL-13. The balance among Th1, Th2, Th17, and regulatory T cells in the early phases of allergen exposure may skew individuals toward an allergic response, a neutrophil-predominant response, or tolerance. The cellular infiltrates associated with allergic pulmonary inflammation are believed to be principle contributors leading to airway obstruction and lung dysfunction. Pulmonary eosinophilia in asthma was noted, even in the earliest studies [14], and the number of airway eosinophils was associated directly with disease severity (reviewed in refs. [15, 16]). Moreover, reduction of airway eosinophils of asthma patients is one of the most reliable indicators of successful treatment of allergen-induced asthma exacerbations [17]. Selective release of eosinophil-derived products, such as cytotoxic (e.g., eosinophil peroxidase and major basic protein-1 and -2) and bronchoactive (leukotrienes) compounds, mediates many aspects of asthma pathology [18–21]. Eosinophil-independent mechanisms exist, and allergen-induced pathologies can develop independently of eosinophil recruitment [19, 22, 23]. Recent studies have also established the connection of eosinophils with the induction and perpetuation of the lung Th2 response driving allergic inflammation [24]. Ablation of the eosinophil-specific sialic acid-binding lectin, Siglec-F, resulted in increased lung eosinophil infiltration upon allergen challenge [25].

Therefore, we asked whether ST6Gal-1 influences eosinophilic allergic lung inflammation. We found that ST6Gal-1 deficiency endows an animal with an unexpected overabundance of eosinophils in elicited inflammation. In experimental models of allergic airway inflammation, *Siat1*ΔP1 and *Siat1* null mice exhibited more severe acute eosinophilic pulmonary inflammation when provoked with allergen compared with wild-type mice with a more pronounced Th2 profile. Further, in wild-type animals, elicitation of acute allergic airway inflammation resulted in depression of P1-mediated ST6Gal-1 expression in the liver and a corresponding depression of secreted ST6Gal-1 in systemic circulation. Together, the data point to a contribution ST6Gal-1 production in eosinophilia and also reveal an unexpected potential role for ST6Gal-1-mediated sialylglycans as regulators of allergic lung inflammation.

MATERIALS AND METHODS

Animals and inflammation models

Generation of the *Siat1*ΔP1 mouse by gene-targeted deletion of the P1 promoter of *Siat1* was described previously [13]. *Siat1*ΔP1 mice were backcrossed 11 successive generations into the C57BL/6 background. *Siat1* null animals [5] were obtained originally from the Consortium for Functional Glycomics, and they have been backcrossed more than six generations into C57BL/6. For all experiments reported here, age- and sex-matched (typically 55- to 70-day-old) C57BL/6 animals were used as wild-type controls. To elicit acute peritonitis, 1 mL 4% w/v thioglycollate (Brewer's yeast thioglycollate, Becton Dickinson Microbiology, Baltimore, MD, USA) solution in PBS was administered i.p. into each recipient animals. At indicated time-points after thioglycollate challenge, animals were killed by CO_2 asphyxiation, and cells were recovered by peritoneal lavage with 6 mL ice-cold PBS. Typically, peritoneal lavage is free of red coloration, indicating the lack of RBC contamination.

For induction of allergic airway inflammation by OVA, mice were sensitized by two i.p. injections of 20 μg OVA (grade IV, Sigma Chemical Co., St. Louis, MO, USA) bound to 2.25 mg Imject Alum [$Al(OH)_3$-$Mg(OH)_2$ (Pierce, Rockford, IL, USA)] in 100 μl saline on Days 0 and 14. Mice were challenged on Days 24–27 by 20-min inhalations of an aerosol generated by nebulization of a 1% OVA solution prepared in saline. Peripheral blood was obtained (typically at approximately 50 μl) by retro-orbital venous plexus sampling in polypropylene tubes containing EDTA, and mice were killed by i.p. injection of 1 ml Avertin (2.5 gr 2,2,2, Tribromethanol, 5 ml 2-methyl-2-butanol in 200 ml distilled water) on Day 29.

ABPA was performed as described in Hogaboam et al. [26]. Briefly, mice received a total of 10 μg *Aspergillus fumigatus* antigens dissolved in 0.2 ml IFA (Sigma Chemical Co.), distributed equally between an i.p. and a s.c. injection. Two weeks later, and for 3 subsequent weeks, mice received a total of 20 μg *A. fumigatus* antigen, dissolved in normal saline, by i.n. installation. One week after the third i.n. challenge, each mouse received 5.0×10^6 live *A. fumigatus* conidia suspended in 30 μl 0.1% Tween-80 via the intratracheal route. Seven days after peripheral blood was obtained by retro-orbital, venous plexus sampling in polypropylene tubes containing EDTA and mice were killed by i.p. injection of 1 ml Avertin.

In both allergy models, BAL was performed. The thoracic cavity was opened, and the trachea was exposed. The trachea was cannulated with a 22-gauge i.v. catheter. PBS (750 μL) was injected and withdrawn from the lung two times using a tuberculin syringe. A white blood cell count of BALF was assessed using a hemocytometer. Cells were then cytocentrifuged onto clean glass slides and stained with the Hema 3® stain set (Fisher Scientific, Pittsburgh, PA, USA), and cell differential counts were obtained. BALF was centrifuged, the supernatant was used for cytokine analysis, and the cells were used for flow cytometry and apoptosis assay. After BAL, lungs were excised and fixed in 10% formaldehyde in PBS. Lungs were then embedded in paraffin, sectioned, and stained with H&E. Lung pathology was evaluated by a pulmonary pathologist blinded to the identity of the slides.

All animal studies presented here have been approved by the Institutional Animal Care and Use Committee of Roswell Park Cancer Institute (Buffalo, NY, USA). Unless otherwise stated, all data here are presented as the mean ± SD. Statistical significance was assessed by a two-sided Student's *t*-test.

Flow cytometry and histopathology

Immunofluorescent staining and flow cytometric analysis of inflammatory cell subsets of BAL and peritoneal cells were performed as follows. Cells

($0.5–2\times10^6$) were washed in PBS containing 0.5% BSA and 0.02% sodium azide. FcR sites were blocked by incubation with goat serum (Gibco, Grand Island, NY, USA) and anti-CD16/32 (FcγRIII/II) Fc Block for 10–15 min at room temperature. Blocked samples were incubated for 30 min on ice with combinations of fluorescently labeled antibodies anti-Ly6G (1A8), anti-CD4 (L3T4), anti-CD8a (Ly-2), anti-CD11c, anti-CD45R/B220, and CCR3 (R&D Systems, Minneapolis MN, USA). Flow cytometric analysis was performed using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Franklin Lakes, NY, USA) and the WinList software package. Unless otherwise stated, all immunoreagents for flow cytometry were from BD PharMingen (San Diego, CA, USA). Pulmonary acute inflammation was evaluated histologically by a trained pulmonary pathologist (P.N.B).

Colony-forming, apoptosis, cytokine, and ST6Gal-1 quantitation

For colony-forming cell assays, a total of 7.5×10^5 bone marrow-nucleated cells in a volume of 0.1 ml was plated in 0.9 ml methylcellulose media (MethoCult 3234, Stem Cell Technologies, Vancouver, BC, Canada), supplemented with 20 ng/ml mouse rIL-5 (R&D Systems), and placed in a humidified incubator with 5% CO_2 at 37°C. Colonies containing at least 50 cells were counted 7 days after incubation. The number of colonies/10^5-nucleated bone marrow cells was calculated. To assay for cell viability, BALF cells were assayed using an Annexin V:FITC apoptosis detection kit (BD PharMingen), according to the manufacturer's instructions. For cytokine assays, BALF supernatant (200 μl) or plasma (50 μl) was subjected to Luminex 100 multiplex assays using a capture bead system developed by Luminex Corp. (Austin, TX, USA). To determine serum Ig levels, OVA (Sigma Chemical Co.) was coated onto ELISA plates at a concentration of 3 μg/ml in coating buffer (0.05 M boric acid in PBS, pH 9·5). Plates were blocked with PBS containing 3% BSA. Serial twofold dilutions of serum (1:200) and BALF (1:20) were prepared in PBS containing 0.05% Tween-20 and allowed to bind to the plate. Mouse Ig was detected by using alkaline phosphatase-conjugated goat anti-mouse second-step reagents specific for IgE, IgG1, IgG2a, or IgG3 (Southern Biotechnology, Birmingham, AL, USA). Alkaline phosphatase was detected with 1 mg/ml *P*-nitrophenylphosphate (Sigma Chemical Co.) in 10 mM diethanolamine buffer (Sigma Chemical Co.), and colorimetric changes were read at a wavelength of 405 nm. The reactive titer was determined as the reciprocal of the dilution, yielding a 0.100 absorbance value over the preimmune serum and BAL sample. Comparisons between groups were performed by using a one-way ANOVA with a multiple comparison post-test (Dunnett test).

Sialyltransferase assays were carried out under incubation conditions as described previously [13, 27, 28]. For the measurement of ST6Gal-1 activity, 10 μl serum was incubated with CMP-^{3}[H]NeuNAc and monitored for the transfer of ^{3}[H]NeuNAc to an exogenously supplied acceptor compound, Galβ4GlcNAcα-o-Bn, or alternatively, GalNAcβ4GlcNAcα-o-Bn—the latter has been shown to be a more efficient and more specific acceptor for ST6Gal-1 (E. V. Chandrasekaran, K. L. Matta, unpublished observations). ^{3}[H]NeuNAc, transferred from CMP-NeuNAc, was measured after recovery and separation of acceptor and ^{3}[H]-sialylated acceptor compounds from unreacted CMP-^{3}[H]NeuNAc and free ^{3}[H]NeuNAc by C_{18} reverse-phase chromatography. ^{3}[H] was quantified by scintillation counting. Liver ST6Gal-1 mRNA was determined by real-time RT-PCR. Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized from 1 μg RNA using the iScript RT kit (Bio-Rad, Hercules, CA, USA), according to the manufacturer's instructions. Real-time PCR reactions, using iQ SYBR-Green Supermix (Bio-Rad), were performed on the My iQ Single-Color Real-Time PCR detection system (Bio-Rad). Primer pairs for each mRNA, available on request, were designed based on sequence information deposited in GenBank. Relative mRNA levels are derived from ΔCt, the difference of the Ct value of the target mRNA and the Ct value for RPL32, a ribosomal protein mRNA used as a reference standard.

RESULTS

Abundant eosinophil counts in thioglycollate-elicited peritonitis in ST6Gal-1-deficient animals

Previously, we reported that ST6Gal-1 deficiency results in overly exaggerated neutrophilic peritonitis [10]. Upon more careful examination, the recruited inflammatory cells in thioglycollate-elicited acute peritonitis also had a surprisingly abundant population of recruited eosinophils, which are distinguished clearly by their eosin staining in cytospin preparations (**Fig. 1A**). We also used flow cytometry and two cell surface markers, CCR3 and Ly6G, which together distinguish two populations of granulocytes: the CCR3^{neg}:Ly6G^{hi} and the CCR3^{hi}:Ly6G^{lo} populations, neutrophils and eosinophils, respectively. Photomicroscopic examination of Wright's stained leukocytes recovered by cell sorting confirmed the identities of these granulocytes (Fig. 1B).

Twenty-four hours after thioglycollate challenge, eosinophils comprised up to 20% of the total peritoneal cellular milieu in *Siat1*ΔP1 and in *Siat1* null animals, which was in threefold excess in comparison with the similarly challenged C57BL/6 (wild-type) animals that have a normal ST6Gal-1 profile (Fig. 1C). Excess peritoneal eosinophilia was similar between *Siat1* null, with an absolute ST6Gal-1 deficiency, and *Siat1*ΔP1, with only a limited ST6Gal-1 deficiency by inactivation of the P1 promoter. Consistent with our earlier observations, the overall total peritoneal infiltrate at 24 h after thioglycollate challenge was 1.5- to 1.7-fold, elevated in *Siat1*ΔP1 and *Siat1* null when compared with wild-type animals. Excess peritoneal eosinophilia in the ST6Gal-1-deficient animals persisted into 48 h and 72 h after thioglycollate challenge (data not shown).

Unexpected prevalence of eosinophils was also observed at baseline in the peritoneum of ST6Gal-1-deficient animals. Without thioglycollate elicitation, the peritoneal cavity lavage contained up to 2.2% eosinophils in the *Siat1*ΔP1 mice, compared with 1.25% eosinophils in wild-type animals (Fig. 1C). Eosinophils in the peritoneal lavage at baseline were confirmed by visual examination of cytospin preparations (data not shown). The majority of the lavage cells from both genotypes at baseline was F4/80^{pos} cells, with low forward- and side-scatter patterns (presumably macrophage); Ly6G^{hi}:CCR3^{neg} neutrophils were not present in lavage at baseline (data not shown). Together, these observations suggest a greater propensity for eosinophilia in ST6Gal-1 deficiency. However, there was no difference in resting circulatory levels of major hematopoietic cell types, including granulocytes, monocytes, and B and T cells among wild-type, *Siat1*ΔP1, and *Siat1* null animals (data shown in Supplemental Fig. 1). This observation recapitulates the hematology phenotype for the *Siat1* null animals reported by the Consortium for Functional Glycomics and available for public access by the web (www.functionalglycomics.org).

More severe acute pulmonary allergic inflammation is associated with ST6Gal-1 deficiency

Eosinophil recruitment is typically associated with Th2 immune responses associated with diseases, such as in allergic asthma. At baseline, wild-type, *Siat1*ΔP1, and *Siat1* null animals had normal lung histology and were generally free of significant inflammatory

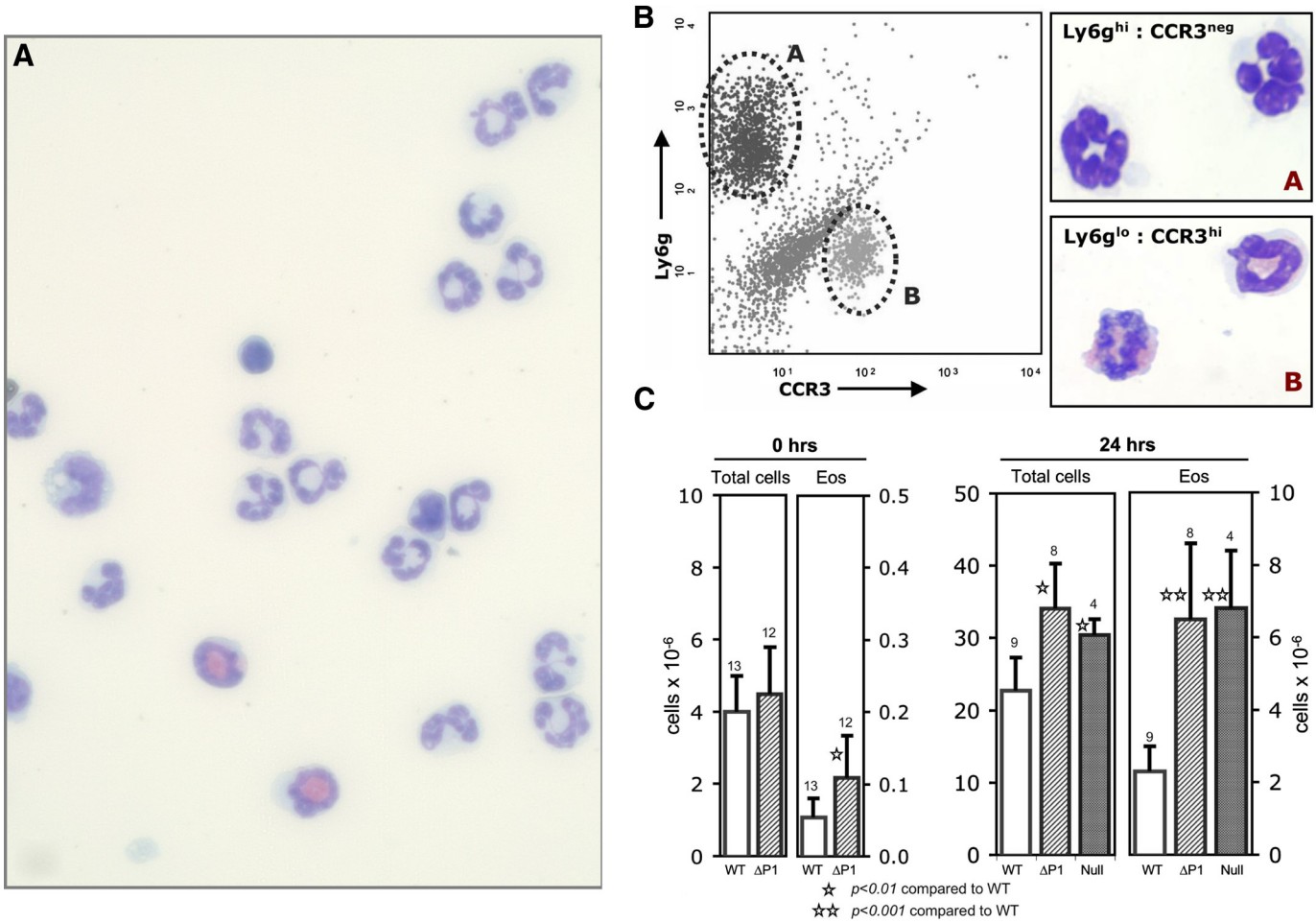


Figure 1. Elevated peritoneal eosinophil abundance in ST6Gal-1-deficient animals. Peritoneal lavage was recovered from *Siat1*ΔP1 mice 24 h after challenge with thioglycollate i.p., as stated in Materials and Methods, immobilized on glass slides by cytospin, and stained by Hema 3® (A). (B) FACS analysis of the elicited peritoneal cells using FITC-anti-CCR3 and PE-Ly6G, where the fractions labeled A and B, representing $Ly6G^{hi}$:$CCR3^{neg}$ and $Ly6G^{lo}$:$CCR3^{hi}$ populations, were flow-sorted and visualized by cytospin. (C) Cells from peritoneal lavage of wild-type (WT, open bars), *Siat1*ΔP1 (ΔP1, hatched bars), or *Siat1* null (Null, shaded bars) were assessed for total cell numbers by hemocytometer and for eosinophil (Eos) numbers by FACS and by visual evaluation of stained cytospin preparations. Peritoneal lavage was harvested at baseline (0 h, without thioglycollate treatment) and at 24 h after thioglycollate elicitation. The numbers above each bar represent the number of animals used for each determination. Statistical significant differences between wild-type and mutant animals are denoted by * ($P<0.01$) or ** ($P<0.002$).

infiltrates (data not shown). When animals were subjected to the OVA model of acute allergic airway challenge, the resultant pulmonary inflammation was generally more severe in ST6Gal-1-deficient compared with wild-type animals. Representative photomicrographs of lung sections from wild-type and *Siat1*ΔP1 mice subjected to the OVA protocol (**Fig. 2, A** and **B**) showed mixed mononuclear and polymorphonuclear composition.

The ABPA model of allergic airway was also used in independent experiments to confirm that ST6Gal-1 insufficiency led to excessive acute allergic inflammation. In the ABPA model, much greater severity of inflammation was observed in the *Siat1*ΔP1 than in identically treated wild-type mice, as illustrated by representative photomicrographs of wild-type and *Siat1*ΔP1 subjected to the ABPA protocol (Fig. 2, C and D). Irrespective of the allergen used, only the ST6Gal-1-deficient mice, but none among the wild-type mice, show the most severe level of acute inflammation, demonstrating inflammatory cells in most or all bronchovascular bundles, infiltration of bronchial epithelium and lumens, spilling of inflammatory cells out of the bronchovascular bundles into adjacent parenchyma, and patchy pleural and septal involvement.

Inflammatory infiltrate in the BALF was used as a quantitative metric for the degree of allergic pulmonary inflammation. As shown in **Figure 3A**, *Siat1* null and *Siat1*ΔP1 mice responded with almost twofold excess BALF cell infiltration than wild-type mice when subjected to OVA provocation. The degree of excess BALF cell infiltration was identical between *Siat1*ΔP1 and *Siat1* null animals. Excess inflammatory cell infiltration into BAL by ST6Gal-1-deficient mice occurred as well in the aspergillosis model (ABPA), and 1.7-fold more cells were recovered in the BALF of *Siat1*ΔP1 mice compared with wild-type mice after the ABPA protocol

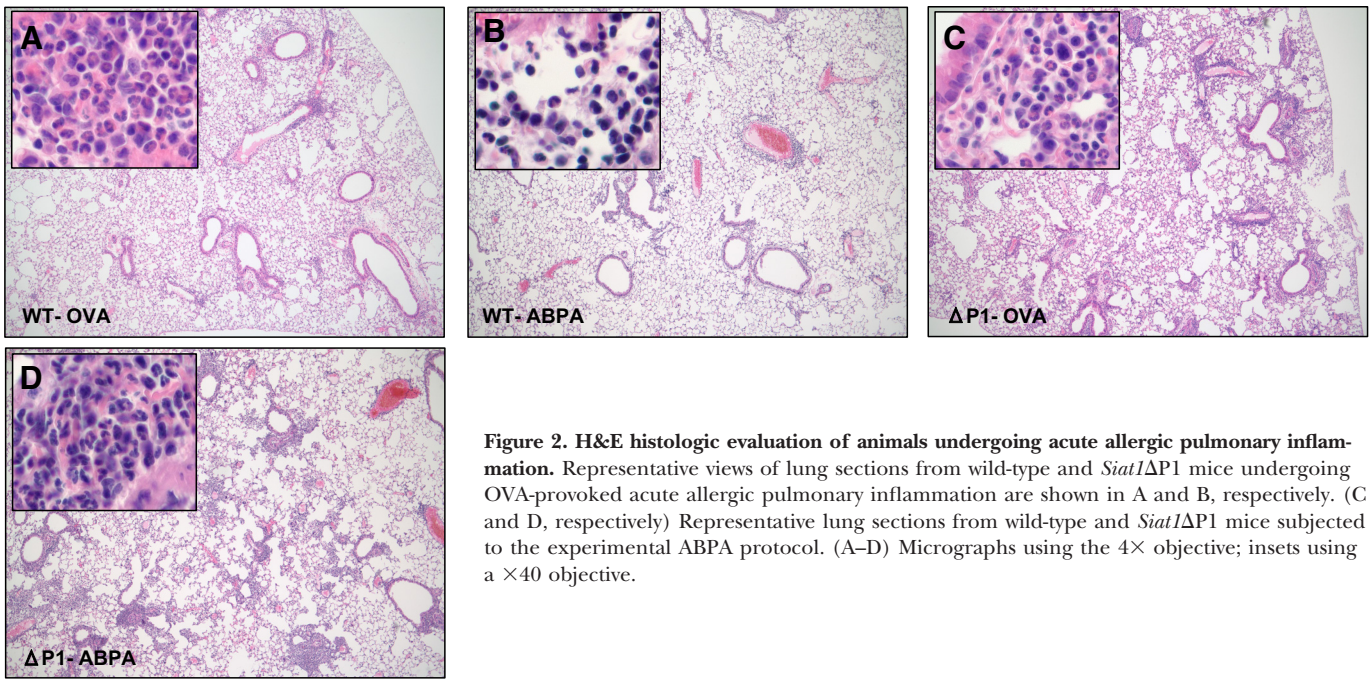


Figure 2. H&E histologic evaluation of animals undergoing acute allergic pulmonary inflammation. Representative views of lung sections from wild-type and *Siat1*ΔP1 mice undergoing OVA-provoked acute allergic pulmonary inflammation are shown in A and B, respectively. (C and D, respectively) Representative lung sections from wild-type and *Siat1*ΔP1 mice subjected to the experimental ABPA protocol. (A–D) Micrographs using the 4× objective; insets using a ×40 objective.

(data not shown). Eosinophils were the principal cells in the BALF of mice undergoing allergic acute airway response. Interestingly, eosinophils comprised >75% of the cells recovered in the lavage fluid (Fig. 3B), suggesting similar Th2 immune responses in both animals. Macrophage/monocyte, DCs, lymphocytes, and a small population of neutrophils were also present.

ST6Gal-1 deficiency is associated with a more pronounced Th2 response

ELISA profiling of circulatory Ig showed more pronounced elevation of sera IgE, IgG1, and IgG3 in the ST6Gal-1-deficient animals, compared with wild-type animals undergoing OVA-challenged acute allergic pulmonary inflammation (**Fig. 4A**). Statistically significant differences were observed in IgE, IgG1, and IgG3 Ig subtypes between OVA-treated wild-type and *Siat1*ΔP1 animals, suggesting an elevated Th2 response in the ST6Gal-1-deficient animals. An elevated Th2 response in ST6Gal-1-deficient animals was recapitulated by serum cytokine analysis, which showed statistically significant differences in circulatory IL-5 and IL-13 between OVA-treated wild-type and *Siat1*ΔP1 mice, with the IL-4 level below our detection limits in all cases (Fig. 4B). Circulatory IL-6 levels were elevated in the *Siat1*ΔP1 mice at baseline

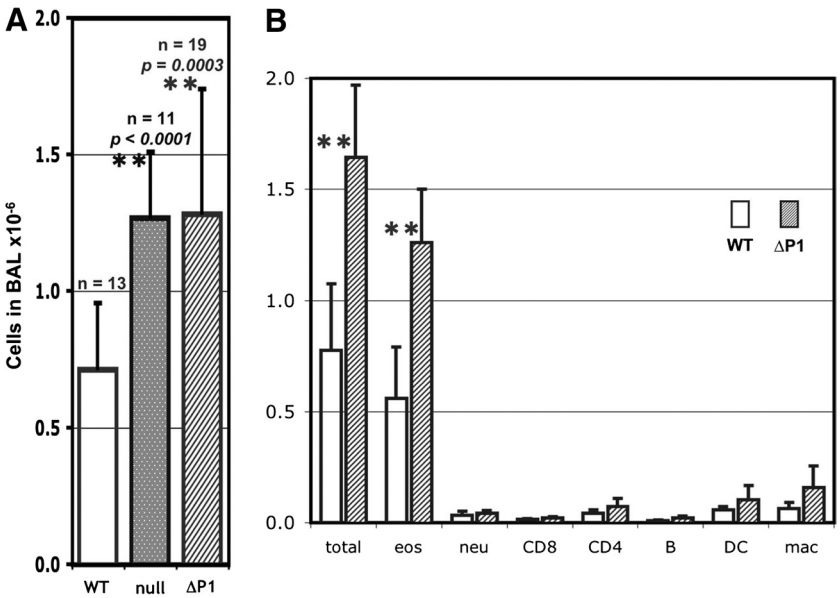


Figure 3. Elevated eosinophil presence in the BAL of ST6Gal-1-deficient mice. BALF was recovered from wild-type, *Siat1*ΔP1, or *Siat1* null animals undergoing acute OVA-provoked allergic pulmonary inflammation. (A) Total cell content, expressed as BAL cells/animal, as determined on a coulter counter. *n* is the number of animals used for each respective data point. (B) BAL cell composition of wild-type (open bars) and *Siat1*ΔP1 (hatched bars), as determined by FACS analysis and outlined in Materials and Methods. The data shown are the mean of four to five wild-type and four to six *Siat1*ΔP1 animals. **, Indicates statistical significance of $P < 0.003$. neu, Neutrophil; mac, macrophage.

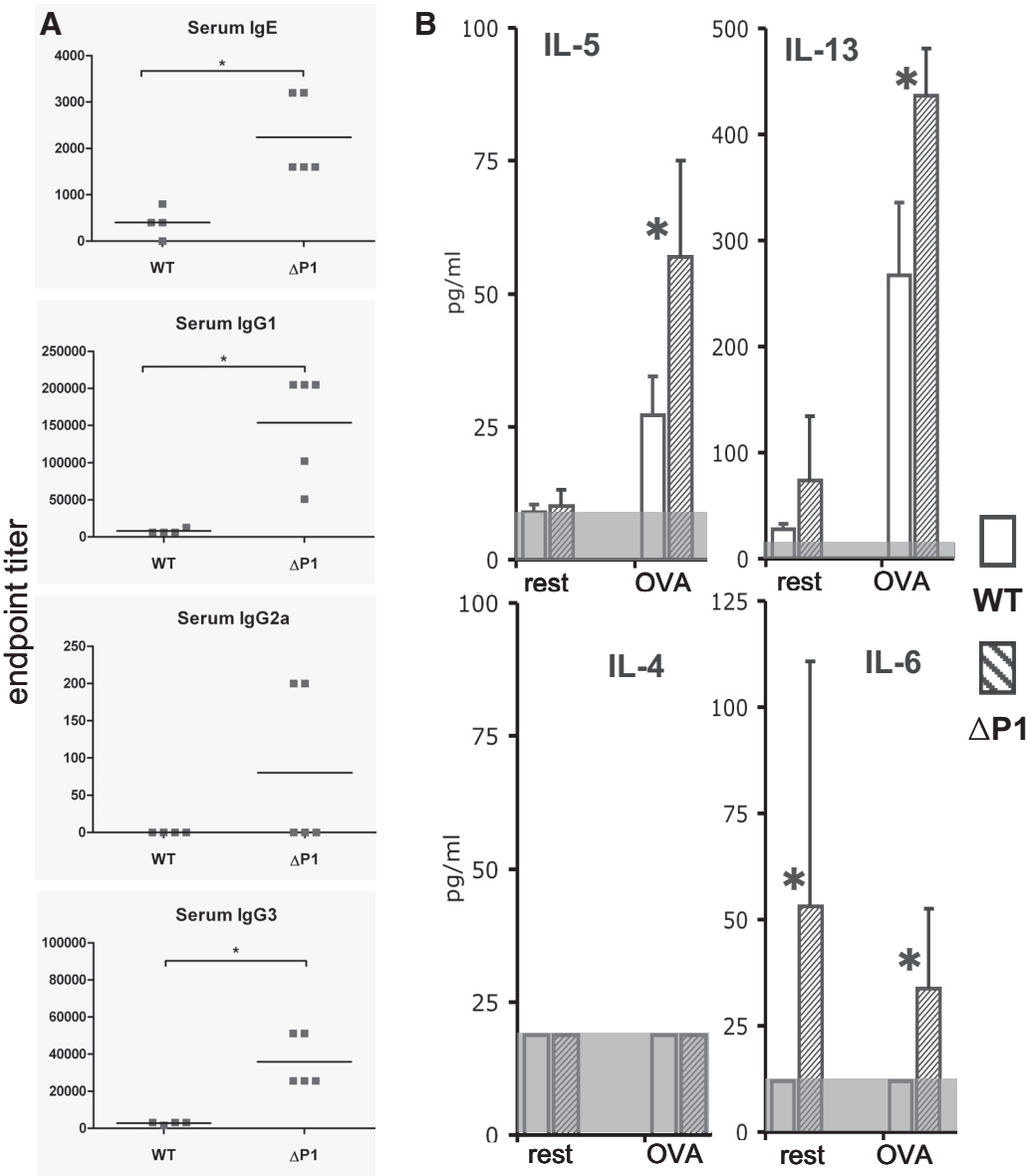


Figure 4. Serum Ig and cytokine profiles of OVA-challenged wild-type and *Siat1*ΔP1 animals. Profiles of IgE, IgG1, IgG2a, and IgG3 (A) and IL-4, IL-5, IL-6, and IL-13 (B) in sera of animals undergoing OVA-provoked allergic airway were determined as outlined in Materials and Methods. End-point titer for corresponding serum IgE, IgG1, IgG2a, and IgG3 was zero in resting (rest) animals in all cases (data not shown). For cytokine assays (B), shaded areas show the lower assayable limit for each cytokine. $n = 4$ for all three genotypes; *, indicates statistical significance of $P < 0.05$.

when compared with wild-type animals, consistent with the proinflammatory tendencies associated with ST6Gal-1 deficiency. OVA challenge did not alter serum IL-6 levels significantly in the *Siat1*ΔP1 mice.

Elevated eosinophil counts associated with increased eosinophil precursors but not extended longevity

Increased eosinophil production and extended longevity of the eosinophils are two mechanisms that may contribute to the more severe pulmonary eosinophilic inflammation in the ST6Gal-deficient animals. To ascertain if the elevated pulmonary eosinophil count is the result of delayed cell death, eosinophils from the BALF of OVA-provoked wild-type and *Siat1*ΔP1 mice were subjected to Annexin-PI analysis to determine the proportion of dying cells. As summarized in **Figure 5A**, the percentage of Annexin-V^{pos}-PI^{neg} cells and the Annexin-V^{pos}-PI^{pos} cells were identical in *Siat1*ΔP1 cells and wild-type cell populations, and roughly one-third of the BALF eosinophils showed Annexin-V reactivity.

As a comparative gauge for the eosinopoietic capacity between wild-type and *Siat1*ΔP1 mice, we measured bone marrow eosinophil progenitor numbers by CFU assays in the presence of IL-5 (CFU-eo) [29, 30]. The results, shown in Figure 5B, demonstrated statistically significant elevation of CFU-eo in marrow of *Siat1*ΔP1 compared with wild-type animals, suggesting a more robust eosinopoietic capacity that is contributing to more severe pulmonary eosinophilia in these animals upon allergen provocation.

Suppression of ST6Gal-1 in liver and in systemic circulation during acute pulmonary allergic inflammation

We have shown that animals with hampered hepatic production of the ST6Gal-1 have excessive eosinophil and neutro-

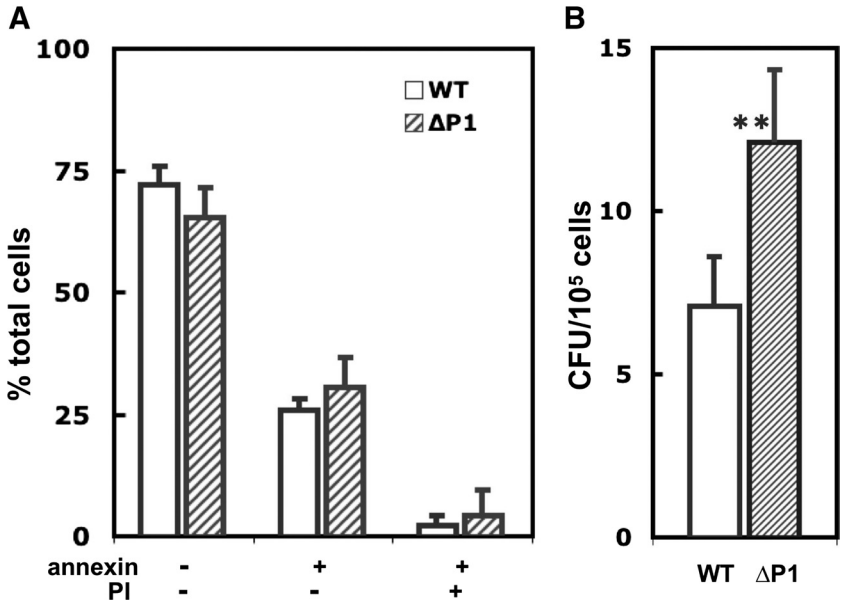


Figure 5. Apoptotic profiles of BAL eosinophils (A) and estimation of eosinophil precursor level cells in bone marrow at baseline (B). Cells from BAL of wild-type (open bar, $n=5$) and *Siat1*ΔP1 (hatched bars, $n=5$) were assayed for viability as described in Materials and Methods. In FACS analysis, granulocytes were gated based on their forward-scatter and side-scatter, and the percentage of different fractions in the gated region was measured (A). Colony-forming cell assay was performed on bone marrow cells as outlined in Materials and Methods. Colonies were counted, and total CFU in 10^5-nucleated bone marrow cells was calculated for WT (open bar, $n=7$) and *Siat1*ΔP1 (hatched bar, $n=7$). **, Indicates statistical significance between WT and *Siat1*ΔP1, $P < 0.0003$ (B).

philic response to acute inflammatory challenges, and we have hypothesized that liver-derived ST6Gal-1 can inversely regulate the magnitude of the inflammatory response. This hypothesis predicts that wild-type animals depress their endogenous liver ST6Gal-1 production when required to undergo acute pulmonary inflammation.

Much of the ST6Gal-1 in systemic circulation originates from the liver. Therefore, circulatory ST6Gal-1 activity can be an accurate monitor of hepatic ST6Gal-1 levels. As shown clearly in **Figure 6A**, wild-type animals, mounting an allergen-dependent airway response, had a nearly 70% decrease in circulatory ST6Gal-1 activity when compared with the blood of wild-type animals at baseline. Depressed circulatory ST6Gal-1 was observed in OVA and ABPA models of allergic airway. In contrast to wild-type animals, *Siat1*ΔP1 animals had the expected low sera ST6Gal-1 levels at baseline, and the levels did not change strikingly or significantly upon allergen-provoked inflammation.

To demonstrate directly depression of hepatic ST6Gal-1 expression, livers were harvested from animals at baseline or during allergen-provoked acute airway inflammation using the OVA or ABPS model. As shown in Figure 6B, wild-type animals suppressed their hepatic ST6Gal-1 mRNA levels by ~50% during allergen provocation. The *Siat1*ΔP1 mice, with an inactivated he-

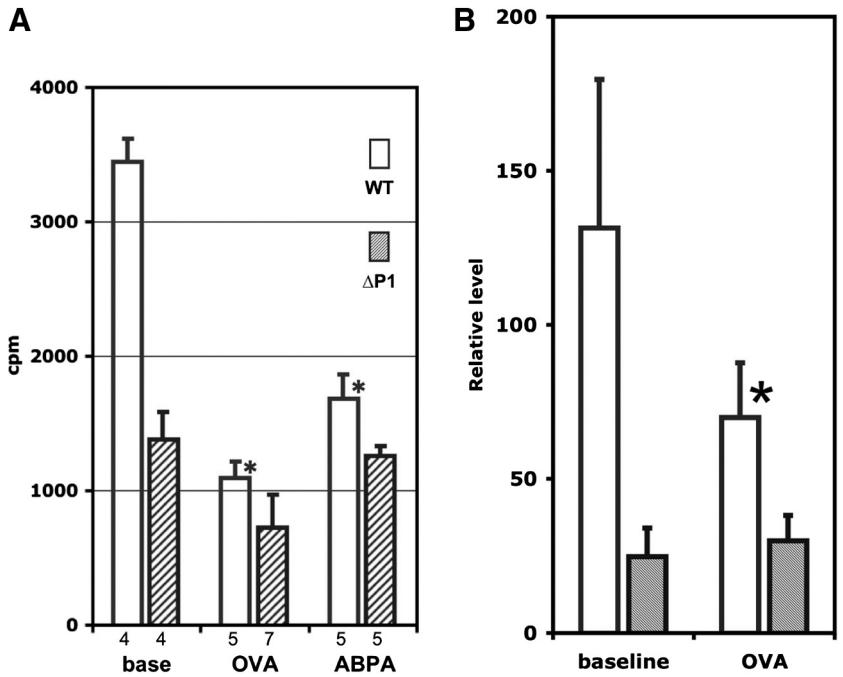


Figure 6. ST6Gal-1 profiles of animals undergoing OVA or ABPA models of allergic pulmonary inflammation. (A) Serum sialyltransferase activity profiles: Serum was harvested from wild-type and *Siat1*ΔP1 mice at rest (base) or undergoing OVA or ABPA protocols of allergic airway inflammation and tested for sialyltransferase activity as described in Materials and Methods. Shown is the ^{3}[H] incorporation into the synthetic acceptor substrate from 30 Ci/mmol CMP-^{3}[H]NeuNAc by 10 μl serum after 2 h incubation at 37°C. The numbers immediately beneath the abscissa indicate the number of animals comprising each data bar. Statistical significance is reached ($P<0.01$) for wild-type undergoing OVA or ABPA when compared with baseline (*) and also for the difference between wild-type and *Siat1*ΔP1 upon OVA provocation. (B) Real-time RT-PCR analysis of liver ST6Gal-1 mRNA in wild-type and *Siat1*ΔP1 mice at rest (baseline) or undergoing the OVA protocol. Open bars represent wild-type ($n=3$), and hatched or shaded bars represent *Siat1*ΔP1 mice ($n=3$). Statistical significance was reached ($P< 0.05$) for wild-type upon OVA provocation compared with baseline.

patic promoter P1, had low hepatic ST6Gal-1 mRNA even at baseline. The level of hepatic ST6Gal-1 mRNA was not changed in *Siat1*ΔP1 animals after airway provocation with allergen.

DISCUSSION

Previously, we have provided the first in vivo evidence for a role of ST6Gal-1 in acute neutrophilic inflammation and in regulating replenishment of neutrophil numbers [10]. The current study demonstrates that ST6Gal-1 may also serve as a regulator in the maintenance of eosinophil numbers. To pursue this hypothesis, we first examined the peritoneum, a tissue regarded as an in vivo reservoir for eosinophils [31]. We showed that unstimulated ST6Gal-1-deficient animals have a twofold increase in the peritoneal eosinophil population compared with wild-type mice. i.p. thioglycollate elicitation augmented the concentration and proportion of peritoneal eosinophils significantly in ST6Gal-1-deficient versus wild-type mice, and mice with ST6Gal-1 deficiency also developed more severe acute pulmonary allergic inflammation, dominated by excess airway eosinophils. The severity of allergic inflammation was similar between *Siat1*ΔP1 and *Siat1* null mice in the OVA and *Aspergillus* allergic models, indicating a role for the P1 promoter in regulating not only neutrophilic but also eosinophilic inflammation. Although it is clear that ST6Gal-1 deficiency resulted in overproduction of inflammatory leukocytes during inflammation, baseline circulatory blood leukocyte counts were surprisingly within normal range despite the ST6Gal-1-deficient animals having more myeloid progenitors in the bone marrow. The lack of an overt phenotype in the absence of challenge probably reflects additional compensatory mechanisms for baseline homeostasis. However, during an inflammatory response, ST6Gal-1 contributes by fine-tuning the rate of inflammatory cell production. It is noteworthy that ablation of Siglec-F, a sialic acid-binding receptor restricted to eosinophils, also generated a similar phenotype of increased lung eosinophil infiltration in the allergen-challenge model [25]. Siglec-F is a CD33rSiglec, expressed not only on mature, circulating mouse eosinophils but also on some myeloid precursors in the bone marrow [32, 33]. However, important differences exist between the two systems. The excess airway eosinophilia in the Siglec-F null model may be explained, at least in part, by delayed resolution of lung eosinophilia and reduced peribronchial cell apoptosis. In contrast, we could not demonstrate altered apoptosis in our ST6Gal-1-deficient models. Rather, the exaggerated lung eosinophilia in ST6Gal-1-deficient mice is apparently driven by an enhanced ability to produce eosinophils, an idea that is supported by the finding of greater eosinophil progenitor numbers in the bone marrow of these animals. The idea of elevated capacity for inflammatory cell production is bolstered further by our previous observation that *Siat1*ΔP1 mice harbor greater numbers of proliferative level myeloid cells in the bone marrow of *Siat1*ΔP1 compared with wild-type animals [10]. Moreover, the α2,6-sialyl structures constructed by ST6Gal-1 are not the preferred ligand for Siglec-F, which has shown a binding preference for α2,3-linked sialic acids [33, 34]. The data strongly suggest multiple mechanisms for sialic acid ligands in governing eosinophilic responses and that the phenotypes generated by ST6Gal-1 deficiency and Siglec-F deficiency result from separate pathways regulating innate inflammatory cell homeostasis.

Among the six known ST6Gal-1 gene promoters, ablation of the P1 promoter alone was sufficient to achieve the magnitude of an eosinophilic response equivalent to that observed in the completely ST6Gal-1 null mouse. These observations further bolster the idea of ST6Gal-1 having multiple physiologic functions, and the synthesis of the ST6Gal-1 for the different roles is regulated by the six known promoters of the ST6Gal-1 gene. The precise mechanism through which ST6Gal-1 drives excess Th1 and Th2 inflammation is currently under investigation. P1 use is apparently restricted to the liver, where it mediates elevated hepatic ST6Gal-1 expression during the acute-phase response [35–37]. P1-mediated ST6Gal-1 mRNA was not detected in wild-type bone marrow cells or in granulocyte populations, despite the highly sensitive RT-PCR approaches that were used [10]. A role for liver-produced ST6Gal-1 in this process is supported further by our observation that wild-type animals depressed their liver ST6Gal-1 mRNA level and the release of ST6Gal-1 into system circulation while experiencing acute page inflammation. Taken together, our data indicate that the pool of ST6Gal-1 produced by the P1 promoter is largely responsible in regulating neutrophilic and eosinophilic inflammation. We have focused on the *Siat1*ΔP1 mouse, as the P1 promoter lesion in the *Siat1*ΔP1 mouse was sufficient to reproduce the full extent of excess eosinophilic inflammation observed in the globally ST6Gal-1 null mouse.

Although it is known that the *Siat1*ΔP1 mouse [13] does not share the attenuated humoral response phenotype of the *Siat1* null animal [5], the marked elevation of all Igs in the *Siat1*ΔP1 compared with the wild-type mouse upon OVA challenge was unexpected. A number of scenarios may explain the more robust cytokine antibody response in the *Siat1*ΔP1 mouse. First, allergen-provoked *Siat1*ΔP1 mice may have increased recruitment of signaling cells, as reflected in the generally more severe mixed-type pulmonary inflammation. Increased recovery of DCs and macrophages in the BALF was also observed (Fig. 3). Increased macrophage recruitment by the *Siat1*ΔP1 mouse was also noted in the thioglycollate model of peritonitis [10]. A second possibility is that the DCs in the ST6Gal-1-deficient background may be more active and have better ability to present antigen. The state of cell-surface sialylation of DCs is already known to contribute to the development of the Th1 proinflammatory response, and enzymatic desialylation of DC surface increased gene expression of specific cytokines and induced a higher proliferative response of T lymphocytes [38, 39]. A third possibility is that DCs in a ST6Gal-1-deficient environment may traffic more efficiently from the lung to draining lymph nodes to more effectively stimulate a robust antibody response. Indeed, ST6Gal-1-mediated sialylation of β1 integrins has been shown to alter integrin-mediated cell migration and cell-adhesive properties of tumor cells and macrophage [40–43]. Whatever the mechanistic explanation, the robust overall response upon allergen challenge strongly suggests that the relevance of the pool of ST6Gal-1 generated from the P1-mediated transcription of the ST6Gal-1 gene extends beyond regulation of inflamma-

tory cell numbers, and it further predicts more severe pathologic consequences for the *Siat1*ΔP1 animals. This hypothesis is currently under investigation using chronic inflammation models.

ST6Gal-1, like other glycosyltransferases, is synthesized as a type II transmembrane protein, with the C terminus catalytic domain preceded by a stem region, a membrane-spanning region, and the cytosolic N terminus [44]. In the native form, ST6Gal-1 is localized in the Golgi, where it participates in the assembly of sialyl-glycoconjugates transiting the secretory apparatus. Thus, based on the canonical understanding of the process of glycosylation, the effect of liver ST6Gal-1 on inflammation should be mediated via the serum sialyl-glycoproteins of hepatic origin that it constructs. Many of the serum glycoproteins of hepatic origin, such as haptoglobin and α1-acid glycoprotein, are not only heavily modified by α2,6-sialic acid structures constructed by ST6Gal-1 but also have known roles in regulation of host immunity and inflammation [45–47]. However, this mechanism is contra-indicated by the earlier observation that the pattern of serum glycoprotein sialylation was unchanged in the *Siat1*ΔP1 animals [13]. Nevertheless, the possibility of an altered *Siat1*ΔP1 serum glycoprotein, present only as a minor component of the overall serum glycoprotein milieu, has yet to be ruled out definitively. An alternative but unconventional explanation is that inflammatory regulation is mediated, not by the secreted serum glycoproteins but directly by the ST6Gal-1 released into circulation. The intact catalytic domain of ST6Gal-1 can be liberated proteolytically by the β-site amyloid precursor protein-cleaving enzyme 1 [48] and released into systemic circulation. It has been generally regarded that the circulatory ST6Gal-1 pool is a byproduct from metabolic inefficiency and without physiologic function, although early studies have pointed to an association between serum ST6Gal-1 levels and inflammatory status [37, 49]. Here, we showed that acute pulmonary inflammation is also accompanied by lowered levels of hepatic ST6Gal-1 mRNA and depression of circulatory levels of ST6Gal-1 (Fig. 6). The idea of a novel, noncanonical pathway for ST6Gal-1, i.e., the ability of circulating sialyltransferases to act at sites distal from their biosynthetic origin, is bolstered by our recent observations that hematopoietic stem and progenitor cells can recruit extrinsically supplied ST6Gal-1 in constructing their cell surface sialylglycans, and ex vivo growth of bone marrow cells in semi-solid media in the presence of IL-3 and G-CSF can be repressed significantly upon addition of recombinant ST6Gal-1 (M. Nasirikenari, Mark B. Jones, J. T. Y. Lau, unpublished observations). The existence of such a noncanonical extrinsic pathway for ST6Gal-1 action, however, is beyond the scope of this present work and will be addressed in a separate manuscript. Whatever the precise mechanism of ST6Gal-1 action, the current data point to the contribution of hepatically derived ST6Gal-1 to the development and management of inflammatory numbers. This hypothesis makes the prediction that animals overexpressing hepatic ST6Gal-1 will have attenuated inflammatory cell numbers upon challenge, which is currently being tested by the construction of a transgenic mouse overexpressing hepatic ST6Gal-1.

ACKNOWLEDGMENTS

This work was supported by NIH AI056082 (J. T. Y. L.) and NIH AI078429. This research used core facilities supported in part by Roswell Park Cancer Institute's National Cancer Institute-funded Cancer Center support grant CA16056.

REFERENCES

1. Frommhold, D., Ludwig, A., Bixel, M. G., Zarbock, A., Babushkina, I., Weissinger, M., Cauwenberghs, S., Ellies, L. G., Marth, J. D., Beck-Sickinger, A. G., Sixt, M., Lange-Sperandio, B., Zernecke, A., Brandt, E., Weber, C., Vestweber, D., Ley, K., Sperandio, M. (2008) Sialyltransferase ST3Gal-IV controls CXCR2-mediated firm leukocyte arrest during inflammation. *J. Exp. Med.* **205,** 1435–1446.
2. Ellies, L. G., Ditto, D., Levy, G. G., Wahrenbrock, M., Ginsburg, D., Varki, A., Le, D. T., Marth, J. D. (2002) Sialyltransferase ST3Gal-IV operates as a dominant modifier of hemostasis by concealing asialoglycoprotein receptor ligands. *Proc. Natl. Acad. Sci. USA* **99,** 10042–10047.
3. Grewal, P. K., Uchiyama, S., Ditto, D., Varki, N., Le, D. T., Nizet, V., Marth, J. D. (2008) The Ashwell receptor mitigates the lethal coagulopathy of sepsis. *Nat. Med.* **14,** 648–655.
4. Priatel, J. J., Chui, D., Hiraoka, N., Simmons, C. J., Richardson, K. B., Page, D. M., Fukuda, M., Varki, N. M., Marth, J. D. (2000) The ST3Gal-I sialyltransferase controls CD8+ T lymphocyte homeostasis by modulating O-glycan biosynthesis. *Immunity* **12,** 273–283.
5. Hennet, T., Chui, D., Paulson, J. C., Marth, J. D. (1998) Immune regulation by the ST6Gal sialyltransferase. *Proc. Natl. Acad. Sci. USA* **95,** 4504–4509.
6. Haas, K. M., Sen, S., Sanford, I. G., Miller, A. S., Poe, J. C., Tedder, T. F. (2006) CD22 ligand binding regulates normal and malignant B lymphocyte survival in vivo. *J. Immunol.* **177,** 3063–3073.
7. Ghosh, S., Bandulet, C., Nitschke, L. (2006) Regulation of B cell development and B cell signaling by CD22 and its ligands {α}2,6-linked sialic acids. *Int. Immunol.* **18,** 603–611.
8. Collins, B. E., Smith, B. A., Bengtson, P., Paulson, J. C. (2006) Ablation of CD22 in ligand-deficient mice restores B cell receptor signaling. *Nat. Immunol.* **7,** 199–206.
9. Seales, E. C., Shaikh, F. M., Woodard-Grice, A. V., Aggarwal, P., McBrayer, A. C., Hennessy, K. M., Bellis, S. L. (2005) A protein kinase C/Ras/ERK signaling pathway activates myeloid fibronectin receptors by altering β1 integrin sialylation. *J. Biol. Chem.* **280,** 37610–37615.
10. Nasirikenari, M., Segal, B. H., Ostberg, J. R., Urbasic, A., Lau, J. T. (2006) Altered granulopoietic profile and exaggerated acute neutrophilic inflammation in mice with targeted deficiency in the sialyltransferase, ST6Gal I. *Blood* **108,** 3397–3405.
11. Dalziel, M., Huang, R. A., Dall'Olio, F., Morris, J. R., Taylor-Papadimitriou, J., Lau, J. T. Y. (2001) Mouse ST6Gal sialyltransferase gene expression during mammary gland lactation. *Glycobiology* **11,** 407–412.
12. Wuensch, S. A., Huang, R. A., Ewing, J., Liang, X. L., Lau, J. T. Y. (2000) Murine B cell differentiation is accompanied by programmed expression of multiple novel β-galactoside α 2,6-sialyltransferase mRNA forms. *Glycobiology* **10,** 67–75.
13. Appenheimer, M. M., Huang, R. Y., Chandrasekaran, E. V., Dalziel, M., Hu, Y. P., Soloway, P. D., Wuensch, S. A., Matta, K. L., Lau, J. T. (2003) Biologic contribution of P1 promoter-mediated expression of ST6Gal I sialyltransferase. *Glycobiology* **13,** 591–600.
14. Huber, H. L., Koessler, K. K. (1922) The pathology of bronchial asthma. *Arch. Intern. Med.* **30,** 689–760.
15. Stelmach, I., Majak, P., Grzelewski, T., Jerzynska, J., Juralowicz, D., Stelmach, W., Borzecka-Podsiadlowicz, M., Korzeniewska, A., Kuna, P. (2004) The ECP/Eo count ratio in children with asthma. *J. Asthma* **41,** 539–546.
16. Berry, M. A., Shaw, D. E., Green, R. H., Brightling, C. E., Wardlaw, A. J., Pavord, I. D. (2005) The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin. Exp. Allergy* **35,** 1175–1179.
17. Venge, P. (2004) Monitoring the allergic inflammation. *Allergy* **59,** 26–32.
18. Lee, J. J., Dimina, D., Macias, M. P., Ochkur, S. I., McGarry, M. P., O'Neill, K. R., Protheroe, C., Pero, R., Nguyen, T., Cormier, S. A., Lenkiewicz, E., Colbert, D., Rinaldi, L., Ackerman, S. J., Irvin, C. G., Lee, N. A. (2004) Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* **305,** 1773–1776.
19. Humbles, A. A., Lloyd, C. M., McMillan, S. J., Friend, D. S., Xanthou, G., McKenna, E. E., Ghiran, S., Gerard, N. P., Yu, C., Orkin, S. H., Gerard, C. (2004) A critical role for eosinophils in pages remodeling. *Science* **305,** 1776–1779.
20. Jacobsen, E. A., Ochkur, S. I., Lee, N. A., Lee, J. J. (2007) Eosinophils and asthma. *Curr. Allergy Asthma Rep.* **7,** 18–26.
21. Ochkur, S. I., Jacobsen, E. A., Protheroe, C. A., Biechele, T. L., Pero, R. S., McGarry, M. P., Wang, H., O'Neill, K. R., Colbert, D. C., Colby, T. V., Shen, H., Blackburn, M. R., Irvin, C. C., Lee, J. J., Lee, N. A. (2007) Coexpression of IL-5 and eotaxin-2 in mice creates an eosinophil-

dependent model of respiratory inflammation with characteristics of severe asthma. *J. Immunol.* **178,** 7879–7889.
22. Henderson Jr., W. R., Chiang, G. K., Tien, Y. T., Chi, E. Y. (2006) Reversal of allergen-induced airway remodeling by CysLT1 receptor blockade. *Am. J. Respir. Crit. Care Med.* **173,** 718–728.
23. Kanehiro, A., Takeda, K., Joetham, A., Tomkinson, A., Ikemura, T., Irvin, C. G., Gelfand, E. W. (2000) Timing of administration of anti-VLA-4 differentiates airway hyperresponsiveness in the central and peripheral airways in mice. *Am. J. Respir. Crit. Care Med.* **162,** 1132–1139.
24. Rothenberg, M. E., Hogan, S. P. (2006) The eosinophil. *Annu. Rev. Immunol.* **24,** 147–174.
25. Zhang, M., Angata, T., Cho, J. Y., Miller, M., Broide, D. H., Varki, A. (2007) Defining the in vivo function of Siglec-F, a CD33-related Siglec expressed on mouse eosinophils. *Blood* **109,** 4280–4287.
26. Hogaboam, C. M., Takahashi, K., Ezekowitz, R. A., Kunkel, S. L., Schuh, J. M. (2004) Mannose-binding lectin deficiency alters the development of fungal asthma: effects on airway response, inflammation, and cytokine profile. *J. Leukoc. Biol.* **75,** 805–814.
27. Appenheimer, M. M., Huang, R. A., Chandrasekaran, E. V., Dalziel, M., Hu, Y. P., Soloway, P. D., Wuensch, S. A., Matta, K. L., Lau, J. T. Y. (2003) Biologic contribution of P1 promoter-mediated expression of ST6Gal I sialyltransferase. *Glycobiology* **13,** 591–600.
28. Chandrasekaran, E. V., Jain, R. K., Larsen, R. D., Wlasichuk, K., Matta, K. L. (1995) Selectin ligands and tumor-associated carbohydrate structures: specificities of α 2,3-sialyltransferases in the assembly of 3′-sialyl-6-sialyl/sulfo Lewis a and x, 3′-sialyl-6′-sulfo Lewis x, and 3′-sialyl-6-sialyl/sulfo blood group T-hapten. *Biochemistry* **34,** 2925–2936.
29. Hogan, M. B., Weissman, D. N., Hubbs, A. F., Gibson, L. F., Piktel, D., Landreth, K. S. (2003) Regulation of eosinophilopoiesis in a murine model of asthma. *J. Immunol.* **171,** 2644–2651.
30. Inman, M. D., Ellis, R., Wattie, J., Denburg, J. A., O'Byrne, P. M. (1999) Allergen-induced increase in airway responsiveness, airway eosinophilia, and bone-marrow eosinophil progenitors in mice. *Am. J. Respir. Cell Mol. Biol.* **21,** 473–479.
31. Ohnmacht, C., Pullner, A., van Rooijen, N., Voehringer, D. (2007) Analysis of eosinophil turnover in vivo reveals their active recruitment to and prolonged survival in the peritoneal cavity. *J. Immunol.* **179,** 4766–4774.
32. Zhang, J. Q., Biedermann, B., Nitschke, L., Crocker, P. R. (2004) The murine inhibitory receptor mSiglec-E is expressed broadly on cells of the innate immune system whereas mSiglec-F is restricted to eosinophils. *Eur. J. Immunol.* **34,** 1175–1184.
33. Angata, T., Hingorani, R., Varki, N. M., Varki, A. (2001) Cloning and characterization of a novel mouse Siglec, mSiglec-F: differential evolution of the mouse and human (CD33) Siglec-3-related gene clusters. *J. Biol. Chem.* **276,** 45128–45136.
34. Tateno, H., Crocker, P. R., Paulson, J. C. (2005) Mouse Siglec-F and human Siglec-8 are functionally convergent paralogs that are selectively expressed on eosinophils and recognize 6′-sulfo-sialyl Lewis X as a preferred glycan ligand. *Glycobiology* **15,** 1125–1135.
35. Wang, X. C., Smith, T. J., Lau, J. T. Y. (1990) Transcriptional regulation of the liver β-galactoside α2,6-sialyltransferase by glucocorticoids. *J. Biol. Chem.* **265,** 17849–17853.
36. Hu, Y. P., Dalziel, M., Lau, J. T. Y. (1997) Murine hepatic β-galactoside α 2,6-sialyltransferase gene expression involves usage of a novel upstream exon region. *Glycoconj. J.* **14,** 407–411.
37. Jamieson, J. C., Lammers, G., Janzen, R., Woloski, B. M. (1987) The acute phase response to inflammation: the role of monokines in changes in liver glycoproteins and enzymes of glycoprotein metabolism. *Comp. Biochem. Physiol. B* **87,** 11–15.
38. Videira, P. A., Amado, I. F., Crespo, H. J., Alguero, M. C., Dall'Olio, F., Cabral, M. G., Trindade, H. (2008) Surface α 2-3- and α 2-6-sialylation of human monocytes and derived dendritic cells and its influence on endocytosis. *Glycoconj. J.* **25,** 259–268.
39. Crespo, H. J., Cabral, M. G., Teixeira, A. V., Lau, J. T. Y., Trindade, H., Videira, P. A. (2009) Effect of sialic acid loss on dendritic cell maturation. *Immunology* **128** (Suppl. 1), e621–e631.
40. Shaikh, F. M., Seales, E. C., Clem, W. C., Hennessy, K. M., Zhuo, Y., Bellis, S. L. (2008) Tumor cell migration and invasion are regulated by expression of variant integrin glycoforms. *Exp. Cell Res.* **314,** 2941–2950.
41. Christie, D. R., Shaikh, F. M., Lucas IV, J. A., Lucas III, J. A., Bellis, S. L. (2008) ST6Gal-I expression in ovarian cancer cells promotes an invasive phenotype by altering integrin glycosylation and function. *J. Ovarian Res.* **1,** 3.
42. Seales, E. C., Jurado, G. A., Brunson, B. A., Wakefield, J. K., Frost, A. R., Bellis, S. L. (2005) Hypersialylation of β1 integrins, observed in colon adenocarcinoma, may contribute to cancer progression by up-regulating cell motility. *Cancer Res.* **65,** 4645–4652.
43. Bellis, S. L. (2004) Variant glycosylation: an underappreciated regulatory mechanism for β1 integrins. *Biochim. Biophys. Acta* **1663,** 52–60.
44. Weinstein, J., Lee, E. U., McEntee, K., Lai, P. H., Paulson, J. C. (1987) Primary structure of β-galactoside α2,6-sialyltransferase. Conversion of membrane-bound enzyme to soluble forms by cleavage of the NH 2-terminal signal anchor. *J. Biol. Chem.* **262,** 17735–17743.
45. Huntoon, K. M., Wang, Y., Eppolito, C. A., Barbour, K. W., Berger, F. G., Shrikant, P. A., Baumann, H. (2008) The acute phase protein haptoglobin regulates host immunity. *J. Leukoc. Biol.* **84,** 170–181.
46. Hochepied, T., Berger, F. G., Baumann, H., Libert, C. (2003) α(1)-Acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties. *Cytokine Growth Factor Rev.* **14,** 25–34.
47. Hochepied, T., Wullaert, A., Berger, F. G., Baumann, H., Brouckaert, P., Steidler, L., Libert, C. (2002) Overexpression of α(1)-acid glycoprotein in transgenic mice leads to sensitization to acute colitis. *Gut* **51,** 398–404.
48. Kitazume, S., Nakagawa, K., Oka, R., Tachida, Y., Ogawa, K., Luo, Y., Citron, M., Shitara, H., Taya, C., Yonekawa, H., Paulson, J.C., Miyoshi, E., Taniguchi, N., Hashimoto, Y. (2005) In vivo cleavage of α2,6-sialyltransferase by Alzheimer's β-secretase. *J. Biol. Chem.* **280,** 8589–8595.
49. Lammers, G., Jamieson, J. C. (1986) Studies on the effect of experimental inflammation on sialyltransferase in the mouse and guinea pig. *Comp. Biochem. Physiol. B* **84,** 181–187.

KEY WORDS:
sialic acid · sialyltransferase · myelopoiesis · Th2 · allergic airway