N-Glycans Differentially Regulate Eosinophil and Neutrophil Recruitment during Allergic Airway Inflammation*5

Received for publication, July 5, 2011, and in revised form, September 9, 2011 Published, JBC Papers in Press, September 12, 2011, DOI 10.1074/jbc.M111.279554

Nooshin S. Bahaei, Bit Na Kang, Elizabeth M. Frenzel, M. Reza Hossein Khan, Xiao Na Ge, Yana Greenberg, Sung Gil Ha, Michael Demetriou, Savita P. Rao, and P. Sriramarao

From the 1Laboratory of Allergic Diseases and Inflammation, Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, Minnesota 55108, the 2Department of Medicine, University of Minnesota, Minneapolis, Minnesota 55455 and the 3Department of Neurology, Microbiology and Molecular Genetics, Institute for Immunology, University of California, Irvine, California 92697

Background: Cell surface-expressed glycans play a role in leukocyte trafficking and recruitment.

Results: Deficiency of MGAT5 causes attenuation of allergen-induced eosinophilia and Th2 cytokines but increases neutrophilic inflammation and airway hyperreactivity.

Conclusion: Recruitment of eosinophils and neutrophils is differentially regulated by MGAT5-modified N-glycans during airway inflammation.

Significance: This study demonstrates a significant role for N-glycans in the development of allergic airway inflammation and asthma.

Allergic airway inflammation, including asthma, is usually characterized by the predominant recruitment of eosinophils. However, neutrophilia is also prominent during severe exacerbations. Cell surface-expressed glycans play a role in leukocyte trafficking and recruitment during inflammation. Here, the involvement of UDP-N-acetylgalactosamineα-6→3-mannoside β1,6-N-acetylgalactosaminyltransferase V (MGAT5)-modified N-glycans in eosinophil and neutrophil recruitment during allergic airway inflammation was investigated. Allergen-challenged Mgat5-deficient (Mgats−/−) mice exhibited significantly attenuated airway eosinophilia and inflammation (decreased Th2 cytokines, mucus production) compared with WT counterparts, attributable to decreased rolling, adhesion, and survival of Mgats−/− eosinophils. Interestingly, allergen-challenged Mgat5−/− mice developed airway neutrophilia and increased airway reactivity with persistent elevated levels of proinflammatory cytokines (IL-17A, TNFα, IFNγ). This increased neutrophil recruitment was also observed in LPS- and thioglycollate (TG)-induced inflammation in Mgat5−/− mice. Furthermore, there was significantly increased recruitment of infused Mgat5−/− neutrophils compared with WT neutrophils in the peritoneal cavity of TG-exposed WT mice. Mgat5−/− neutrophils demonstrated enhanced adhesion to P-selectin as well as increased migration toward keratinocyte-derived chemokine compared with WT neutrophils in vitro along with increased calcium mobilization upon activation and expression of elevated levels of CXCR2, which may contribute to the increased neutrophil recruitment. These data indicate an important role for MGAT5-modified N-glycans in differential regulation of eosinophil and neutrophil recruitment during allergic airway inflammation.

ALLERGIC AIRWAY INFLAMMATION, INCLUDING asthma, is usually characterized by the predominant recruitment of eosinophils. However, neutrophilia is also prominent during severe exacerbations. Cell surface-expressed glycans play a role in leukocyte trafficking and recruitment during inflammation. Here, the involvement of UDP-N-acetylgalactosamineα-6→3-mannoside β1,6-N-acetylgalactosaminyltransferase V (MGAT5)-modified N-glycans in eosinophil and neutrophil recruitment during allergic airway inflammation was investigated. Allergen-challenged Mgat5-deficient (Mgats−/−) mice exhibited significantly attenuated airway eosinophilia and inflammation (decreased Th2 cytokines, mucus production) compared with WT counterparts, attributable to decreased rolling, adhesion, and survival of Mgats−/− eosinophils. Interestingly, allergen-challenged Mgat5−/− mice developed airway neutrophilia and increased airway reactivity with persistent elevated levels of proinflammatory cytokines (IL-17A, TNFα, IFNγ). This increased neutrophil recruitment was also observed in LPS- and thioglycollate (TG)-induced inflammation in Mgat5−/− mice. Furthermore, there was significantly increased recruitment of infused Mgat5−/− neutrophils compared with WT neutrophils in the peritoneal cavity of TG-exposed WT mice. Mgat5−/− neutrophils demonstrated enhanced adhesion to P-selectin as well as increased migration toward keratinocyte-derived chemokine compared with WT neutrophils in vitro along with increased calcium mobilization upon activation and expression of elevated levels of CXCR2, which may contribute to the increased neutrophil recruitment. These data indicate an important role for MGAT5-modified N-glycans in differential regulation of eosinophil and neutrophil recruitment during allergic airway inflammation.

Allergic airway inflammation, including asthma, is characterized by the influx of inflammatory leukocytes to the airways (1). Although eosinophils are the predominant cells recruited, there is increasing evidence for the significant involvement of neutrophils in asthma (2, 3). Several studies have indicated that cell surface-expressed glycans play an important role in trafficking, migration and recruitment of leukocytes during inflammation by virtue of their ability to bind to selectins (4–6). A classic example is the role played by N-glycans in L-selectin-mediated lymphocyte homing and recruitment (7). However, the role played by N-glycans in eosinophil and neutrophil recruitment, particularly in the context of allergic airway inflammation, is not known.

In addition to L-selectin and α4 integrins (8), a significant role has been demonstrated for galectin-3 in mediating eosinophil rolling and adhesion by binding to α4 integrins (9) and in promoting allergic airway inflammation, including airway remodeling, in vivo (10). Galectins bind to N-glycan residues of cell surface glycoproteins in a manner proportional to the number of N-glycan residues and the degree of N-acetylgalactosamine branching. This results in a molecular lattice that controls the concentration of surface glycoproteins to effect signaling, cell growth, differentiation, and disease states (11, 12). The direct relevance of dysregulated N-glycosylation to human disease was recently confirmed in the inflammatory demyelinating disease multiple sclerosis (13). UDP-N-acetylgalactosamineα-6→3-mannoside β1,6-N-acetylgalactosaminyltransferase V (MGAT5)2 modifies N-glycans by transferring N-acetyllactosamine to the OH on carbon 6 of the α1,6-linked mannose, which are then preferentially extended with poly-N-acetyllac-
MGAT5-modified N-Glycans and Leukocyte Recruitment
tosamine units, resulting in high affinity ligands for galectins, particularly galectin-3 (14). Previous studies have demonstrated that MGAT5-modified N-glycans regulate T cell inflammatory responses (15, 16) and promote Th2 over Th1 differentiation (17). Mice deficient for Magt5 (Magt5−/−) display more severe delayed type hypersensitivity reactions, a greater tendency for autoimmune kidney disease, and susceptibility to experimental autoimmune encephalomyelitis (16–18). Because MGAT5-modified N-glycans have the capacity to regulate Th1/Th2 polarization as well as function as ligands for galectin-3 (14), which promotes eosinophil trafficking and recruitment (9, 10), MGAT5 activity may well play a role in the regulation of allergic inflammation including asthma. Using Magt5−/− mice, we examined in detail the contribution of MGAT5-modified N-glycans to trafficking and recruitment of eosinophils and neutrophils during the development of allergic airway inflammation.

EXPERIMENTAL PROCEDURES

Mouse Model of Allergic Airway Inflammation—Magt5−/− mice on a C57BL/6 background were obtained from the Consortium for Functional Glycomics and bred in-house. Offspring were genotyped by PCR using genomic DNA isolated from tail clippings. Age- and gender-matched Magt5−/− and WT C57BL/6 mice were sensitized with 20 μg of ovalbumin (OVA, Grade V, Sigma) in 2 mg of aluminum hydroxide gel intraperitoneally. Control mice received aluminum hydroxide gel alone. Sixteen days later, control and OVA-sensitized mice were exposed to aerosolized saline alone or OVA in saline (20 mg/ml), respectively, for 5 days via a nebulizer. Mice were sacrificed 18–24 h after the last challenge. All animal studies were performed following standards and procedures approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

Measurement of Airway Hyperresponsiveness (AHR)—Eighteen to twenty-four hours after the last aerosol challenge, AHR was measured in allergen-challenged and control mice using a single chamber, whole-body plethysmograph (Buxco, Wilmington, NC) as described previously (19). The enhanced pause in breathing after each nebulization of methacholine (Mch, from 3–25 mg/ml in saline, Sigma) was monitored, and results were expressed as a percentage of baseline enhanced pause values following exposure to saline.

Bronchoalveolar Fluid (BALF) Analysis—Total cells and differential cell counts based on morphologic and histologic criteria after staining with Hema 3 System (Thermo Fisher Scientific Co., Pittsburgh, PA) were determined. Phascolonus vulgaris leucoagglutinin (PHA-L) binding by gated eosinophils in the BALF after 30 min was analyzed using FITC-PHA-L (1 μg/ml, Vector Laboratories, Burlingame, CA) or FITC-BSA (as control) with a FACSscan flow cytometer (BD Biosciences) and FlowJo flow cytometry analysis software (Tree Star, Ashland, OR) for data acquisition.

Lung Immunohistochemistry—Paraformaldehyde-fixed, paraffin-embedded lung tissue sections (4 μm) were used for most analyses. VECTASTAIN ABC kits containing appropriate biotinylated secondary antibodies and avidin-biotin HRP complex (Vector Laboratories) and the Peroxidase AEC substrate kit (Vector Laboratories) were used for detection. Stained slides were evaluated using a Nikon Microphot EPI-FL microscope (Nikon Instruments, Inc., Melville, NY). Images were captured with an Olympus DP71 camera and quantitated by image analysis with ImageJ software (20). Sections were stained with H&E (Thermo Fisher Scientific) to determine cellular infiltration. Infiltrated eosinophils were assessed after staining with rat mAb against murine major basic protein (MBP) (21). Expression and quantitation of FIZZ1 (found in inflammatory zone 1) and α-smooth muscle actin was performed as described previously (10). Intelectin-1 expression was evaluated using sheep anti mouse intelectin-1 (0.8 μg/ml, R&D Systems, Minneapolis, MN), and results were expressed as μm2 intelectin-1 positive area/100 μm basement membrane length. Airway mucus secretion was assessed after periodic acid-Schiff’s (PAS) staining as described (10). Neutrophils within blood vessels were assessed in cryopreserved lung sections using NIMP-R14 rat mAbs specific for murine neutrophils (Abcam, Cambridge, MA), whereas peribronchial neutrophils were detected in paraffin-embedded lung sections by immunofluorescence staining after antigen retrieval with proteinase K using FITC-conjugated goat anti-rat IgG (7.5 μg/ml, Jackson ImmunoResearch Laboratories, West Grove, PA) as the secondary antibody. DAPI was used to visualize the cells. Slides were observed at ambient temperature using a Fluoview FV1000/BX61 confocal laser scanning biological microscope equipped with an UPlanSApo lens (20×/numerical aperture 0.85 (oil)) and a PlanApo N lens (60×/numerical aperture 1.42 (oil)). FV10-ASW 2.0 software was used for image acquisition (Olympus, Melville, NY).

Gr-1 Levels in Lung Tissue by Western Blot—Lung tissue lysates (50 μg protein) were evaluated by Western blots using anti-mouse Ly-6G (alternate name Gr-1, 1:500, eBioscience, San Diego, CA) followed by HRP-conjugated goat anti-rat IgG (Cell Signaling Technology, Danvers, MA) as the secondary antibody. HRP-conjugated anti-mouse β-actin (Santa Cruz Biotechnology) was used to monitor levels of β-actin expression as an internal control. Protein bands detected with ECL™ Western blotting Detection Reagents (Millipore, Bedford, MA) were visualized on x-ray films, which were scanned and analyzed using ImageJ software to quantitate integrated density (pixels) of the bands. Density of Gr-1 bands in lung tissue lysates were normalized against β-actin.

Measurement of Lung Cytokines and Chemokines—Cytokine and chemokine levels in lung lysate supernatants were measured using Flex Set kits (BD Biosciences) for Th1 (IL-2, IFN-γ)/Th2 (IL-4, IL-5) cytokines (catalog no. 551527), IL-13 (catalog no. 558349), IL-17A (catalog no. 560283), and keratinocyte-derived chemokine (KC; catalog no. 558340) according to the manufacturer using the same amount of protein for each sample with a FACScan flow cytometer and FCAP Array™ Software (BD Biosciences). KC levels were expressed as pg/ml lung lysate, whereas levels of all other cytokines were expressed as pg cytokine/mg protein.

Induction of Pulmonary and Peritoneal Neutrophilic Inflammation—Peripheral blood was first collected from the facial vein of Magt5−/− and WT mice to determine baseline differential cell counts. For pulmonary inflammation, mice were exposed to a single dose (10 μg in 50 μl of saline) of LPS
(Sigma) administered intranasally (4). Three hours after LPS exposure, BALF and blood (by cardiac puncture) were collected, and post-challenge total and differential cells counts were determined. For peritoneal inflammation, mice were injected intraperitoneal with 1 ml of 3% thioglycollate (TG) (22), and the peritoneal cavity was lavaged with 5 ml of sterile PBS after 6 h to determine total and differential cell counts.

Mouse Eosinophils and Neutrophils—Mouse eosinophils were obtained from cultured BM of naïve Mgat5<sup>−/−</sup> and WT mice as described previously (23). Cells between day 11–13 of culture that were 99% Hema 3-positive (Fisher Scientific) and expressed both MBP (by confocal microscopy) and Siglec-F (by flow cytometry using phycoerythrin-conjugated rat anti-mouse Siglec-F at 5 μg/ml, BD Biosciences) were used in studies. Mature neutrophils were isolated from BM of naïve WT and Mgat5<sup>−/−</sup> mice by discontinuous Percoll gradient (82%/65%/55%) centrifugation. The 82%/65% interface of the gradient containing mature neutrophils was collected and demonstrated to be positive for Gr-1, a marker for murine neutrophils (24), using FITC-conjugated rat anti mouse Gr-1 (5 μg/ml, BD Biosciences) by flow cytometry. More than 85% of cells isolated were neutrophils as assessed by Hema 3 staining and cell morphology. Viability by trypan blue exclusion was >95%. PHA-L binding by eosinophils and neutrophils was assessed by flow cytometry as described in an earlier section.

Flow Chamber Studies—Rolling and adhesion of murine eosinophils and neutrophils from WT and Mgat5<sup>−/−</sup> mice on endothelial adhesion molecules under conditions of flow was evaluated in an in vitro parallel plate flow chamber as described in our previous studies (9). Coverslips treated with PBS were used to assess background rolling and adhesion. Interaction of eosinophils (1×10<sup>6</sup>) with recombinant murine galectin-3 (10) and recombinant murine VCAM-1 (10) and of neutrophils (1×10<sup>6</sup>) with recombinant murine P-selectin (R&D Systems) at a flow rate of 1 ml/min (wall shear stress, 1.0–2.0 dynes/cm<sup>2</sup>) was evaluated and analyzed as described previously (9). The number of adherent neutrophils is expressed as a fold increase in adhesion compared with background (adhesion to PBS-treated).

Static Adhesion Studies—WT and Mgat5<sup>−/−</sup> neutrophils were allowed to adhere to P-selectin-coated cover-slips for 5 min, washed, fixed, mounted in mounting medium with DAPI, and evaluated by confocal microscopy. The number of cells adhered in six fields were enumerated per coverslip for each cell type.

Expression of Adhesion Molecules—Expression of PSGL-1 by WT and Mgat5<sup>−/−</sup> neutrophils (4×10<sup>6</sup> cells) was assessed by flow cytometry with phycoerythrin-conjugated anti mouse PSGL-1 (5 μg/ml, BD Biosciences) after initially fixing the cells (4% paraformaldehyde) using FlowJo software for data analysis. Expression of α4 (CD49d), LFA-1 (CD11a), Mac-1 (CD11b), Siglec-F, and L-selectin (CD62L) by eosinophils was assessed using rat mAbs against murine α4 (PS/2) (25), CD11a (eBiosciences), CD11b (eBiosciences), Siglec-F (BD Biosciences), and CD62L (BD Biosciences). Depending on the mAb, rat IgG1, -2a, or -2b was used as the isotype-matched control.

Chemotaxis Assay and Expression of Chemokine Receptors—Eosinophils or neutrophils obtained from BM of WT and Mgat5<sup>−/−</sup> mice were suspended in DMEM and added to the upper wells (5×10<sup>5</sup>/well in 50 μl) of 96-well Transwell<sup>®</sup> Chambers. Murine eotaxin (CCL11, 100 nM from PeproTech for eosinophil chemotaxis), murine KC (CXCL1, 100 nM from PeproTech for neutrophil chemotaxis), or medium alone was added to the lower wells, and the plates were incubated at 37 °C for 3 h. The number of cells that migrated in each case was evaluated under an Olympus CK2 inverted microscope using a 40× objective, and cells in three-seven different fields of each well were counted. The assay was performed two to three times in duplicate or triplicate, and results are expressed as the average number of cells/field. Expression of CCR3 (CD193), the receptor for eotaxin-1, on WT and Mgat5<sup>−/−</sup> eosinophils was assessed using FITC-conjugated anti-mouse CCR3 (R&D Systems) with FITC-conjugated rat IgG2a as the isotype control.

In Vivo Migration Studies—To evaluate migration/recruitment of WT versus Mgat5<sup>−/−</sup> neutrophils or eosinophils in vivo, the TG-induced peritoneal inflammation model described earlier was used. BM collected from the femur and tibia of WT and Mgat<sup>−/−</sup> mice was exposed to hypotonic shock to remove red blood cells and used as a source of neutrophils. Carboxyfluorescein succinimidyl ester (Invitrogen) and CellTracker Orange (CMRA, Invitrogen) were used at 5 μM to label WT and Mgat5<sup>−/− </sup> cells, respectively, or vice versa for 15 min at room temperature. After washing, an equal number of labeled WT and Mgat5<sup>−/−</sup> neutrophils (2.3×10<sup>5</sup>) were infused simultaneously via tail vein injection into WT recipient mice immediately after they were administered TG. Peritoneal fluid was collected from the TG-exposed mice after 6 h, and total and differential cell counts were determined. Cells in the peritoneal fluid were analyzed by flow cytometry after gating for neutrophils by staining with allopurinocyanin-conjugated mAbs against mouse Gr-1 (BD Biosciences). To evaluate eosinophil recruitment, WT and Mgat5<sup>−/−</sup> eosinophils from BM cultures were labeled as described above for neutrophils and infused (1×10<sup>5</sup> cells) 13 h after administration of TG. Peritoneal fluid was collected after 2 h, i.e. 15 h after TG, based on previous studies that indicate that WT mice develop transient eosinophilic infiltration in the peritoneal cavity 12 to 24 h after TG injection (26). Cells were analyzed by flow cytometry after gating for eosinophils with mAbs against Siglec-F followed by allopurinocyanin-conjugated anti-rat antibodies (Jackson ImmunoResearch Laboratories). The number of carboxyfluorescein succinimidyl ester versus CMRA-labeled neutrophils or eosinophils in the peritoneal fluid in each case was determined.

Measurement of Intracellular Calcium [Ca<sup>2+</sup>]<i>i</i>, Concentration—[Ca<sup>2+</sup>]<i>i</i>, in BM-derived eosinophils and neutrophils was determined using the permeant Ca<sup>2+</sup> indicator dye Fura-2 AM (Molecular Probes, Invitrogen) as described previously for airway smooth muscle cells (27) but with minor mod-
MGAT5-modified N-Glycans and Leukocyte Recruitment

ifications. Briefly, eosinophils or neutrophils attached to poly-
l-lysine-coated (10 μg/ml) glass coverslips were incubated with 5 μM Fura-2 AM for 30 min at 37 °C and 5% CO₂. Coverslips were washed gently, perfused with HBSS containing 10 mM HEPES, 11 mM glucose, 2.5 mM CaCl₂, and 1.2 mM MgCl₂ (pH 7.4), and mounted on the stage of a Nikon Diaphot inverted microscope (Nikon Instruments, Inc.) in an open slide chamber. Fura-2 AM-loaded cells were alternately excited at 340 and 380 nm with a Lambda DG-4 high speed wavelength switcher (Sutter Instrument Co., Novato, CA). Basal [Ca²⁺], was measured by real-time digital video fluorescence imaging using NIS-Elements imaging software (Nikon Instruments, Inc.). After 1 min, neutrophils were stimulated with 100 nM KC (Peprotech) and evaluated for an additional 2 min to assess agonist-induced [Ca²⁺] responses.

**Assessment of Neutrophil and Eosinophil Survival—**Neutrophils obtained from the peritoneal cavity of TG-treated Mgat5⁻/⁻ and WT mice as well as eosinophils cultured from BM of Mgat5⁻/⁻ and WT mice were cultured in medium containing reduced serum (5%, to exclude effects of any growth factors) in 24-well plates (1 × 10⁶ cells/ml/well) previously coated with poly-2-hydroxyethyl methacrylate (Sigma) to prevent cells from adhering as described previously (22). At various time points ranging from 2–24 h after culture, cells were assayed for apoptosis by flow cytometry based on FITC-annexin V binding by gated neutrophils or eosinophils using apoptosis detection kits according to the manufacturer’s instructions (eBiosciences). Results are expressed as percent annexin V-positive cells.

**Statistical Analysis—**Results are expressed as mean ± S.E. Statistical significance was determined by one-tailed or two-tailed unpaired Student’s t test. A p value <0.05 was considered as significant.

**RESULTS**

**Allergen-challenged Mgat5⁻/⁻ Mice Exhibit Reduced Eosinophilic Airway Inflammation—**To examine the role of MGAT5-modified N-glycans in allergic inflammation, BALF and lung tissue from control and allergen-challenged Mgat5⁻/⁻ and WT mice were evaluated for cellular infiltration. Significantly fewer cells were present in the BALF (Fig. 1A, left panel) and lung tissue (Fig. 1B) of allergen-challenged Mgat5⁻/⁻ mice compared with WT counterparts. BALF differential cell counts indicated a significant reduction in the number of eosinophils (p < 0.01), monocytes/macrophages and lymphocytes (p < 0.05). Assessment of lung tissue eosinophils by immunohistology also revealed a decreased number of MBP-positive cells in allergen-challenged Mgat5⁻/⁻ mice than in WT mice (Fig. 1C, left), with few to no MBP-positive cells in control mice of both groups (data not shown). BALF eosinophils from allergen-challenged WT mice exhibited strong binding to PHA-L compared with control (BSA), thus confirming surface expression of branched N-glycans, whereas residual eosinophils in BALF of allergen-challenged Mgat5⁻/⁻ mice demonstrated only marginal binding to PHA-L relative to control (Fig. 1C, right). Evaluation of lung eotaxin-1 expression by immunohistology indicated no difference between allergen-challenged WT and Mgat5⁻/⁻ mice (supplemental Fig. 1), suggesting that the reduced eosinophil recruitment observed in allergen-challenged Mgat5⁻/⁻ mice is most likely regulated by factors other than eotaxin-1 levels.

Assessment of mucus production by goblet cells exhibited significantly increased number of PAS-positive mucus-producing cells in the airways of allergen-challenged WT mice compared with the small number observed in Mgat5⁻/⁻ counterparts (Fig. 1D). Little to no positive PAS staining was observed in lung sections of control mice (data not shown). Expression of FIZZ1, which is associated with early stage remodeling events in allergic airways (28), by type II alveolar and airway epithelial cells was also markedly reduced in allergen-challenged Mgat5⁻/⁻ mice compared with WT mice (Fig. 1E). Furthermore, expression of interlectin-1, another proinflammatory molecule induced during allergic inflammation (29), was significantly inhibited in secretory cells of the airway epithelium in allergen-challenged Mgat5⁻/⁻ mice (Fig. 1F). No expression of interlectin-1 was observed in control mice (data not shown). These data suggest that MGAT5 deficiency results in attenuated inflammatory responses that promote allergen-induced airway eosinophilic inflammation and remodeling.

**Mgat5⁻/⁻ Eosinophils Exhibit Reduced Trafficking and Survival—**MGAT5 is involved in the generation of N-glycan ligands for galectin-3, which supports eosinophil trafficking and recruitment (9, 10). Furthermore, eosinophils are known to roll and adhere on VCAM-1 (30), a key adhesion molecule up-regulated during allergic inflammation. Therefore, we evaluated the interaction of BM-cultured eosinophils from WT and Mgat5⁻/⁻ mice with immobilized galectin-3 and VCAM-1 under conditions of physiologic flow in vitro. Expression of branched N-glycans by cultured WT eosinophils and the lack of N-glycan expression by Mgat5⁻/⁻ eosinophils was first confirmed based on PHA-L binding (supplemental Fig. 2). Rolling and adhesion of Mgat5⁻/⁻ eosinophils on galectin-3 and VCAM-1 was significantly inhibited (to baseline levels) compared with WT eosinophils (p < 0.05) (Fig. 2, A and B). Flow cytometry revealed a small difference in expression of LFA-1 and Mac-1 but no difference in expression of α4 or Siglec-F between WT and Mgat5⁻/⁻ eosinophils. However, a reduction in L-selectin expression by a population of Mgat5⁻/⁻ eosinophils was observed (supplemental Fig. 3A). Although lung eotaxin-1 levels were similar in allergen-challenged Mgat5⁻/⁻ and WT mice (supplemental Fig. 1), we investigated whether the ability of Mgat5⁻/⁻ eosinophils to migrate in response to this chemokine was compromised. WT and Mgat5⁻/⁻ eosinophils exhibited comparable migration toward eotaxin (Fig. 2C). Furthermore, there was no difference in the level of expression of the eotaxin receptor CCR3 between WT and Mgat5⁻/⁻ eosinophils (supplemental Fig. 3B). The reduced adhesive interactions of Magt5⁻/⁻ eosinophils was also associated with significantly lower basal intracellular Ca²⁺ ([Ca²⁺]i) levels compared with WT eosinophils (p < 0.05) (Fig. 2D). Interestingly, Mgat5⁻/⁻ eosinophils cultured up to 24 h demonstrated significantly increased apoptosis from 9 h onwards compared with WT eosinophils (p < 0.05) (Fig. 2E). These data indicate an important role for MGAT5-modified N-glycans not only in supporting eosinophil rolling and adhesion but also survival. Thus, in allergen-challenged Mgat5⁻/⁻ mice, despite their ability to
undergo normal chemotaxis, reduced adhesive interactions with the vascular endothelium may result in decreased recruitment of eosinophils to the airways (Fig. 1). In addition, the decreased survival of eosinophils may contribute to reduced airway eosinophilia in allergen-challenged mice.

**Th2 Cytokines Are Decreased but Proinflammatory Cytokines Remain Elevated in Lungs of Allergen-challenged Mgat5−/− Mice**—Although allergen challenge-induced expression of IL-4, IL-5, and IL-13 in lungs of WT and Mgat5−/− mice relative to corresponding control mice (Table 1), the levels of each of these Th2 cytokines were significantly lower in allergen-challenged Mgat5−/− mice compared with WT mice. With Th1 cytokines, there was no change in IL-2 levels in response to allergen challenge, whereas IFNγ levels increased in WT and Mgat5−/− mice compared with respective control mice but without any significant difference between the allergen-challenged groups. Allergen challenge also induced IL-17A and TNFα in WT and Mgat5−/− mice, albeit with no statistically significant difference between the two groups. Overall, although allergen-challenged Mgat5−/− mice exhibit a signifi-
MGAT5-modified N-Glycans and Leukocyte Recruitment

FIGURE 2. MGAT5-modified N-glycans are essential for eosi
nophil trafficking and survival. Rolling (A) and adhesion (B) of WT and Mgat5−/− eosinophils on immobilized VCAM-1 or galectin-3 (Gal-3) under conditions of flow. Rolling on PBS was considered as background. C, chemotaxis of WT and Mgat5−/− eosinophils in response to eotaxin (100 nM) or medium alone (control) in vitro. Combined data of two independent experiments performed in triplicate for each condition with eosinophils from two different mice for each group is shown. Data represent mean ± S.E. of two independent experiments performed in triplicate for each group. D, Eosinophil survival evaluated by flow cytometry based on FITC-annexin V binding by gated eosinophils. Combined data of three experiments with eosinophils from three different mice for each group is shown. Data represent mean ± S.E. in A–E.* p < 0.05 for comparison of WT versus Mgat5−/− eosinophils.

TABLE 1

| Cytokine | Control (pg/mg protein) | OVA (pg/mg protein) | Control (pg/mg protein) | OVA (pg/mg protein) |
|----------|-------------------------|---------------------|-------------------------|---------------------|
| IL-4     | 4.97 ± 0.21             | 9.27 ± 1.04         | 4.39 ± 0.12             | 6.64 ± 0.53         |
| IL-5     | 4.18 ± 0.3              | 14.33 ± 1.42        | 3.86 ± 0.19             | 7.03 ± 0.35         |
| IL-13    | 3.81 ± 0.87             | 49.41 ± 9.72        | 3.85 ± 0.36             | 14.78 ± 2.65a       |
| IFNγ     | 0.44 ± 0.16             | 12.99 ± 2.26a       | 0.01 ± 0.01             | 9.08 ± 3.16         |
| IL-2     | 9.0 ± 0.99              | 7.02 ± 0.98         | 10.48 ± 1.16            | 7.42 ± 0.48         |
| IL-17A   | ND                      | 60.63 ± 21.58       | ND                      | 41.89 ± 2.94        |
| TNFα     | 15.73 ± 1.45            | 207.38 ± 35.39      | 24.81 ± 7.07            | 144.75 ± 17.46a     |

*a p < 0.05 control versus OVA-challenged groups for WT and Mgat5−/− mice. 

The increased airway reactivity in Mgat5−/− mice is not likely due to exaggerated smooth muscle hypertrophy.

However, allergen-challenged Mgat5−/− mice exhibited a significant increase (2-fold) in the number of neutrophils recruited to the airways compared with WT mice (p < 0.05) (Fig. 1A), which was even more profound when expressed as a percentage of total cells (3-fold higher, p < 0.01) (supplemental Fig. 4A). This was further examined in lung tissue as tissue neutrophilia could potentially contribute to the increased AHR. Lung tissue of allergen-challenged Mgat5−/− mice demonstrated a significant increase in Gr-1 expression, a marker for neutrophils, not only compared with control Mgat5−/− mice, but also allergen-challenged WT mice (p < 0.05), which mount only a modest neutrophil response compared with corresponding unchallenged mice (Fig. 3B). Interestingly, lung tissue Gr-1 expression in control Mgat5−/− mice was higher than in control WT mice and may explain the higher AHR observed in these mice compared with WT counterparts (Fig. 3A). Confocal microscopy with another neutrophil-specific mAb (NIMP-R14) also demonstrated increased neutrophils in lung sections of allergen-challenged Mgat5−/− mice compared with WT counterparts (Fig. 3C). Surprisingly, despite increased neutrophils in BALF and lung tissue, KC levels in the lungs of allergen-challenged Mgat5−/− mice were substantially lower than in WT mice (p < 0.05) (Fig. 3D), suggesting that the increased airway neutrophilia observed in these mice may not be driven by KC levels.

MGAT5 Deficiency Enhances Neutrophilic Inflammation in Response to LPS and TG—In two different models of inflammation that predominantly induce a neutrophilic response, Mgat5−/− mice demonstrated significantly increased neutro-

significant reduction in Th2 cytokines compared with WT mice, the levels of proinflammatory cytokines, IFNγ, IL-17A, and TNFα remain elevated and are nearer to the levels observed in allergen-challenged WT mice.

Allergen-challenged Mgat5−/− Mice Exhibit Increased AHR and Neutrophilic Inflammation—Despite the significant reduction in eosinophilic inflammation (Fig. 1) and Th2 cytokine levels (Table 1) in OVA-challenged Mgat5−/− mice, these mice displayed elevated AHR to inhaled MCh (25 mg/ml) relative to OVA-challenged WT mice (p < 0.05) as assessed by whole body plethysmography (Fig. 3A). Interestingly, airway responsiveness to MCh in control Mgat5−/− mice was higher than in control WT mice, although the difference between these two groups was not found to be statistically significant (p = 0.1 at 25 mg/ml MCh). Immunohistology studies to detect smooth muscle hypertrophy revealed no difference in airway α-smooth muscle actin between control and allergen-challenged WT and Mgat5−/− mice (data not shown), suggesting that the increased airway reactivity in Mgat5−/− mice is not likely due to exaggerated smooth muscle hypertrophy.

However, allergen-challenged Mgat5−/− mice exhibited a significant increase (2-fold) in the number of neutrophils recruited to the airways compared with WT mice (p < 0.05) (Fig. 1A), which was even more profound when expressed as a percentage of total cells (3-fold higher, p < 0.01) (supplemental Fig. 4A). This was further examined in lung tissue as tissue neutrophilia could potentially contribute to the increased AHR. Lung tissue of allergen-challenged Mgat5−/− mice demonstrated a significant increase in Gr-1 expression, a marker for neutrophils, not only compared with control Mgat5−/− mice, but also allergen-challenged WT mice (p < 0.05), which mount only a modest neutrophil response compared with corresponding unchallenged mice (Fig. 3B). Interestingly, lung tissue Gr-1 expression in control Mgat5−/− mice was higher than in control WT mice and may explain the higher AHR observed in these mice compared with WT counterparts (Fig. 3A). Confocal microscopy with another neutrophil-specific mAb (NIMP-R14) also demonstrated increased neutrophils in lung sections of allergen-challenged Mgat5−/− mice compared with WT counterparts (Fig. 3C). Surprisingly, despite increased neutrophils in BALF and lung tissue, KC levels in the lungs of allergen-challenged Mgat5−/− mice were substantially lower than in WT mice (p < 0.05) (Fig. 3D), suggesting that the increased airway neutrophilia observed in these mice may not be driven by KC levels.

MGAT5 Deficiency Enhances Neutrophilic Inflammation in Response to LPS and TG—In two different models of inflammation that predominantly induce a neutrophilic response, Mgat5−/− mice demonstrated significantly increased neutro-
phosphatidylinositol 3-kinase (PI3K) and Akt phosphorylation, which are crucial for the migration and function of neutrophils.

Migration along with Elevated CXCR2 Expression

Migration of neutrophils to the peritoneal cavity was assessed using chemotaxis assays. WT neutrophils demonstrated a 1.3-fold increase compared with Mgat5−/− neutrophils. However, Mgat5−/− neutrophils showed a 4-fold increase in adhesion to P-selectin, whereas WT neutrophils demonstrated only a 1.3-fold increase compared with baseline adhesion under flow conditions (Fig. 4B).

Migration of neutrophils to P-selectin was evaluated using confocal microscopy studies. Mgat5−/− neutrophils exhibited a 4-fold increase in adhesion to P-selectin, whereas WT neutrophils demonstrated only a 1.3-fold increase compared with baseline adhesion under flow conditions (Fig. 5B). Confocal microscopy studies confirmed increased adhesion of Mgat5−/− neutrophils to P-selectin even under static conditions (Fig. 5C), although no difference was detected between Mgat5−/− and WT neutrophils in the expression of PSGL-1 by flow cytometry (supplemental Fig. 4C). Indeed, an increased number of neutrophils recruited to the peritoneum of Mgat5−/− mice was not significantly reduced compared with WT eosinophils (p = 0.19). These findings are similar to those observed in TG-exposed Mgat5−/− versus WT mice (Fig. 4C) and further indicate a differential role for MGAT5-modified N-glycans in eosinophil versus neutrophil recruitment.

Mgat5−/− Neutrophils Exhibit Increased Adhesion and Migration along with Elevated CXCR2 Expression—The basis for the increased recruitment of neutrophils in Mgat5−/− mice was further evaluated. First, WT BM neutrophils exhibited strong binding to FITC-PHA-L compared with the control (FITC-BSA), indicating the expression of branched N-glycans. Mgat5−/− neutrophils also exhibited PHA-L binding relative to control but to a much lesser extent compared with WT cells (supplemental Fig. 4B). This residual binding of Mgat5−/− neutrophils to PHA-L relative to the control could be due to interaction of PHA-L with N-glycans other than MGAT5-modified N-glycans expressed by neutrophils. Next, unlike the decreased rolling observed with Mgat5−/− eosinophils, rolling of Mgat5−/− neutrophils on immobilized P-selectin was similar to that of WT neutrophils under conditions of flow (Fig. 5A).

However, contrary to the decreased adhesion of Mgat5−/− eosinophils (Fig. 2B), Mgat5−/− neutrophils demonstrated a 4-fold increase in adhesion to P-selectin, whereas WT neutrophils demonstrated only a 1.3-fold increase compared with baseline adhesion under flow conditions (Fig. 5B). Confocal microscopy studies confirmed increased adhesion of Mgat5−/− neutrophils to P-selectin even under static conditions (Fig. 5C), although no difference was detected between Mgat5−/− and WT neutrophils in the expression of PSGL-1 by flow cytometry (supplemental Fig. 4C). Indeed, an increased number of neutrophils recruited to the peritoneum of Mgat5−/− mice was not significantly reduced compared with WT eosinophils (p = 0.19). These findings are similar to those observed in TG-exposed Mgat5−/− versus WT mice (Fig. 4C) and further indicate a differential role for MGAT5-modified N-glycans in eosinophil versus neutrophil recruitment.

Mgat5−/− Neutrophils Exhibit Increased Adhesion and Migration along with Elevated CXCR2 Expression—The basis for the increased recruitment of neutrophils in Mgat5−/− mice was further evaluated. First, WT BM neutrophils exhibited strong binding to FITC-PHA-L compared with the control (FITC-BSA), indicating the expression of branched N-glycans. Mgat5−/− neutrophils also exhibited PHA-L binding relative to control but to a much lesser extent compared with WT cells (supplemental Fig. 4B). This residual binding of Mgat5−/− neutrophils to PHA-L relative to the control could be due to interaction of PHA-L with N-glycans other than MGAT5-modified N-glycans expressed by neutrophils. Next, unlike the decreased rolling observed with Mgat5−/− eosinophils, rolling of Mgat5−/− neutrophils on immobilized P-selectin was similar to that of WT neutrophils under conditions of flow (Fig. 5A).

However, contrary to the decreased adhesion of Mgat5−/− eosinophils (Fig. 2B), Mgat5−/− neutrophils demonstrated a 4-fold increase in adhesion to P-selectin, whereas WT neutrophils demonstrated only a 1.3-fold increase compared with baseline adhesion under flow conditions (Fig. 5B). Confocal microscopy studies confirmed increased adhesion of Mgat5−/− neutrophils to P-selectin even under static conditions (Fig. 5C), although no difference was detected between Mgat5−/− and WT neutrophils in the expression of PSGL-1 by flow cytometry (supplemental Fig. 4C). Indeed, an increased number of neutrophils recruited to the peritoneum of Mgat5−/− mice was not significantly reduced compared with WT eosinophils (p = 0.19). These findings are similar to those observed in TG-exposed Mgat5−/− versus WT mice (Fig. 4C) and further indicate a differential role for MGAT5-modified N-glycans in eosinophil versus neutrophil recruitment.
CXCR2 (KC receptor) expression by flow cytometry indicated that MGAT5-elaborated N-glycans and Leukocyte Recruitment. Neutrophils were found to be adhered to the wall of blood vessels in lung sections from allergen-challenged Mgat5<sup>−/−</sup> mice as opposed to WT mice by immunohistology (data not shown). In addition to increased adhesion, Mgat5<sup>−/−</sup> neutrophils also demonstrated significantly increased migration toward KC compared with WT neutrophils (p < 0.05) (Fig. 5D). Analysis of CXCR2 (KC receptor) expression by flow cytometry indicated that after treatment with KC (agonist-induced), Mgat5<sup>−/−</sup> neutrophils demonstrated increased expression of CXCR2 compared with WT neutrophils (Fig. 5E, right panel) and a significantly larger percentage of Mgat5<sup>−/−</sup> neutrophils were positive for CXCR2 expression after treatment with KC compared with WT neutrophils (Fig. 5F), correlating with their increased ability to migrate toward KC. Furthermore, although stimulation with KC resulted in an increase in [Ca<sup>2+</sup>], levels in WT and Mgat5<sup>−/−</sup> neutrophils, stimulated [Ca<sup>2+</sup>], in Mgat5<sup>−/−</sup> neutrophils was significantly higher than in WT neutrophils (p < 0.05) (Fig. 5G). Finally, in contrast to the increased apoptosis of Mgat5<sup>−/−</sup> eosinophils, annexin V staining demonstrated no difference in apoptosis between WT and Mgat5<sup>−/−</sup> neutrophils from TG-exposed mice up to 24 h in culture (Fig. 5H), suggesting that MGAT5-elaborated N-glycans are not critical for survival of recruited neutrophils.

**DISCUSSION**

Whereas the role of eosinophils in the pathogenesis of allergic airway inflammation including asthma is more established (2, 33), several lines of evidence suggest that neutrophils also accumulate in the airways in asthmatic patients with more severe airflow obstruction and in airway secretions during acute severe asthma exacerbations (2) with neutrophil numbers correlating with severity of the disease (3). However, factors regulating the recruitment of eosinophils relative to neutrophils during allergen-induced airway inflammation are unclear. Our studies demonstrate an important role for MGAT5 synthesized N-glycans in regulation of allergen-induced airway inflammation by exerting opposing effects on eosinophil versus neutrophil recruitment. Exposure of Mgat5<sup>−/−</sup> mice to allergen challenge resulted in attenuation of eosinophilic inflammation, lung Th2 cytokines, airway mucus secretion, and expression of proinflammatory molecules. However, AHR remained elevated in these mice with aberrant N-glycan expression and was associated with increased airway neutrophilia, which may be a contributory factor for the increased airway reactivity.

Galectin-3, which binds to N-glycan ligands elaborated by MGAT5 activity (14), has been shown to mediate eosinophil rolling and adhesion by binding to α4 integrins in a carbohydrate-dependent manner (9). Integrins contain multiple MGAT5-modified N-linked glycosylation sites that play a role in regulating integrin clustering, adhesion, and migration (34–37). Alterations in glycan expression on α4 integrins in the absence of MGAT5 may not alter the level of expression of α4 but affect binding of α4 to VCAM-1 or galectin-3 resulting in reduced eosinophil rolling, adhesion, and infiltration of Mgat5<sup>−/−</sup> eosinophils in allergen-challenged airways. Because LFA-1 and Mac-1 do not play a role in mediating rolling and/or adhesion on VCAM-1, the small difference observed between WT and Mgat5<sup>−/−</sup> eosinophils in expression of these receptors is not likely to impact rolling and adhesion on VCAM-1 but may modulate eosinophil trafficking in vivo. Likewise, the reduced expression of L-selectin on Mgat5<sup>−/−</sup> eosinophils...
Mgat5−/− neutrophils exhibit increased adhesion and migration along with elevated CXCR2 expression. Rolling (A) and adhesion (B) of WT and Mgat5−/− neutrophils on immobilized P-selectin under conditions of flow. Adhesion is expressed as fold increase over background (adhesion to PBS). Combined data of three independent experiments performed in duplicate for each condition are shown for WT and Mgat5−/− neutrophils adhered to P-selectin under static conditions. Adherent cells in six fields were counted for each cell type, and combined data of two independent experiments are shown. D, migration of WT and Mgat5−/− neutrophils in response to KC (100 nM) or medium alone (control). Combined data of two experiments performed in triplicate for each condition with neutrophils from two different mice for each group is shown. E, quantitation of WT and Mgat5−/− neutrophils adhered to P-selectin under static conditions. Combined data of three independent experiments performed in duplicate for each condition are shown for WT and Mgat5−/− neutrophils by flow cytometry with PerCP/Cy5.5 anti-mouse CD182 (5 μg/ml) before and after activation with KC (100 ng/ml). Histograms shown are representative of three independent experiments. F, relative expression of CXCR2 on WT versus Mgat5−/− neutrophils by flow cytometry after activation with KC as percent cells positive for CXCR2. Combined data of three independent experiments performed in triplicate for each condition are shown for WT and Mgat5−/− neutrophils by digital videofluorescence imaging with Fura-2 AM. Combined data of three pooled experiments with Mgat5−/− and WT neutrophils (∼500 cells for each group) from three different mice are shown. H, neutrophil survival evaluated flow cytometry based on FITC-annexin V binding by gated neutrophils. Combined data of three independent experiments with neutrophils from three different mice are shown. Data represent mean ± S.E. in A–D and F–H, *p < 0.05 for Mgat5−/− versus WT neutrophils in B, C, D, and F, and versus respective control in A (rolling on PBS) and G (without KC). #, p < 0.05 for Mgat5−/− versus WT neutrophils with KC treatment in G.

could also lead to decreased trafficking and recruitment in vivo. Along with this, levels of [Ca²⁺]i, an important regulator of integrins and cytoskeleton structure in the stabilization of cell adhesion (38), were significantly lower in non-activated Mgat5−/− eosinophils. Finally, decreased survival of Mgat5−/− eosinophils could also contribute to the reduced airway eosinophilia.

Decreased eosinophilic inflammation in allergen-challenged Mgat5−/− mice was associated with decreased lung Th2 cytokines (IL-4, IL-5, and IL-13). This is most likely due to the decreased recruitment of lymphocytes to the airways of allergen-challenged Mgat5−/− mice compared with WT mice (Fig. 1A) leading to inhibition of Th2 responses. Furthermore, mucus secretion and expression of proinflammatory molecules (FIZZ1 and interleukin-1) were also reduced in these mice. Despite this, AHR remained significantly elevated in allergen-challenged Mgat5−/− mice compared with WT mice. With no evidence of exaggerated smooth muscle hypertrophy that could account for the increased airway reactivity in these mice, an alternate and conceivable explanation may be the increased presence of airway neutrophils along with elevated levels of the proinflammatory cytokines IFNγ, IL-17A, and TNFα. The increased neutrophilia in allergen-challenged Mgat5−/− mice was reproducible in other models of neutrophilic inflammation induced by LPS or TG, suggesting that overall MGAT5-elaborated N-glycans may negatively regulate neutrophil recruitment.

Neutrophilia in allergen-challenged Mgat5−/− mice can be attributed to increased adhesion of Mgat5−/− neutrophils to P-selectin. Although the level of expression of PSGL-1 in Mgat5−/− neutrophils was similar to WT neutrophils, cell surface distribution of PSGL-1 or signaling events upon activation may be altered in Mgat5−/− neutrophils and may cause increased adhesion. Alternatively, residual PHA-L binding observed in Mgat5−/− neutrophils in contrast to eosinophils is suggestive of the expression of non-Mgat-5 modified N-glycans.
in the former, thereby promoting neutrophil over eosinophil recruitment. Increased neutrophilia in allergen-challenged Mgat5−/− mice may also be a consequence of the increased ability of Mgat5−/− neutrophils to migrate in response to KC compared with WT neutrophils. Moreover, enhanced migration may be facilitated by the significant increase in KC-induced calcium flux observed in Mgat5−/− neutrophils relative to WT neutrophils. The calcium-signaling pathway plays an important role in neutrophil adhesion and is critical for inflammatory recruitment (38). Increased chemotaxis of Mgat5−/− neutrophils in response to KC may be facilitated by the activation-induced cell surface expression and potentially retention of CXCR2 compared with WT neutrophils. Inhibition of MGAT5 is not limited to leukocytes in Mgat5−/− mice, it is possible that other cell types may also contribute to the attenuated airway inflammation observed in these mice. However, differences in rolling and adhesion on endothelial adhesion molecules in vitro together with the differential recruitment of infused Mgat5−/− neutrophils and eosinophils to the peritoneal cavity of recipient WT mice compared with WT cells in vivo clearly indicate that N-glycans expressed on eosinophils and neutrophils play a significant role in mediating trafficking and migration of these cells during inflammation. Furthermore, these studies provide novel insights into the role of N-glycans in the development of allergic airway inflammation and asthma.

Acknowledgments—We thank Cari M. Calhoun for excellent technical assistance and Dr. Riaz I. Zuberi (Torrey Pines Institute for Molecular Studies, San Diego, CA) for helpful discussions.

REFERENCES

1. Galli, S. J., Tsai, M., and Piliponsky, A. M. (2008) Nature 454, 445–454
2. Fehy, J. V. (2009) Proc. Am. Thorac. Soc. 6, 256–259
3. Alcorn, J. F., Crowe, C. R., and Kolis, J. K. (2010) Ann. Rev. Physiol. 72, 495–516
4. Broide, D. H., Miller, M., Castaneda, D., Nayar, J., Cho, J. Y., Roman, M., Ellis, L. G., and Sriramarao, P. (2002) Am. J. Physiol. Lung Cell Mol. Physiol. 282, L259–266
5. Sperandio, M., Gleissner, C. A., and Ley, K. (2009) Immunol. Rev. 230, 97–113
6. Tanino, Y., Coombe, D. R., Gill, S. E., Kett, W. C., Kajikawa, O., Proudfoot, A. E., Wells, T. N., Parks, W. C., Wight, T. N., Martin, T. R., and Frevert, C. W. (2010) J. Immunol. 184, 2677–2685
7. Mitoma, J., Yao, X., Petryanik, B., Schaeili, P., Gauguet, J. M., Yu, S. Y., Kawashima, H., Saito, H., Ohtsubo, K., Marsh, J. D., Khoo, K. H., von Andrian, U. H., Lowe, J. B., and Fukuda, M. (2007) Nat. Immunol. 8, 409–418
8. Sriramarao, P., von Andrian, U. H., Butcher, E. C., Bourdon, M. A., and Broide, D. H. (1994) J. Immunol. 53, 4238–4246
9. Rao, S. P., Wang, Z., Zuberi, R. I., Sikora, L., Babaie, N. S., Zuraw, B. L., Liu, E. T., and Sriramarao, P. (2007) J. Immunol. 179, 7800–7807
10. Ge, N. H., Babaie, N. S., Kang, B. N., Hosseinkhani, M. R., Ha, S. G., Frenzel, E. M., Liu, F. T., and Sriramarao, P. (2010) J. Immunol. 185, 1205–1214
11. Dennis, J. W., Nabi, I. R., and Demetriou, M. (2009) Cell 139, 1229–1241
12. Grigorian, A., Torossian, S., and Demetriou, M. (2009) Immunol. Rev. 230, 232–246
13. Mkhikian, H., Grigorian, A., Li, C. F., Chen, H. L., Newton, B., Zhou, R. W., Beeton, C., Torossian, S., Tatarian, G. G., Lee, S. U., Lau, K., Walker, E., Simonovitch, K. A., Chandy, K. G., Yu, Z., Dennis, J. W., and Demetriou, M. (2011) Nat. Commun. 2, 334
14. Dennis, J. W., Pawling, J., Cheung, P., Partridge, E., and Demetriou, M. (2002) Biochim. Biophys. Acta 1573, 414–422
15. Lau, K. S., Partridge, E. A., Grigorian, A., Silvescu, C. I., Reinhold, V. N., Demetriou, M., and Dennis, J. W. (2007) Cell 129, 123–134
16. Demetriou, M., Granovsky, M., Quaggan, S., and Dennis, J. W. (2001) Nature 409, 733–739
17. Morgan, R., Gao, G., Pawling, J., Dennis, J. W., Demetriou, M., and Li, B. (2004) J. Immunol. 173, 7200–7208
18. Lee, S. U., Grigorian, A., Pawling, J., Chen, I. J., Gao, G., Mozaffar, T., McKerlie, C., and Demetriou, M. (2007) J. Biol. Chem. 282, 33725–33734
19. Miller, M., Sung, K. L., Muller, W. A., Cho, J. Y., Roman, M., Castaneda, D., Nayar, J., Condon, T., Kim, J., Sriramarao, P., and Broide, D. H. (2001) J. Immunol. 167, 2292–2297
20. Abramoff, M. D., Magalhaes, P. J., and Ram, S. J. (2004) Biophotonics International 11, 36–42
21. Zuberi, R. I., Ge, X. N., Jiang, S., Babaie, N. S., Kang, B. N., Hosseinkhani, R. M., Frenzel, E. M., Fuster, M. M., Esko, J. D., Rao, S. P., and Sriramarao,
MGAT5-modified N-Glycans and Leukocyte Recruitment

P. (2009) ImmunoL. 183, 3971–3979
22. Coxon, A., Rieu, P., Barkalow, F. J., Askari, S., Sharpe, A. H., von Andrian, U. H., Arnaout, M. A., and Mayadas, T. N. (1996) Immunity 5, 653–666
23. Dyer, K. D., Moser, J. M., Czapiga, M., Siegel, S. J., Percopo, C. M., and Rosenberg, H. F. (2008) J. Immunol. 181, 4004–4009
24. Abbitt, K. B., Cotter, M. J., Ridger, V. C., Crossman, D. C., Hellewell, P. G., and Norman, K. E. (2009) J. Leukoc. Biol. 85, 55–63
25. Miyake, K., Medina, K., Ishihara, K., Kimoto, M., Auerbach, R., and Kincaide, P. W. (1991) J. Cell Biol. 114, 557–565
26. Louahed, J., Zhou, Y., Maloy, W. L., Rani, P. U., Weiss, C., Tomer, Y., Vink, A., Renaud, J., Van Snick, J., Nicolaides, N. C., Levitt, R. C., and Haczku, A. (2001) Blood 97, 1035–1042
27. Deshpande, D. A., Dogan, S., Walseth, T. F., Miller, S. M., Amrani, Y., Panettieri, R. A., Kannan, M. S. (2004) Am. J. Respir. Cell Mol. Biol. 31, 36–42
28. Dong, L., Wang, S. J., Camoretti-Mercado, B., Li, H. J., Chen, M., Bi, W. X. (2008) J. Asthma 45, 648–653
29. Gu, J., Isaji, T., Sato, Y., Kariya, Y., Fukuda, T. (2009) Biol. Pharm Bull 32, 780–785
30. Sriramarao, P., DiScipio, R. G., Cobb, R. R., Cybulsky, M., Stachnick, G., Castaneda, D., Elices, M., and Broide, D. H. (2000) Blood 95, 592–601
31. Partridge, E. A., Le Roy, C., Di Guglielmo, G. M., Pawling, J., Cheung, P., Granovsky, M., Nabi, I. R., Wrana, J. L., and Dennis, J. W. (2004) Science 306, 120–124
32. Marley, S. B., Hadley, C. L., and Wakelin, D. (1994) Infect. Immun. 62, 4304–4309
33. Lee, J. I., Dimina, D., Macias, M. P., Ochuk, S. I., McGarry, M. P., O’Neill, K. R., Brotheroe, C., Pero, R., Nguyen, T., Cormier, S. A., Lenkiewicz, E., Colbert, D., Rinaldi, L., Ackerman, S. J., Irvin, C. G., Lee, N. A. (2004) Science 305, 1773–1776
34. Demetriou, M., Nabi, I. R., Coppolino, M., Dedhar, S., Dennis, J. W. (1995) J. Cell Biol. 130, 383–392
35. Granovsky, M., Fata, J., Pawling, J., Muller, W. J., Khokha, R., and Dennis, J. W. (2000) Nat. Med. 6, 306–312
36. Gu, J., Isaji, T., Sato, Y., Kariya, Y., Fukuda, T. (2009) Biol. Pharm Bull 32, 780–785
37. Janik, M. E., Lityńska, A., and Vereecken, P. (2010) Biochim. Biophys. Acta 1800, 545–555
38. Simon, S. I., Sarantos, M. R., Green, C. E., and Schaff, U. Y. (2009) Clin. Exp. Pharmacol. Physiol. 36, 217–224
39. Guo, H. B., Johnson, H., Randolph, M., Lee, I., and Pierce, M. (2009) Glycobiology 19, 547–559
40. Suzuki, H., Prado, G. N., Wilkinson, N., Navarro, J. (1994) J. Biol. Chem. 269, 18263–18266
41. Combadiere, C., Ahuja, S. K., and Murphy, P. M. (1995) J. Biol. Chem. 270, 16491–16494
42. Ouyang, W., Kolls, J. K., Zheng, Y. (2008) Immunity 28, 454–467
43. Thomas, P. S. (2001) Immunol. Cell Biol. 79, 132–140
44. Li, L., Huang, L., Vergis, A. L., Ye, H., Bajwa, A., Narayan, V., Strieter, R. M., Rosin, D. L., and Okusa, M. D. (2010) J. Clin. Invest. 120, 331–342
45. Feiken, E., Rømer, J., Eriksen, J., and Lund, L. R. (1995) J. Invest. Dermatol. 105, 120–123
46. Nocker, R. E., Ouyang, W., Kolls, J. K., Zheng, Y. (2006) Chest 129, 1405–1411
47. Taube, C., Dakhama, A., Takeda, K., Nick, J. A., and Gelfand, E. W. (2003) Chest 123, 4105–4115
48. Tomkinson, A., Cieslewicz, G., Duez, C., Larson, K. A., Lee, J. I., and Gelfand, E. W. (2001) Am. J. Respir. Crit Care Med. 163, 721–730
49. Monteserin, J. (2009) J. Investig. Allergol. Clin. Immunol. 19, 340–354
50. Suzuki, T., Wang, W., Lin, J. T., Shirato, K., Mitsuhashi, H., Inoue, H. (1996) Am. J. Respir. Crit Care Med. 153, 1405–1411
51. Eynott, P. R., Groneberg, D. A., Caramori, G., Adcock, I. M., Donnelly, L. E., Karitnov, S., Barnes, P. J., and Chung, K. F. (2002) Eur. J. Pharmacol. 452, 123–133