Siglec-F Inhibition Reduces Esophageal Eosinophilia and Angiogenesis in a Mouse Model of Eosinophilic Esophagitis

*Eitan Rubinstein, †Jae Youn Cho, †Peter Rosenthal, †James Chao, †Marina Miller, †Alexa Pham, †Seema S. Aceves, †Ajit Varki, and †David H. Broide

ABSTRACT

Objectives: Eosinophilic esophagitis (EoE) is a disorder characterized histologically by tissue eosinophilia. Sialic acid–binding immunoglobulin-like lectin (Siglec-F) is a receptor highly expressed on mouse eosinophils and mediates eosinophilic apoptosis. We investigated whether administration of an anti-Siglec-F Ab would reduce esophageal eosinophilic inflammation and remodeling in a mouse model of egg ovalbumin (OVA)–induced EoE.

Subjects and Methods: Three groups of mice were studied (no OVA, OVA + anti-Siglec-F Ab, and OVA + isotype control Ab). Mice were sensitized intraperitoneally and then challenged chronically with intraesophageal OVA. Levels of esophageal eosinophils and features of remodeling (angiogenesis, vascular endothelial growth factor expression, deposition of fibronectin, basal zone hyperplasia, and fibrosis) were quantitated by immunohistochemistry and image analysis.

Results: Administration of an anti-Siglec-F Ab to OVA-challenged mice significantly reduced levels of esophageal eosinophils, down to levels noted in non-OVA-challenged mice. The anti-Siglec-F Ab also reduced features of OVA-induced remodeling, including angiogenesis, basal zone hyperplasia, and fibronectin deposition. The reduced angiogenesis in anti-Siglec-F Ab-treated mice was associated with reduced numbers of vascular endothelial growth factor–positive cells in the esophagus. The anti-Siglec-F antibody did not significantly reduce esophageal fibrosis as assessed by trichrome staining.

Conclusions: Administration of an anti-Siglec-F antibody significantly decreased the number of eosinophils in the esophagus in a mouse model of OVA-induced EoE. The reduction in eosinophilic inflammation was associated with a significant decrease in levels of angiogenesis, deposition of fibronectin, and basal zone hyperplasia. Studies in this preclinical model of EoE suggest that Siglec-F (and its human paralog Siglec-8) may be novel therapeutic targets to reduce eosinophilic inflammation in EoE.

Key Words: basal zone hyperplasia, eosinophil, fibronectin, Siglec-8, vascular endothelial cell growth factor

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Eosinophilic esophagitis (EoE) is a clinicopathologic disorder characterized histologically by a dense esophageal eosinophilia (>15 eos/Hpf) (1–3). In addition to the prominent levels of eosinophilic inflammation, features of remodeling have been noted in EoE, including basal zone hyperplasia, angiogenesis, deposition of extracellular matrix components such as fibronectin, and fibrosis. The eosinophil likely contributes to remodeling in EoE through expression of cytokines such as transforming growth factor-β1 (TGF-β1) (4) and vascular endothelial growth factor (VEGF) (5). The importance of interleukin-5 (IL-5) and eosinophils to esophageal remodeling in EoE is indeed suggested from mouse and human studies (6). In a mouse model of EoE induced by intranasal administration of *Aspergillus fumigatus*, IL-5-deficient mice had significantly less esophageal eosinophilic inflammation as well as basal layer thickness and fibrosis compared to wild-type (WT) mice (6). Similarly, in a placebo-controlled study of anti-IL-5 in patients with EoE, the anti-IL-5-treated group had significantly reduced esophageal eosinophils as well as levels of the extracellular matrix protein tenascin and the growth factor TGF-β1 (7).

Targeting IL-5 (an eosinophil growth factor) is one mechanism of reducing eosinophilic inflammation in EoE; another potential strategy would be to target sialic acid–binding immunoglobulin-like lectin (Siglec)-8 (or its murine isofunctional paralog Siglec-F) (8,9), a receptor highly expressed on eosinophils and which mediates apoptosis and clearance of eosinophils (10,11). We have developed a mouse model of egg (ie, OVA [ovalbumin])-induced esophageal eosinophilia associated with esophageal remodeling, which has allowed us to investigate whether targeting Siglec-F would reduce levels of eosinophilic inflammation and esophageal remodeling. Remodeling is the term that refers to structural changes in the esophagus in EoE (basal zone hyperplasia, angiogenesis, fibrosis) that may be the result of persistent inflammation and/or aberrant tissue repair mechanisms (2,6). Previous studies have demonstrated that antibody-mediated cross-linking of Siglec-F induces eosinophil apoptosis (12) and that administration of an anti-Siglec-F antibody (Ab) to mice reduces levels of eosinophilic inflammation in the lung (13), blood (13–15), and jejunum (14,15). Eosinophils express growth factors and mediators that may contribute to angiogenesis, deposition of extracellular matrix proteins, basal zone hyperplasia, and fibrosis. Thus, we hypothesized that targeting Siglec-F in a mouse model of EoE could induce eosinophil apoptosis, reduce the number of esophageal

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From the *Division of Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, University of California, San Diego, and the †Department of Medicine, University of California, San Diego.

Address correspondence and reprint requests to David Broide, MB, ChB, University of California, San Diego, Biomedical Sciences Building, Room 5090, 9500 Gilman Dr, La Jolla, CA 92093-0635 (e-mail: dbroide@ucsd.edu).

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eosinophils expressing these growth factors and mediators, and as a consequence, reduce levels of esophageal remodeling.

SUBJECTS AND METHODS

Oral OVA Allergen–induced Esophageal Eosinophilic Inflammation

Eight-week-old female BALB/c mice (8 mice per group unless otherwise noted; Charles River Laboratory, Wilmington, MA) were sensitized intraperitoneally (ip) on day 0 and 14 (50 μg of OVA adsorbed to 1 mg of aluminum hydroxide adjuvant in phosphate-buffered saline (PBS); Sigma-Aldrich, St Louis, MO) and challenged intraesophageally 3 times per week for 4 weeks with 10 mg OVA suspended in 100 μL PBS on days 28, 30, 32, 35, 37, 39, 42, 44, 46, 49, 51, and 53 (see protocol Fig. 1) (Cho et al, unpublished manuscript). OVA was administered through an intragastric feeding needle (20-gauge, 1.5-inch; Pepper and Sons, New Hyde Park, NY). Mice were sacrificed 24 hours after the last administration of intraesophageal OVA (day 54). Control BALB/c mice were neither sensitized nor challenged. The esophagus was removed in its entirety and fixed with 4% paraformaldehyde solution (Electron Microscopy Sciences, Hatfield, PA) for 24 hours, oriented, and embedded in 1% agarose (Invitrogen, Carlsbad, CA), and then sectioned (upper, middle and lower) and embedded in paraffin. Five-micron esophageal sections were then prepared from each layer and equivalent numbers of sections from each layer were included in every experiment for analysis. Results in each group are presented as a combined score of the 3 layers analyzed (upper, middle, lower).

Therapeutic Intervention With Anti-Siglec-F or Control Antibody

Different groups of OVA-challenged mice were pretreated with either an anti-Siglec-F or control Ab. The anti-Siglec-F Ab + OVA group (n = 8 mice unless otherwise noted) were administered 10 μg of a purified rat anti-mouse Siglec-F IgG2a antibody (BD Pharminogen, San Jose, CA) in 100 μL of PBS by intraperitoneal injection 1 hour before each of the 12 OVA intraesophageal challenges. The control Ab + OVA group (n = 8 mice unless otherwise noted) were administered 10 μg of a purified rat immunoglobulin G2a isotype control antibody (BD Pharminogen) in 100 μL PBS by intraperitoneal injection 1 hour before each of the 12 OVA intraesophageal challenges. The non-OVA control group received neither OVA nor an Ab. As previously reported in pilot studies, we demonstrated that this dose of anti-Siglec-F antibody was sufficient to bind all eosinophil Siglec-F in blood and bone marrow (13).

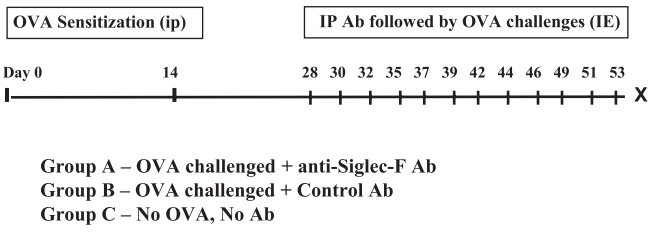

FIGURE 1. Experimental egg OVA EoE protocol. Mice were sensitized intraperitoneally (ip) on day 0 and day 14 and challenged intraesophageally (IE) on days 28, 30, 32, 35, 37, 39, 42, 44, 46, 49, 51, and 53. One hour before each OVA challenge, an anti-Siglec-F or isotype control antibody was administered ip. Mice were sacrificed 24 hours after last administration of intraesophageal OVA (day 54) and their esophagi were analyzed.

Quantitation of Major Basic Protein–positive Esophageal Eosinophils

Eosinophils were detected in esophageal tissue by immunohistochemistry using an anti-mouse major basic protein (MBP) antibody (provided by James Lee, PhD, Mayo Clinic, Scottsdale, AZ). Quantitation of the number of eosinophils was performed using a light microscope attached to an image-analysis system with the entire cross-section of the esophagus visualized. The area of the esophageal lamina propria (LP) analysis was outlined and this area was determined by the image analysis software (Image-Pro Plus; Media Cybernetics, Bethesda, MD). Results are expressed as the number of eosinophils per square millimeter of LP.

Peripheral Blood and Bone Marrow Eosinophil Quantification

Peripheral blood and bone marrow cell counts were performed on Wright-Giemsa-stained slides as previously described in this laboratory (12).

Effect of Anti-Siglec-F Antibody on Apoptosis

The number of TUNEL-positive cells (ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit; Chemicon, Temecula, CA), which were also MBP positive, were quantitated in bone marrow or esophagus in anti-Siglec-F Ab and control Ab-treated mice chronically challenged with oral OVA as previously described (13).

Angiogenesis and VEGF Quantitation

Blood vessels in esophageal tissue were identified by immunohistochemistry using a rat anti-mouse platelet endothelial cell adhesion molecule (PECAM) monoclonal antibody (BD Bioscience, San Jose, CA), which detects the blood vessel adhesion molecule PECAM, as previously described in this laboratory (16). To enhance the ability to detect new vessels, only those vessels $\leq$5 μm were counted as previously described in this laboratory (16). In addition we quantitated the number of VEGF-positive cells using an anti-VEGF primary Ab (Santa Cruz Biotechnology, Santa Cruz, CA). Results are expressed as the number of PECAM-1–positive vessels per square millimeter of LP, and the number of VEGF-positive cells per square millimeter of LP. In selected experiments we examined the relation between expression of PECAM and MBP with immunofluorescence microscopy as previously described (12) using the anti-PECAM Ab and anti-MBP Ab. The anti-PECAM Ab was detected with an HRP-labeled secondary Ab (alexa 488, green color), whereas the anti-MBP Ab was detected with a different HRP-labeled secondary Ab (alexa 546, red color). Cells coexpressing PECAM and MBP would have a merged yellow color.

Quantitation of TGF-β1, Fibronectin, and Fibrosis

Esophageal tissue sections were processed for immunohistochemistry using a primary mAb directed against either TGF-β1 (Santa Cruz Biotechnology) or fibronectin (Abcam, Cambridge, MA) as described above. Results are expressed as TGF-β1-positive

cells per square millimeter of LP and the area of fibronectin immunostaining area per area of LP ($\mu m^2/\mu m^2$).

The area of trichrome staining in paraffin-embedded esophagus was outlined and quantified using a light microscope attached to an image analysis system as previously described (6). Results are expressed as the area of trichrome staining per micron length of basement membrane.

Basal Zone Thickness

The epithelial basal zone thickness was assessed in esophageal sections stained with hematoxylin and eosin using a light microscope attached to an image-analysis system. The maximal thickness of the basal layer in each slide was recorded in microns.

Data Analysis

Results were compared by a Mann-Whitney test using a statistical software package (GraphPad Prism, San Diego, CA). P values < 0.05 were considered statistically significant. Results are presented as the mean $\pm$ SEM.

RESULTS

Anti-Siglec-F Antibody Reduces Esophageal Eosinophilia

The number of eosinophils in the esophageal LP increased significantly in the mice challenged with OVA compared with non-OVA–challenged mice (320 ± 61 vs 118 ± 36 eosinophils/mm^2; $P < 0.0001$) (Figs. 2 and 3A). In OVA-challenged mice, the administration of an anti-Siglec-F antibody significantly reduced the level of esophageal eosinophilia compared to OVA-challenged mice administered a control antibody (96 ± 11 vs 320 ± 61 eosinophils/mm^2; $P = 0.003$) (Figs. 2 and 3A). The anti-Siglec-F antibody reduced levels of eosinophils in the esophagus in OVA-challenged mice to levels similar to that observed in non-OVA–challenged mice (Figs. 2 and 3A).

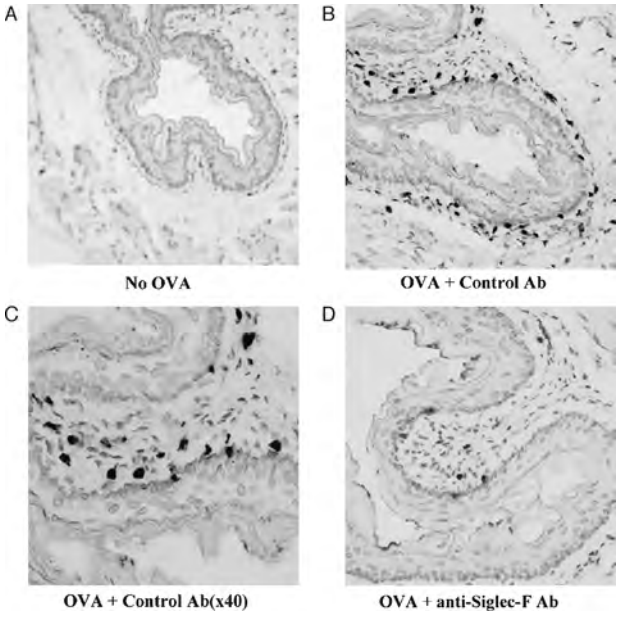

FIGURE 2. Eosinophils in the esophagus. Hematoxylin and anti-mouse major basic protein immunostain of esophagus. A, No OVA. B, OVA + control Ab. C, OVA + control Ab (40× magnification of panel B). D, OVA + anti-Siglec-F Ab.

Anti-Siglec-F Antibody Reduces Blood and Bone Marrow Eosinophils

The percentage of eosinophils in the peripheral blood was increased in mice challenged with OVA compared to non-OVA-challenged mice ($7.5\% \pm 1.1\%$ vs $4.6\% \pm 0.7\%$; $P = 0.01$) (Fig. 3B). In OVA-challenged mice, administration of an anti-Siglec-F antibody significantly reduced the levels of peripheral blood eosinophilia compared to OVA-challenged mice administered a control antibody ($4.8\% \pm 0.7\%$ vs $7.5\% \pm 1.1\%$; $P = 0.04$) (Fig. 3B) ($n = 16$ mice/group).

The number of eosinophils in the bone marrow also was increased in the mice challenged with OVA (and a control antibody) compared to non-OVA-challenged mice ($10.1\% \pm 0.8\%$ vs $5.9\% \pm 0.4\%$; $P = 0.0002$) (Fig. 3C). In OVA-challenged mice, administration of an anti-Siglec-F antibody significantly reduced the levels of bone marrow eosinophilia compared to OVA-challenged mice administered a control antibody ($4.9\% \pm 0.4\%$ vs $10.1\% \pm 0.8\%$; $P = 0.0002$) (Fig. 3C).

Effect of Anti-Siglec-F Antibody on Apoptosis

The number of TUNEL-positive/MBP-positive eosinophils in mice chronically challenged with oral OVA was significantly increased in the bone marrow of anti-Siglec-F Ab compared with control Ab-treated mice ($P = 0.02$) (Fig. 4). There was no difference in the number of apoptotic cells in the esophagus of anti-Siglec-F Ab compared with control Ab-treated mice (data not shown).

Effect of Anti-Siglec-F Antibody on Features of Esophageal Remodeling

Angiogenesis and VEGF Expression

Mice challenged with OVA developed a significant increase in the number of small blood vessels within the esophageal LP as quantitated by PECAM staining (186 ± 18 vs 29 ± 6 small blood vessels/mm^2; $P < 0.001$) (Figs. 5 and 6). Immunofluorescence microscopy of esophageal sections demonstrated that there was no overlap of PECAM-positive cells with MBP-positive cells (Fig. 6A–C).

Administration of an anti-Siglec-F Ab to OVA-challenged mice significantly reduced the number of small blood vessels within the esophageal LP (64 ± 7 vs 186 ± 18 small blood vessels/mm^2; $P < 0.001$) (Figs. 5 and 6D).

To determine whether the anti-Siglec-F antibody influenced levels of angiogenic cytokines, we quantitated the number of VEGF-positive cells within the LP. Mice challenged with OVA had a significantly increased number of VEGF-positive cells in the LP (67 ± 12 vs 18 ± 7 VEGF-positive cells/mm^2; $P = 0.004$) (Fig. 6E). Administration of an anti-Siglec-F Ab to OVA-challenged mice significantly reduced the number of VEGF-positive cells in the LP (28 ± 7 vs 67 ± 12 VEGF-positive cells/mm^2; $P = 0.02$) (Fig. 6E).

Extracellular Matrix Fibronectin, TGF-β1, and Fibrosis

Levels of fibronectin, an extracellular matrix protein, were significantly increased in the LP of mice administered OVA as compared with non-OVA-challenged mice (1.60 ± 0.37 vs

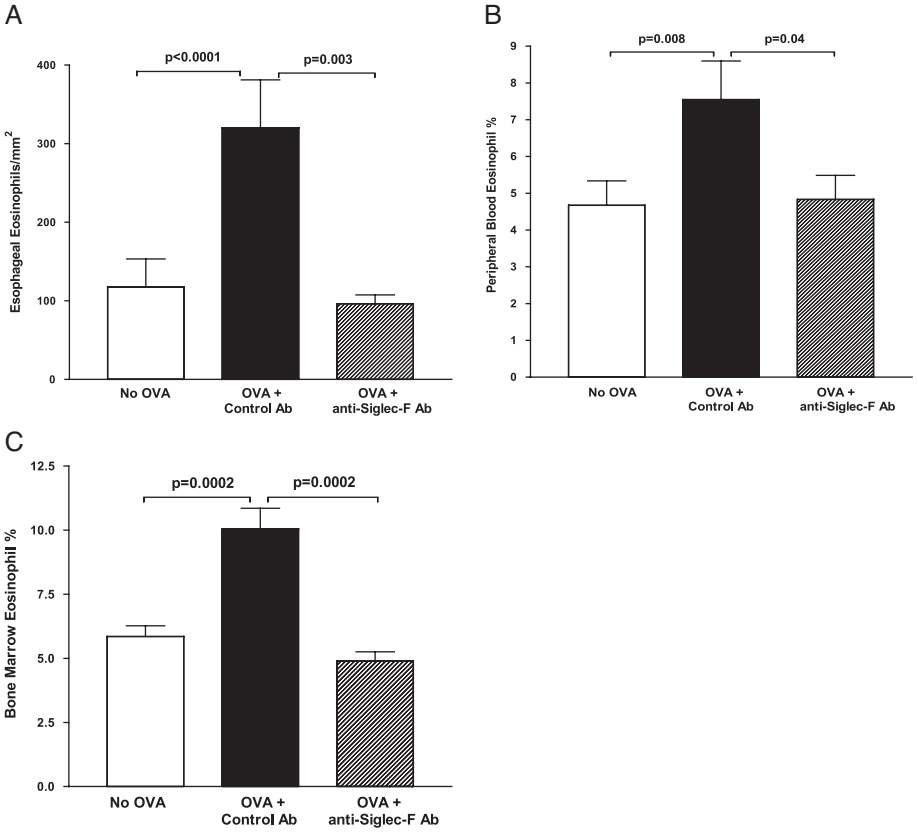

FIGURE 3. Eosinophil quantitation in esophagus, peripheral blood, and bone marrow. A, Esophagus. The number of eosinophils per square millimeter of esophageal lamina propria was quantitated. Intraesophageal OVA challenge induced a significant accumulation of eosinophils (OVA vs no OVA, $P < 0.0001$). Administration of an anti-Siglec-F antibody to OVA-challenged mice significantly reduced the number of esophageal eosinophils ($P = 0.003$) to levels similar to those in the no-OVA group (n = 8 mice/group). B, Peripheral blood. The number of eosinophils was quantitated in Wright-Giemsa-stained peripheral blood. A significant increase in the percentage of eosinophils was noted in mice challenged with OVA compared to the non-OVA–exposed group ($P = 0.0006$). The administration of an anti-Siglec-F Ab to OVA-challenged mice significantly reduced the degree of peripheral blood eosinophilia compared to the group challenged with OVA and administered a control Ab ($P = 0.04$) (n = 16 mice/group). C, Bone marrow. The number of eosinophils was quantitated in Wright-Giemsa-stained bone marrow. A significant increase in the percentage of eosinophils was noted in OVA-challenged mice compared to the non-OVA-exposed group ($P = 0.0002$). The administration of an anti-Siglec-F Ab to OVA-challenged mice significantly reduced the degree of bone marrow eosinophilia compared to the group challenged with OVA and administered a control Ab ($P = 0.0002$) (n = 8 mice/group).

1.26 ± 0.49; $P = 0.049$) (Fig. 7A). Administration of an anti-Siglec-F Ab to OVA-challenged mice significantly reduced levels of fibronectin deposition (1.12 ± 0.42 vs 1.60 ± 0.37; $P = 0.0005$) (Fig. 7A).

OVA-challenged mice had a significant increase in the number of TGF-β1-positive cells compared to the non-OVA group (523 ± 39 vs 287 ± 44 positive cells/mm^2; $P < 0.0001$) (Fig. 7B). Administration of an anti-Siglec-F antibody to OVA-challenged mice induced a trend to reduce the number of TGF-β1-positive cells, but this was not statistically significant (452 ± 43 vs 523 ± 39; $P = 0.18$) (Fig. 7B).

Administration of an anti-Siglec-F Ab to OVA-challenged mice induced a small reduction in the area of trichrome staining, but this was not statistically significant (data not shown).

Epithelial Basal Zone Thickness

OVA-challenged mice had a trend for increased maximal basal zone thickness, which was not statistically significant compared with non-OVA-challenged mice (12.5 ± 3.5 vs 14.4 ± 4.8 μm; $P = 0.25$); however, administration of the anti-Siglec-F antibody to OVA-challenged mice significantly reduced the maximal basal zone thickness (10.7 ± 3.0 vs 14.4 ± 4.8 μm; $P = 0.006$).

DISCUSSION

Our study aimed at determining whether targeting Siglec-F, a receptor highly expressed on mouse eosinophils, would reduce levels of eosinophilic inflammation and remodeling in a mouse model of food allergen–induced eosinophilic esophagitis. We demonstrated that administration of an anti-Siglec-F Ab was indeed able to significantly reduce levels of esophageal eosinophils to levels noted in the esophagus of non-OVA-challenged mice, suggesting that targeting Siglec-F can significantly reduce eosinophil levels in a mouse model of EoE. Although previous studies have demonstrated that targeting Siglec-F influences eosinophil

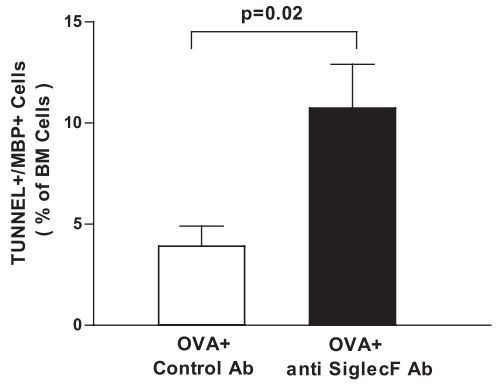

FIGURE 4. Effect of anti-Siglec-F antibody (Ab) on apoptosis. Bone marrow from mice chronically challenged with oral ovalbumin (OVA) and treated with either an anti-Siglec-F or control Ab was processed for TUNEL staining and the number of TUNEL-positive cells quantitated by immunohistology. The number of TUNEL-positive cells in mice chronically challenged with oral OVA was significantly increased in the bone marrow of anti-Siglec-F Ab compared to control Ab-treated mice ($P = 0.02$).

levels in mouse models of asthma (12,13), the hypereosinophilic syndrome (14), and small bowel eosinophilic inflammation (14,15), this is the first study to demonstrate that targeting Siglec-F plays an important role in reducing eosinophil levels in a preclinical model of EoE induced by administration of an egg allergen.

The anti-Siglec-F Ab also significantly reduced selected features of esophageal remodeling including angiogenesis, extracellular matrix deposition of fibronectin, and the thickness of the epithelial basal zone, all features of remodeling noted in EoE (6,17). Angiogenic vessels are known to exhibit increased expression of adhesion molecules (18) and may contribute to increased inflammation through increased recruitment of eosinophils into the esophagus. Studies in EoE have demonstrated increased angiogenic blood vessels with increased levels of expression of the adhesion molecule vascular cell adhesion molecule-1 (17), which binds the $\alpha 4\beta 1$ ligand expressed by eosinophils. Thus, the ability of the anti-Siglec-F Ab to reduce the number of small blood vessels in the LP of the esophagus may contribute to the reduced numbers of eosinophils being recruited to the esophagus, as demonstrated in this study. The mechanism by which the anti-Siglec-F Ab reduces levels of angiogenesis is suggested from our novel observation that the anti-Siglec-F Ab reduces the number of cells in the esophagus expressing the proangiogenic cytokine VEGF. VEGF is produced by cell types including eosinophils (5) and macrophages (19), which both express Siglec-F. Thus, the anti-Siglec-F Ab could be reducing VEGF levels by direct effects on eosinophils or macrophages or through indirect effects of eosinophils or macrophages on alternate cell types, which are expressing VEGF but do not express Siglec-F.

In addition to significantly reducing levels of angiogenesis, the anti-Siglec-F Ab significantly reduced extracellular matrix fibronectin deposition and the thickness of the epithelial basal zone, all features of remodeling noted in EoE. Increased levels of deposition of the extracellular matrix protein tenascin have been noted in remodeling in EoE (7) and can contribute to eosinophil activation. For example, eosinophil adhesion to fibronectin is mediated by $\alpha 4\beta 1$ ligand with resultant increased eosinophil viability and enhanced degranulation, which can contribute to the proinflammatory effects of eosinophils in EoE (20). Thus, the ability of the anti-Siglec-F Ab to reduce levels of fibronectin can contribute to reduced eosinophil viability and activation. The anti-Siglec-F Ab also reduced the thickness of the surface epithelial basal zone. Because a variety of proinflammatory cytokines, chemokines, and mediators are produced by the esophageal epithelium, reducing the thickness of this proinflammatory cellular layer may contribute to reduced inflammation and remodeling in EoE. The basal zone epithelium do not express Siglec-F receptors,

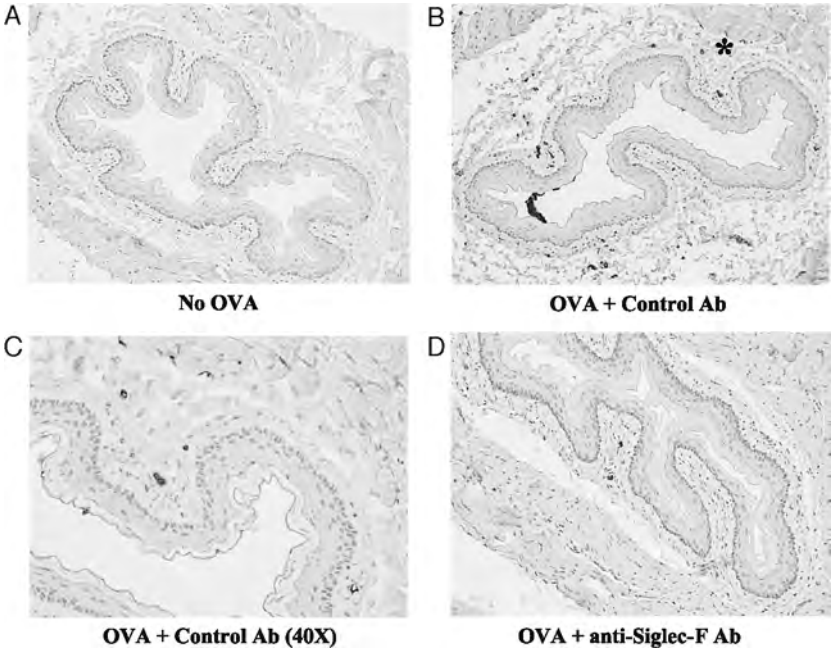

FIGURE 5. Angiogenesis in the esophagus. Hematoxylin and PECAM immunostain of esophagus. A, No OVA. B, OVA + control Ab. C, OVA + control Ab (40× magnification of panel B). D, OVA + anti-Siglec-F Ab.

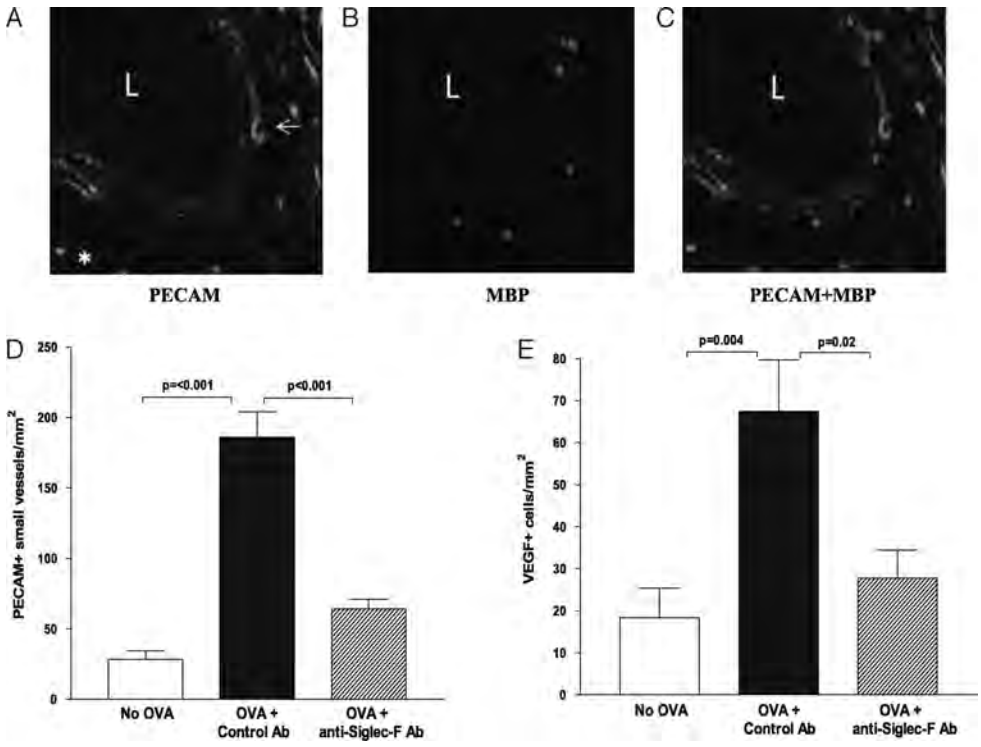

FIGURE 6. OVA-induced angiogenesis and vascular endothelial growth factor (VEGF) expression in the esophagus. A–C, Detection of cells expressing PECAM and major basic protein (MBP). Esophageal sections from wild-type mice that had been subjected to chronic oral OVA challenge were immunostained with both an anti-PECAM Ab (immunofluoresce green) and an anti-MBP Ab (immunofluoresce red). L indicates esophageal lumen. Arrow in A points to PECAM-positive blood vessel and ∗ indicates PECAM-positive cells, which do not colocalize with MBP (B and C). D, Angiogenesis. A significant increase in the number of small blood vessels was noted in the lamina propria of mice challenged with OVA compared to the non-OVA-exposed group ($P < 0.001$). The administration of an anti-Siglec-F Ab to OVA-challenged mice significantly reduced the number of small vessels compared with mice challenged with OVA and administered a control Ab ($P < 0.001$). E, VEGF-positive cells. A significant increase in the number of VEGF-positive cells was noted in OVA-challenged mice compared to the non-OVA-exposed group ($P < 0.004$). The administration of an anti-Siglec-F Ab to OVA-challenged mice significantly reduced the number of VEGF-positive cells compared with the group challenged with OVA and administered a control Ab ($P < 0.02$) (n = 8 mice/group).

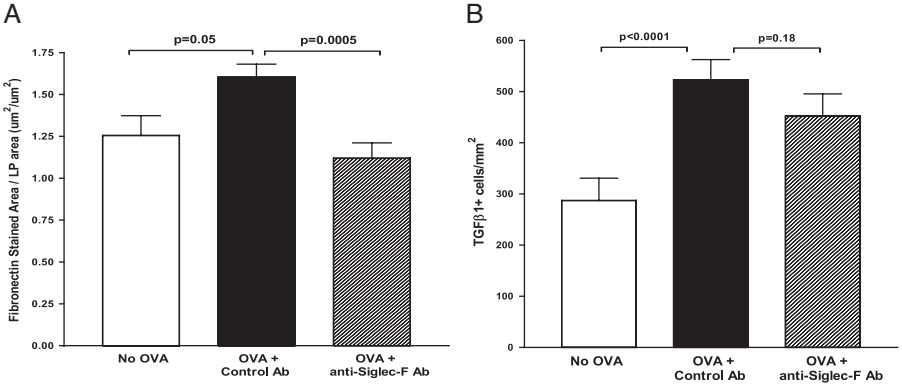

FIGURE 7. Esophageal fibronectin deposition and TGF-β1-positive cells. A, Fibronectin. A significant increase in lamina propria fibronectin deposition was noted in OVA-challenged mice compared to the non-OVA-exposed group ($P = 0.05$). The administration of an anti-Siglec-F Ab to OVA-challenged mice significantly reduced the amount of fibronectin deposition compared with the group challenged with OVA and administered a control Ab ($P = 0.0005$). B, TGF-β1-positive cells. A significant increase in the number of TGF-β1-positive cells was noted in OVA-challenged mice compared with the non-OVA-exposed group ($P = 0.0001$). The administration of an anti-Siglec-F Ab to OVA-challenged mice resulted in a nonsignificant trend to reduced numbers of TGF-β1-positive cells ($P = 0.18$) (n = 8 mice/group).

and thus the ability of the Siglec-F Ab to reduce the thickness of the basal zone epithelium is likely mediated indirectly through effects on inhibiting release of mediators, which influence basal zone thickness from Siglec-F-positive cells such as eosinophils and macrophages.

Previous in vitro and in vivo studies have investigated potential mechanisms by which targeting Siglec-F may reduce levels of eosinophils. Siglecs are single-pass type I transmembrane proteins that recognize and bind sialic acid–containing glycans (21). Siglec-F consists of 4 immunoglobulin-like domains, a transmembrane domain and a tyrosine-based inhibitory motif in the cytoplasmic tail, which suggests that it may have an inhibitory role in cell signaling (8,22,23). In vitro studies have demonstrated that incubating eosinophils with an anti-Siglec-F Ab induces eosinophil apoptosis (12,14), whereas in vivo studies have detected increased numbers of apoptotic eosinophils following administration of an anti-Siglec-F Ab (13–15). In this study we confirmed that the bone marrow is a significant site for induction of eosinophil apoptosis following the administration of the anti-Siglec-F Ab. Administration of the anti-Siglec-F Ab did not significantly increase the number of apoptotic cells in the esophagus; however, because apoptotic cells are rapidly removed in tissues, it is possible that rapid clearance of apoptotic cells in the esophagus precluded us detecting these apoptotic cells. The administration of Fab fragments of the anti-Siglec-F Ab in vivo has similar effects to the intact Ab, making it less likely that eosinophils tagged with the anti-Siglec-F Ab are being cleared by the Fc portion of the anti-Siglec-F Ab or via complement activation (13). When administered in vivo the anti-Siglec-F Ab reduces levels of bone marrow eosinophils as well as peripheral blood eosinophils (13–15), as we have confirmed in this study. Administration of the anti-Siglec-F Ab also has reduced levels of TGF-β1-positive cells as well as levels of fibrosis in the lung (13). In this study in EoE, administration of the anti-Siglec-F Ab demonstrated a trend to reduce levels of TGF-β1-positive cells and levels of fibrosis in the esophagus, which was not statistically significant. Thus, the anti-Siglec-F Ab was able to reduce many important features associated with EoE (eosinophilic inflammation, angiogenesis, VEGF expression, basal zone hyperplasia), but did not influence levels of TGF-β1-positive cells or fibrosis. Whether differences in response to anti-Siglec-F in the lung and esophagus reflect differences in mechanisms of remodeling in different organs or alternate explanations is at present unknown.

As with any animal model of human disease, it is unclear how the results we have observed with a mouse model of EoE will translate into humans with EoE. The mouse model does demonstrate several features noted in human EoE, including infiltration of the esophagus with eosinophils, basal zone hyperplasia, angiogenesis, and fibrosis. An advantage of this mouse model of EoE over alternative models of EoE (24) is that it is dependent on administration of a food allergen to the esophagus. As subjects with EoE demonstrate significant improvements on elemental diets (25), using a food-dependent mouse model of EoE has advantages. Indeed, egg is one of the most common inciting antigens in human EoE (26). Although Siglecs are expressed on eosinophils in mice and humans, in humans Siglec-8 is the predominant Siglec expressed by eosinophils. Siglec-F in the mouse is the functional convergent paralog of human Siglec-8 because they are both predominantly expressed on eosinophils and both have specificity for the same ligand (6′-sulfo-sialyl Lewis X) (8,23). Also, in vitro studies have demonstrated that activation of Siglec-8 in vitro induces eosinophilic apoptosis (10,11,27), suggesting that both Siglec-F and Siglec-8 may have the same function in vivo. Studies in Siglec-F-deficient mice (12) demonstrate that they have enhanced lung eosinophilic inflammation following allergen challenge, suggesting that Siglec-F normally functions to down-regulate levels of eosinophilic inflammation.

In summary, we have demonstrated that in a mouse model of OVA, food-induced EoE administration of an anti-Siglec-F Ab significantly reduces levels of esophageal eosinophilic inflammation to levels noted in non-OVA control mice. In addition, we demonstrated that the anti-Siglec-F Ab reduced levels of esophageal angiogenesis, VEGF expression, basal zone hyperplasia, and deposition of the extracellular matrix protein fibronectin. These observations suggest that targeting Siglec-F may significantly reduce both levels of esophageal eosinophilic inflammation and several aspects of esophageal remodeling (though not all aspects as it did not inhibit fibrosis). The use of anti-Siglec–based therapies in humans are being pursued in oncology and autoimmunity (ie, a toxin conjugated anti-Siglec-3 antibody is currently approved to treat acute myeloid leukemia; a toxin conjugated anti-Siglec-2 is currently undergoing clinical trials for the treatment of B cell non-Hodgkin lymphoma and autoimmune diseases) (28). IVIG, which is widely used as therapy for a multitude of inflammatory condition, has been shown to have naturally occurring anti-Siglec-8 and anti-Siglec-9 autoantibodies (29). In one study, such autoantibodies were responsible for inducing eosinophil apoptosis in vitro (29). Further studies are needed to determine whether targeting Siglec-8 (the human paralog of Siglec-F) will influence levels of eosinophilic inflammation or remodeling in human subjects with EoE.

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