

Inhibitory receptor gp49B regulates eosinophil infiltration during allergic inflammation

Hillary H. Norris,* Mary E. Peterson,[†] Chris C. Stebbins,[†] Brittany W. McConchie,*
Virgilio G. Bundoc,* Shweta Trivedi,* Marcus G. Hodges,* Robert M. Anthony,[‡]
Joseph F. Urban Jr.,[‡] Eric O. Long,[†] and Andrea M. Keane-Myers*,¹

Laboratories of *Allergic Diseases and [†]Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, USA; and [‡]Nutrient Requirements and Functions Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, USA

Abstract: gp49B, an Ig-like receptor, negatively regulates the activity of mast cells and neutrophils through cytoplasmic immunoreceptor tyrosine-based inhibition motifs. To characterize the role of gp49B further in vivo, gp49B-deficient mice were tested in two allergic models. Responses to ragweed (RW) challenge in the lung and conjunctiva were assessed in models of allergic inflammation and during an infection with parasitic larvae of the nematode *Ascaris suum*. Infiltration by inflammatory cells into the lung during allergic responses was under negative control of the inhibitory receptor gp49B. Furthermore, an increase in conjunctival inflammation with a predominance of eosinophils, neutrophils, and degranulated mast cells was observed in RW-sensitized, gp49B-deficient mice, which had been challenged in the eye, as compared with C57BL/6 wild-type (WT) controls. Finally, an increase in allergic inflammation in the lungs of *A. suum*-infected, RW-sensitized mice was observed upon RW challenge, as compared with C57BL/6 WT controls. The observed influx of eosinophils into mucus membranes is characteristic of allergic asthma and allergic conjunctivitis and may contribute to airway hyper-responsiveness, airway remodeling, and mucus production. Expression of gp49B was detected on peripheral eosinophils of control mice and on eosinophils from lungs of mice treated with RW, suggesting a role for gp49B on eosinophils in dampening allergic inflammatory responses. *J. Leukoc. Biol.* 82: 000–000; 2007.

Key Words: mouse asthma model · helminth

INTRODUCTION

Allergic conjunctivitis is characterized by itching, swelling, and redness on the mucosal surface of the eye in response to allergen challenge and is one of the most common, initial symptoms reported by allergic patients [1]. Allergic asthma is a closely related, chronic inflammatory lung disease characterized by reversible airway hyper-responsiveness (AHR), mu-

cus secretion, and infiltration of eosinophils [2–6]. The course of asthmatic disease involves an initial influx of inflammatory cells responsible for the tissue remodeling, which leads to long-term airway dysfunction.

Mast cells, neutrophils, and eosinophils play major roles in the development and propagation of the allergic cascade in the lung and eye [1]. A number of inhibitory receptors modulate the activation of these cells (see reviews, refs. [7, 8]). One example of such an inhibitory receptor, the murine gp49B protein, has been shown to negatively regulate mast cells [9–14] during delayed-type hypersensitivity reactions and animal models of synovitis. Neutrophils and NK cells have also been shown to express gp49B [15, 16], which is a transmembrane protein whose cytoplasmic tail contains two immunoreceptor tyrosine-based inhibition motifs [17, 18]. Castells et al. [19], using an in vitro cell-binding assay, determined that the widely expressed integrin $\alpha_3\beta_3$ is a ligand for mouse gp49B on the surface of antigen-induced, activated mast cells.

Rojo et al. [16] demonstrated that naïve NK cells and mast cells from mice genetically depleted (knockout) of gp49B (gp49BKO) develop normally and display similar in vitro activation profiles as cells from wild-type (WT) mice. However, in vivo IgE-dependent mast cell activation in a passive cutaneous anaphylaxis reaction is under negative control by gp49B [12], as mast cells in the gp49BKO mice were hyper-reactive, as shown by increased degranulation following sensitization and challenge. Hyperactive degranulation was also observed in a model of active, cutaneous inflammation, and the study concluded that the activity of mast cells involves a balance between IgE activation and inhibition by the gp49B inhibitory receptor [12]. The role of gp49B in additional models of allergic disease, including airway hyper-reactivity and allergic conjunctivitis, has not been established.

Neutrophils, which are generally the first cells recruited during an inflammatory cascade, infiltrate the lungs in severe

¹ Correspondence: Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Twinbrook II, Room 125, 12441 Parklawn Drive, Rockville, MD 20852, USA. E-mail: akeane@niaid.nih.gov

Received November 9, 2006; revised July 24, 2007; accepted July 25, 2007.
doi: 10.1189/jlb.1106667

cases of allergic asthma, as well as the conjunctiva in allergic eye disease [20, 21]. Zhou et al. [15] detected gp49B recently on the surface of neutrophils and showed increased sensitivity to LPS in vivo in gp49BKO mice. Although this study established that vascular injury in response to LPS was inhibited by gp49B, it is not yet clear whether gp49B inhibits neutrophil infiltration during allergic disease. Furthermore, the role of gp49B in the regulation of eosinophil activity has not been addressed yet.

Eosinophils normally comprise only 1–3% of total blood leukocytes [22]; however, this percentage can increase dramatically in allergic and parasitic disease states [23]. A dynamic equilibrium of eosinophils in the circulation and tissues is maintained by eosinophil production in the bone marrow. Continuous production of eosinophils in the bone marrow is essential for the maintenance of this cell population, as the half-life of eosinophils in the blood is approximately 8 h [24–26]. Mice congenitally deficient of eosinophils revealed a requirement for eosinophils in pulmonary mucus accumulation and AHR in response to allergen challenge [27]. As these mice had a normal complement of mast cells, NK cells, and neutrophils, these studies underscore the importance of the eosinophil in allergic disease manifestations. Studies using a different set of eosinophil KO mice suggest that eosinophils may also be important for the tissue remodeling and restructuring seen in chronic asthma [28]. Once in tissues, murine eosinophils can be activated through a number of receptors, including receptors for IgG [29]. Exposure to soluble stimuli such as cytokines (GM-CSF and IL-5) and eosinophil granule proteins also results in eosinophil activation [30–33]. Although the mechanism of receptor-mediated activation of eosinophils has been studied extensively, the role of inhibitory receptors on eosinophils in dampening allergic inflammatory responses continues to be explored.

In these studies, we used a mouse model of allergic inflammation to determine the role of gp49B in regulating infiltration of inflammatory cells into the conjunctiva and lungs following ragweed (RW) allergen sensitization and challenge. Increased neutrophil and eosinophil infiltration, mast cell degranulation in the conjunctiva, as well as an overall increase in lung inflammation characterize the response of gp49BKO mice. In addition, we show here that mouse eosinophils express the gp49B inhibitory receptor on their surface, suggesting that this inhibitory receptor may play an important role in eosinophil regulation.

MATERIALS AND METHODS

Mice

All mice were housed and maintained in the Comparative Medicine Branch of the National Institute of Allergy and Infectious Diseases [NIAID; National Institutes of Health (NIH), Twinbrook II Facility, Rockville, MD, USA]. C57BL/6 control mice were obtained from Taconic (Germantown, NY, USA) or were gp49B^{+/+} littermate control mice. gp49BKO mice [16] were backcrossed to homogeneity with C57BL/6 mice for 10 generations. An IL-5 transgenic mouse line (IL-5 Tg; NJ.1638), which produces high levels of IL-5 from their T cells and has eosinophils, as over 60% of the circulating leukocytes, were a generous gift of James J. Lee (Mayo Clinic College of Medicine, Rochester, MN, USA) [34]. Male IL-5 Tg mice were bred with female gp49BKO, and

offspring were screened for the IL-5 Tg and for the lack of the gp49B inhibitory receptor. These studies conformed to the principles for laboratory animal research outlined by the Animal Welfare Act and were approved by the NIAID Animal Care and Use Committee. Each group contained five to 10 mice, and each experiment was repeated two to three times.

Ascaris suum infection

A. suum eggs were obtained from adult worms isolated from infected pigs, and the eggs were developed to the infective stage in vitro [35]. To model the chronic exposure to infective eggs, which occurs in patients in *Ascaris*-endemic countries, and to enhance eosinophil production substantially, mice were infected with *A. suum* eggs two to three times per week from Day –44 until Day 0 and then sensitized concurrently with an i.p. injection of 50 µg RW (1 mg/ml) in 100 µl aluminum hydroxide (alum; *A. suum*+RW group) [36]. Control mice were sensitized with PBS + alum (PBS group), infected with *A. suum* and sham-sensitized (*A. suum* group), or given RW sensitization without *A. suum* infection (RW group).

Antigen challenge

RW sensitization and challenge were used to elicit an ocular and/or bronchial allergic response. *A. suum*-infected WT and gp49BKO mice were sensitized on Days 0 and 5 with a 200-µl i.p. injection containing 50 µg RW extract (Greer Laboratories, Lenoir, NC, USA), emulsified with an equal volume (100 µl/antigen injection) of alum (Pierce, Rockford, IL, USA) using a method, which has been published previously [37]. Control mice were sensitized i.p. with the same quantity of alum mixed with PBS vehicle to control for any nonspecific responses to alum administration. The mice were subsequently challenged on Days 14 and 15 with 1 mg RW extract in PBS drop-wise in the eye (5 µl/eye) and 50 µg RW extract in PBS by intratracheal (i.t.) or intranasal (i.n.) inoculation (30 µl). Mice were killed 45 min postsecondary eye challenge to evaluate the degranulation of mast cells and 72 h postchallenge to evaluate cellular infiltration into the conjunctiva. In all cases, mice were killed by anesthesia with ketamine (Fort Dodge Animal Health, Fort Dodge, IA, USA)/xylazine (Phoenix Pharmaceuticals, St. Joseph, MO, USA; 100 mg/kg and 10 mg/kg, respectively), followed by exsanguination.

Cytokine analysis

For restimulation assays, following lysis of RBC, 2×10^6 splenocytes were added to a 24-well plate and incubated in 2 ml media alone, media, and Con A (2.5 µg/ml, Sigma Chemical Co., St. Louis, MO, USA) as a positive control for T cell stimulation or media and RW (100 µg/ml) for 48 h at 37°C at 5% CO₂. The media used consisted of RPMI 1640 with glutamax, gentamicin (20 µg/ml), 0.01 M HEPES, and β-ME (all from Invitrogen, Carlsbad, CA, USA) and 10% FBS (Hyclone, Logan, UT, USA). At the 48-h time-point, 500 µl aliquots were removed from the plate, and the supernatant was collected following centrifugation at 2700 g for 5 min. The in vitro levels of IL-5 and IL-13 were measured using the Biosource Multiplex assay kit (Biosource International, Camarillo, CA, USA) with the Luminex reader (Qiagen, Valencia, CA, USA). For cytokine analysis in the bronchoalveolar lavage fluid (BALF), the lungs were washed with 500 µl PBS + 1% FBS, and the supernatant was collected following centrifugation at 2700 g for 5 min. The levels of IL-4, IL-13, and IFN-γ in the BALF were measured using the Lincplex Multiplex assay kit (Millipore, Billerica, MA, USA) with the Luminex reader (Qiagen).

Quantitative measurement of sera antibody isotypes by luminex technology

Antibody isotypes (IgG1, IgG2a, and IgE) were measured using the Beadlyte mouse Ig isotyping kit (Upstate, Temecula, CA, USA), as per the manufacturer's instructions. All samples, including standards, were assayed in duplicate. Fluorescence was measured by using the Luminex reader (Qiagen).

Histology

To assess the cellular infiltrate in the conjunctiva and surrounding tissue, the eyes and attached lids were removed intact from killed mice and immediately fixed in 10% neutral-buffered formalin (EMD Chemicals, Gibbstown, NJ, USA). This process was performed 45 min postchallenge to assess mast cell

degranulation and 72 h postchallenge to assess eosinophil and neutrophil infiltration. Fixed tissues were embedded in paraffin and stained by American Histolabs (Gaithersburg, MD, USA) with giemsa or H&E to assess general inflammation using a methodology, which has been published previously by different authors [36, 37]. Slides were masked, and five sections of conjunctiva from each mouse were scored by a single examiner in four nonoverlapping fields (including the conjunctival fornix and the presence of goblet cells as anatomical landmarks) at 40× magnification [36]. To assess lung inflammation, the lungs were removed and fixed in 10% neutral-buffered formalin. Slides were stained with H&E for generalized inflammation or periodic-acid Schiff (PAS) for determination of mucus production. The slides were masked, and lung slides from a minimum of five mice per group were examined and assigned an inflammatory lung score (**Table 1**) [38]. Slides were examined and photographed at high (20×) and low (5×) power to allow for evaluation of overall lung pathology and infiltration of specific cell types.

Eosinophil enrichment

Peripheral blood was collected from naïve C57BL/6 mice by exsanguination, added to Percoll E (Amersham Biosciences, Piscataway, NJ, USA), and centrifuged at 1500 *g* for 45 min to enrich for peripheral eosinophils [39]. The buffy coat was removed, and remaining RBC were lysed using RBC lysis buffer (eBiosciences, San Diego, CA, USA). The cells were incubated with anti-CD45 beads (B220), anti-CD90 beads (Thy1.2), and anti-TER119 beads (Miltenyi Biotec, Auburn, CA, USA) to remove B cells, T cells, and any residual RBC by AutoMACS depletion (Miltenyi Biotec).

Flow cytometry, cell sorting, and fixed cell staining

For FACS analysis on whole lung homogenates, the following antibodies were used: anti-Ly-6G allophycocyanin (APC)-conjugated (Gr-1; clone RB6-3C5, BD Biosciences, San Jose, CA, USA), anti-Siglec-F PE-conjugated (clone E50-2440, BD Biosciences), and rabbit anti-mouse gp49 [16] detected with donkey anti-rabbit-FITC (Jackson ImmunoResearch, West Grove, PA, USA). The isotype control antibody was rabbit anti-human killer inhibitory receptor used at the same concentration. To enhance the number of eosinophils found in the circulation of naïve mice, the cells used (see Fig. 5, A–C) were IL-5 Tg (denoted as IL-5tg WT), and the gp49KO cells were extracted from IL-5 Tg × gp49BKO (denoted as IL-5tg/BKO). The circulating cells (see Fig. 5, D and E) were extracted from naïve WT C57BL/6 mice using the eosinophil enrichment protocol and were sorted for Siglec-F⁺ staining. Once the cells were sorted, they were stained with H&E and shown to be enriched to 92% eosinophils (data not shown).

Whole lung homogenates were processed from WT and gp49BKO mice after sensitization and challenge with RW. Briefly, lung lobes were excised, minced, and digested in RPMI-1640 media supplemented with 2% FCS, 1 mg/ml collagenase A, and 50 units/ml DNase for 1 h at 37°C. Digested lung fragments were then strained through mesh screens to obtain single cell suspensions. The lung digests were incubated with Fc block and labeled with the appropriate fluorochrome-labeled antibodies, and the cells were then analyzed by flow cytometry [40]. Data were collected using a FACSCaliber flow cytometer (BD Biosciences). Data analysis was performed using CELLQuest software (BD Biosciences) and FlowJo software (Tree Star, Inc., Ashland, OR, USA). Cell sorting of the Siglec-F⁺ cells was performed using the FACS Aria flow cytometer/cell sorter (BD Biosciences).

TABLE 1. Scoring Chart for Lung Inflammation

Score	Description
0	Normal lung, minor inflammation, no goblet cells
1	Minor PVC ^a
2	Moderate PVC and PBC ^b
3	Increased PVC and PBC, increased goblet cells, smaller airways
4	Severe PVC and PBC, goblet cell infiltration into small and large airways

^a Perivascular cuffing; ^b peribronchial cuffing.

Western blot

Siglec-F⁺ cells (2×10⁷) from WT mice and the equivalent protein from 6-week-old, in vitro-cultured bone marrow-derived mast cells (BMMC), derived from gp49BKO and WT mice, were lysed in 1 ml each lysis buffer (0.5% Triton X, 150 mM NaCl, 50 mM Tris, pH 7.4, 2 mM EGTA, and 1 mM PMSF, Sigma Chemical Co.). Protein concentration was performed prior to Protein G Sepharose (PGS) isolation. Nuclei were pelleted, and the lysate was incubated with PGS for 1 h to remove the Siglec-F antibody. Comparable amounts of protein (determined by dendritic cell protein assay, Bio-Rad, Hercules, CA, USA) were incubated with 1 µg hamster anti-mouse gp49 (BD Biosciences; clone H1.1) and fresh PGS for 1 h. Sepharose beads were washed two times in lysis buffer. Protein was eluted in 1× NuPAGE lithium dodecyl sulfate sample buffer with 1× reducing agent (Invitrogen). Lysates were incubated at 70°C for 10 min and run on a 10% NuPAGE Bis Tris gel (Invitrogen) at 180 volts. Proteins were transferred to membrane (Immobilon-P, Millipore, Bedford, MA, USA) for 1 h at 30 volts in 1× NuPAGE transfer buffer. Blot was blocked in blocking buffer (5% BSA/0.1% Tween 20/1×PBS) for 30 min. Polyclonal rabbit anti-gp49B tail antibody was diluted (1 µg/ml) in blocking buffer and incubated with blot for 1 h. After 3- to 5-min washes, HRP-conjugated anti-rabbit Ig (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was diluted 1/10,000 and incubated for 30 min. After 3- to 5-min washes, blot was incubated in 3 ml Super Signal West Pico solution (Pierce) and exposed to film (Biomax MR, Kodak, Rochester NY, USA). The blot for gp49A was run in parallel with the gp49B blot. gp49A antisera were used at 1 µg/ml and were developed as stated above for gp49B.

Statistical analysis

Values are expressed as the mean ± SEM. Unless otherwise specified, differences among groups were analyzed using ANOVA, followed by a post-hoc Tukey's Multiple Comparison test. For specific comparisons between two groups, sera (IgE, IgG1, and IgG2a) were analyzed using a Mann-Whitney *t*-test. In all cases, *P* values <0.05 were considered significant by Graphpad Prism 4 software (Graphpad, Inc., San Diego, CA, USA).

RESULTS

Increased ocular neutrophilia, eosinophilia, and mast cell degranulation in gp49BKO mice following RW sensitization and challenge

An 18-day RW sensitization and challenge protocol [36] was used to elicit an ocular allergic response and to verify the role of gp49B in allergic inflammation (**Fig. 1A** and Materials and Methods). The number of intact and degranulated mast cells, which reacted to the allergen prior to neutrophil and eosinophil infiltration, was determined 45 min after ocular challenge with RW. Consistent with published data using passive and active cutaneous inflammation models [12], a significant increase in the number of degranulated mast cells was observed in the RW-treated gp49BKO mice as compared with the RW-treated WT controls (Fig. 1B). The role of gp49B, however, had not been shown previously in an allergen-induced model of pulmonary hypereosinophilia or ocular allergic disease.

Conjunctiva were also examined for cellular infiltration, 72 h post-RW challenge. A marked difference was observed in the number of infiltrating neutrophils into the conjunctiva of WT and gp49BKO mice, which had been sham-treated with PBS. RW-treated WT mice did not have any significant increases in neutrophils at 72 h post-RW challenge compared with sham-sensitized WT mice. Similar to what occurs in human disease, in our mouse conjunctivitis model, neutrophils infiltrate the conjunctiva during the early response (within 6 h) but are not

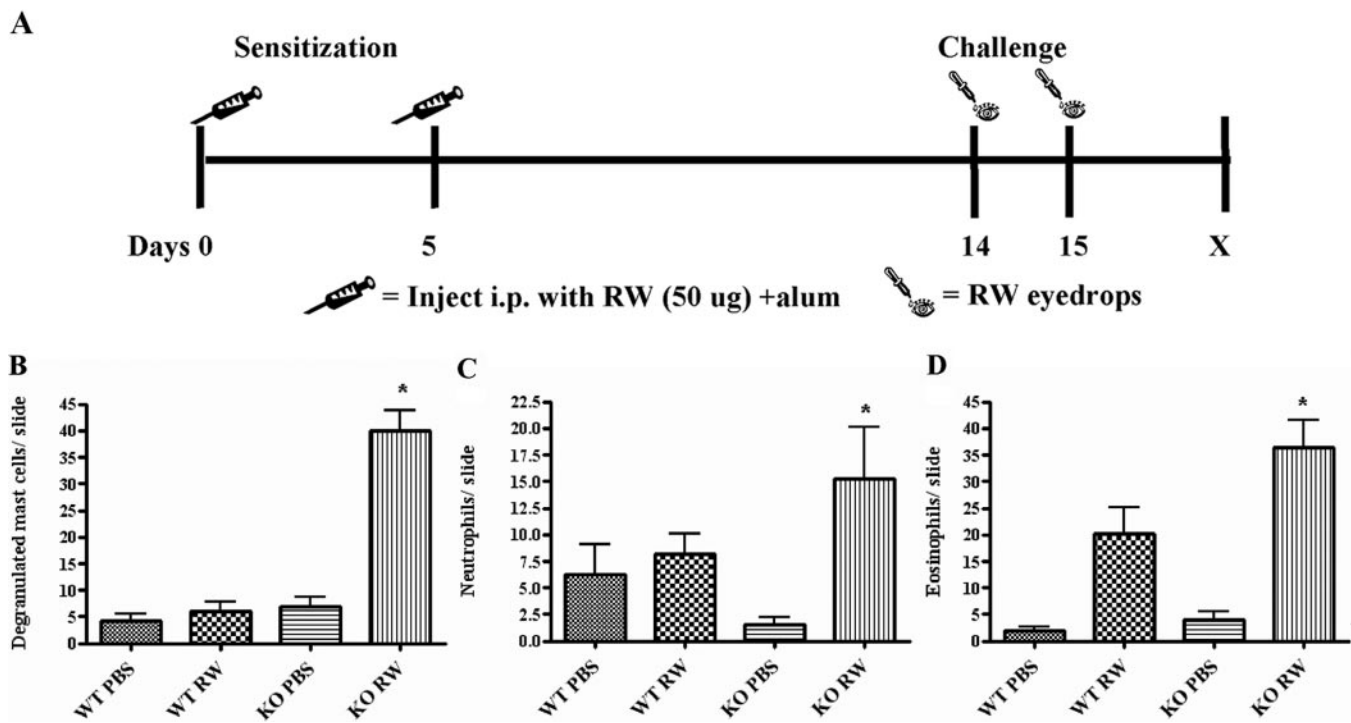


Fig. 1. Conjunctival infiltration following RW sensitization and challenge. (A) RW sensitization and challenge protocol in the conjunctiva. (B) The total number of degranulated mast cells was determined by examining the conjunctival matrix under Wright's-Giemsa stain, 45 min postchallenge. A significant increase in the number of degranulated mast cells was observed in the RW-treated gp49BKO mice as compared with the RW-treated WT mice ($P < 0.001$). A significant increase in the number of (C) neutrophils ($P < 0.05$) and (D) eosinophils ($P < 0.001$) was observed in the conjunctiva of the RW-treated gp49BKO mice compared with WT RW-treated mice, 72 h postallergen challenges. Results are expressed as the number of neutrophils or eosinophils, which had infiltrated the conjunctival matrix per slide. This is a representative of three separate experiments.

present in significant numbers during the late-phase response. As neutrophils are not normally present in increased numbers in the conjunctiva 72 h after challenge in WT mice, the significant increase in the number of neutrophils observed in the conjunctiva of the RW-treated gp49BKO mice compared with RW-treated WT mice was particularly striking (Fig. 1C). These data demonstrate that gp49B inhibits neutrophil recruitment in an ocular allergy model as well as suppressing mast cell degranulation during the immediate phase of the allergic response.

Examination of eosinophil infiltration 72 h after allergen challenge revealed an increase in eosinophil migration in RW-treated WT mice over of the PBS WT control mice (Fig. 1D). The increase in eosinophil migration in response to allergen challenge was enhanced further in the conjunctiva of gp49BKO mice as compared with RW-treated WT mice. These data imply that in addition to regulatory roles in mast cell and neutrophil activity, gp49B may act as an inhibitory receptor on eosinophils, which have been shown to be a key effector cell in the allergic cascade [3], suggesting that the expression of an inhibitory receptor on eosinophils would be essential for modulating ligand-regulated, allergic responses.

In vitro cytokine levels in gp49BKO mice

Development, activation, and infiltration of immune effector cells depend on the production of cytokines produced by several types of immune cells including dendritic cells, T cells, and B cells. To determine if cytokine-producing lymphocytes

were altered systemically following RW sensitization and challenge of gp49BKO mice, splenocytes from WT and gp49BKO mice were restimulated in vitro with RW. There was no significant difference in the production of the allergy-promoting cytokines IL-5 and IL-13 [41, 42], measured between the WT and the gp49BKO mice in response to RW restimulation (data not shown), suggesting the observed differences were not a result of T cell-derived cytokines.

Lung inflammation following RW sensitization and challenge

An 18-day RW sensitization and challenge protocol for the lung was used to elicit a bronchial allergic response and to test for a role of gp49B in allergic inflammation (Fig. 2A and Materials and Methods). Lung inflammation was evaluated 72 h after challenge. Lungs were removed from the PBS or RW-treated WT and gp49BKO mice, and PAS and H&E-stained histology sections were evaluated using an inflammatory lung score on a scale of 0–4 [38]. Table 1 lists the criteria used for scoring inflammation. Lung inflammation in WT and gp49BKO mice treated with PBS was minimal [lung scores of 0.19 ($SE \pm 0.12$) and 0 ($SE 0$), respectively], and WT RW-treated mice showed clear inflammation [lung score of 2.65 ($SE \pm 0.19$); Table 2]. Lungs from gp49BKO mice demonstrated higher inflammation as compared with the WT controls following RW challenge [lung score of 3.22 ($SE \pm 0.21$)] however, the increase inflammatory scores did not reach statistical significance ($P > 0.05$).

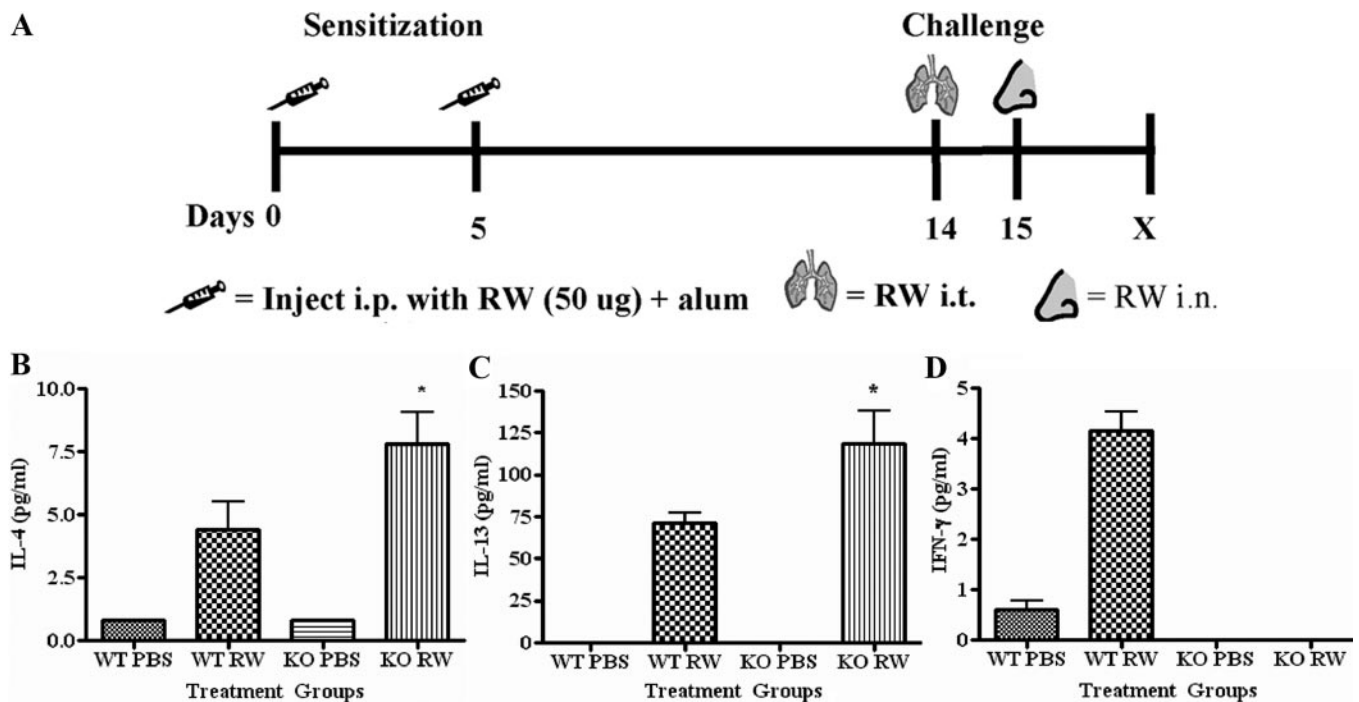


Fig. 2. Allergen-induced lung pathology. (A) RW sensitization and challenge protocol in the lung. BALF was collected in 500 μ l PBS + 1% FBS, 72 h postchallenge from PBS and RW-treated WT and gp49BKO mice. (B) IL-4, (C) IL-13, and (D) IFN- γ levels were measured. RW sensitization and challenge increased the production of IL-4 ($P < 0.05$) and IL-13 ($P < 0.01$) significantly in the BALF of RW-treated gp49BKO mice versus WT control mice. The level of IFN- γ in the BALF from RW-treated WT animals was increased significantly over PBS-treated WT controls ($P < 0.001$). However, the level of IFN- γ in gp49BKO PBS or gp49BKO RW animals was below the level of assay detection. Each group contained $n = 5$ –8. This is a representative example of three independent experiments.

Cytokine levels were measured ex vivo from the BALF in mice after allergen challenge. The Th1 and Th2 cytokines were detected in the BALF of mice, which had been lavaged with 500 μ l PBS + 1% FBS. We have found this lavage volume necessary to achieve a consistent sample size between animals. However, this volume significantly dilutes the cytokine concentration in the samples. gp49BKO mice displayed significantly higher levels of IL-4 (Fig. 2B) and IL-13 (Fig. 2C) detected in the BALF following RW sensitization and challenge, as compared with the similarly treated WT control mice. However, the concentration of IL-4 was low in all samples. Eosinophils have been shown previously to increase the production of IL-13 in BALF in models of eosinophil pulmonary inflammation [43, 44]. In addition, eosinophils themselves have been found recently to be a significant source of IL-13 in a mouse model of liver fibrosis as a result of infection with the helminth *Schistosoma mansoni* [45]. The level of the Th1 cytokine IFN- γ (Fig. 2D) in the BALF from WT RW animals

was increased significantly in WT RW mice over WT PBS controls. However, the level of IFN- γ in gp49BKO PBS or gp49BKO RW animals was below the level of assay detection. This finding should not be construed as saying the animals did not make any IFN- γ in the lung, just that the mice likely had an exceedingly low concentration of IFN- γ in the lung lumen and that the dilution volume prohibited the assessment of the cytokine with the assay used. Overall, these findings suggest that gp49 expression may be important for local expression of Th1 cytokines in response to antigen challenge

Analysis of antibody isotypes from sera

The potent Type 2 cytokine response associated with allergen sensitization and challenge is known to induce isotype class-switching in B cells and the induction of polyclonal IgG1 and IgE [46]. As the production of these antibody isotypes are dependent on B cell exposure to IL-4 and IL-13, the concentration of these polyclonal antibodies can be used as an indirect measure of Th2 cytokine production. There was a significant increase in the concentration of IgG1 (Fig. 3A, $P < 0.001$) and IgE (Fig. 3B, $P < 0.001$) in the sera of WT and gp49BKO mice exposed to RW compared with PBS-treated control mice. However, RW-exposed WT mice had significantly higher IgE ($P < 0.0001$) compared with RW-exposed gp49BKO mice. There was no significant difference in the IFN- γ -dependent antibody isotype IgG2a in any of the mouse groups examined (Fig. 3C, $P > 0.05$), suggesting that although the local IFN- γ response in the BALF was reduced in

TABLE 2. Lung Inflammatory Scores following RW Sensitization and Challenge

Mice	Treatment	Inflammatory lung score	+/- SEM
WT	PBS	0.19	0.12
gp49BKO	PBS	0	0
WT	RW	2.65	0.19
gp49BKO	RW	3.22; $P > 0.5$	0.21

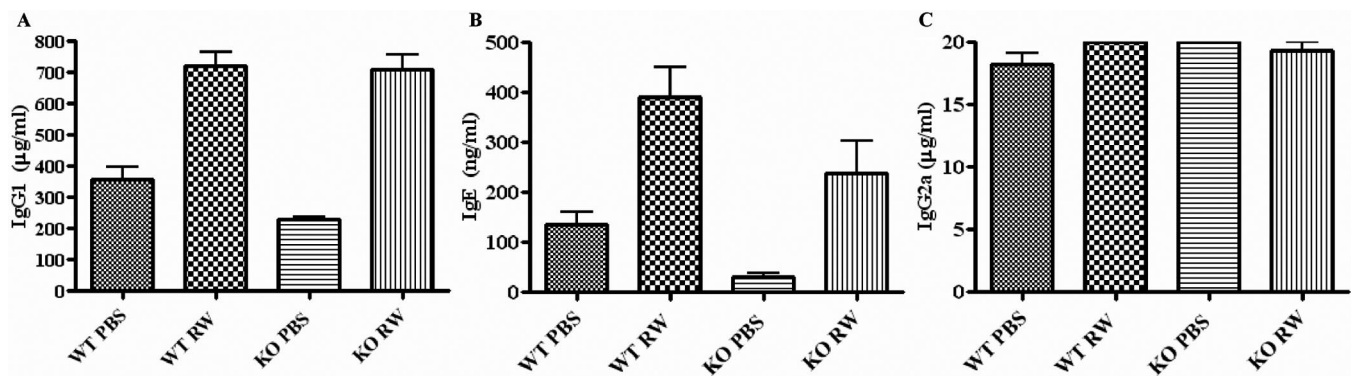


Fig. 3. Antibody isotypes in the sera from WT and gp49BKO mice treated with RW and PBS. Antibody isotypes (IgG1, IgG2a, and IgE) were measured in the sera of mice collected 72 h postallergen sensitization and challenge in the lung and eye. RW sensitization and challenge in WT mice significantly increased the level of (A) IL-4-dependent polyclonal IgG1 ($P < 0.001$) and (B) IgE ($P < 0.05$) but not (C) IFN- γ -dependent IgG2a ($P > 0.05$) compared with WT PBS control mice. RW sensitization and challenge also increased the concentration of (A) Th2 cytokine-dependent polyclonal IgG1 ($P < 0.001$) and (B) IgE ($P < 0.05$) but not (C) Th1 cytokine-dependent IgG2a ($P > 0.05$) in gp49BKO mice compared with gp49BKO PBS control mice. There was no significant difference between RW-treated WT and gp49BKO mice in any of the antibody isotypes.

gp49BKO mice in response to RW challenge, the systemic expression of IFN- γ was not altered.

Lung inflammation following parasite-mediated response

Previous studies from our group demonstrated that RW-exposed mice infected with *A. suum* had an enhanced eosinophil and allergic response in the conjunctiva following an ocular challenge with RW [36]. In the current experiments, this model (Fig. 4A) was used to amplify the number of eosinophils infiltrating the lung of WT (Fig. 4, B and C) and gp49BKO mice (Fig. 4, D and E). Lungs were removed from the PBS or *A. suum* + RW-treated mice at 72 h after RW challenge. Minimal

inflammation occurred in the lungs WT and gp49BKO mice treated and exposed to PBS (Fig. 4, B and C, respectively). In contrast, extensive inflammation and a preponderance of eosinophil and neutrophil infiltration were observed in lungs of WT mice infected with *A. suum* and treated with RW (Fig. 4, D, 200 $\times$, and F, 400 $\times$). RW-exposed and -challenged gp49BKO mice infected with *A. suum*, however, had an even further increase in inflammatory cell infiltrates (as evidenced by PVC and PBC) as well as enhanced interstitial inflammation (Fig. 4, E, 200 $\times$, and G, 400 $\times$). Pulmonary inflammation and infiltration in the RW + *A. suum*-treated gp49BKO mice included an abundance of plasma cells and macrophages, increased mucus production, and a significant increase in lung

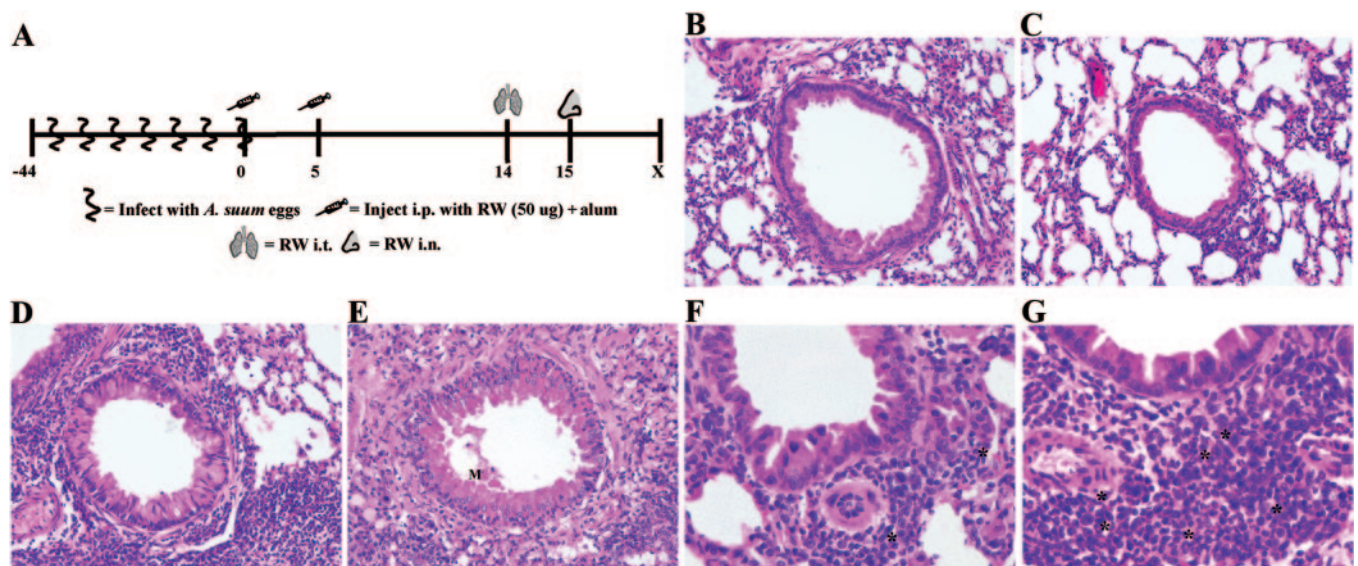


Fig. 4. *A. suum* and RW model of allergic inflammation. (A) *A. suum* and RW sensitization and challenge protocol in the lung. (B–G) PAS-stained sections of (B) WT lungs treated with PBS (200 $\times$), (C) gp49BKO lungs treated with PBS (200 $\times$), (D and F) *A. suum*-infected, RW-challenged WT lungs (D, 200 $\times$, and F, 400 $\times$), and (E and G) *A. suum*-infected, RW-challenged gp49BKO lungs (E, 200 $\times$, and G, 400 $\times$). The *A. suum*-infected, RW-challenged gp49BKO lungs demonstrated increased lung pathology post-RW treatment, including increased mucus production (indicated by M) and increased eosinophil infiltration (indicated by *) compared with *A. suum*-infected, RW-treated WT control mice ($P < 0.01$). These data are a representative of two independent experiments. Each group contained $n = 8$ –10.

TABLE 3. *A. suum* and RW Allergic Inflammation Lung Scores

Mice	Treatment	Inflammatory lung score	+/- SEM
WT	PBS	0.81	0.26
gp49BKO	PBS	1.06	0.16
WT	<i>A. suum</i> + RW	2.64	0.17
gp49BKO	<i>A. suum</i> + RW	3.82; $P < 0.01$	0.05

scores compared with similarly treated WT mice. Histology sections were evaluated and assigned an inflammatory lung score on a scale of 0–4 (Table 3).

Eosinophils isolated from WT naïve peripheral blood express gp49B

Expression of Siglec-F on the surface of mouse peripheral blood eosinophils provides a means of distinguishing eosino-

phils from neutrophils by flow cytometry [47]. The increased infiltration of eosinophils into the conjunctiva of gp49BKO mice suggested that eosinophils may express inhibitory gp49B. To test this possibility, WT IL-5 Tg mice were used to increase the number of naïve eosinophils in the circulation, and eosinophils were enriched further from the peripheral blood by Percoll E gradient and RBC lysis [34]. In a representative experiment, ~50% of the eosinophil-enriched population from naïve IL-5 Tg mice was forward-scatter (FSC)^{hi} and Siglec-F⁺ (Fig. 5A, upper panel). To generate sufficient numbers of naïve eosinophils deficient in the gp49B, IL-5 Tg mice were crossed with gp49BKO mice. In a representative experiment, ~54% of the eosinophil-enriched population from these eosinophil-enriched gp49BKO mice was FSC^{hi} and Siglec-F⁺ (Fig. 5A, lower panel). Eosinophils from IL-5 Tg/gp49BKO mice were negative for gp49, as they did not stain for the gp49 antibody compared with staining with an isotype control anti-

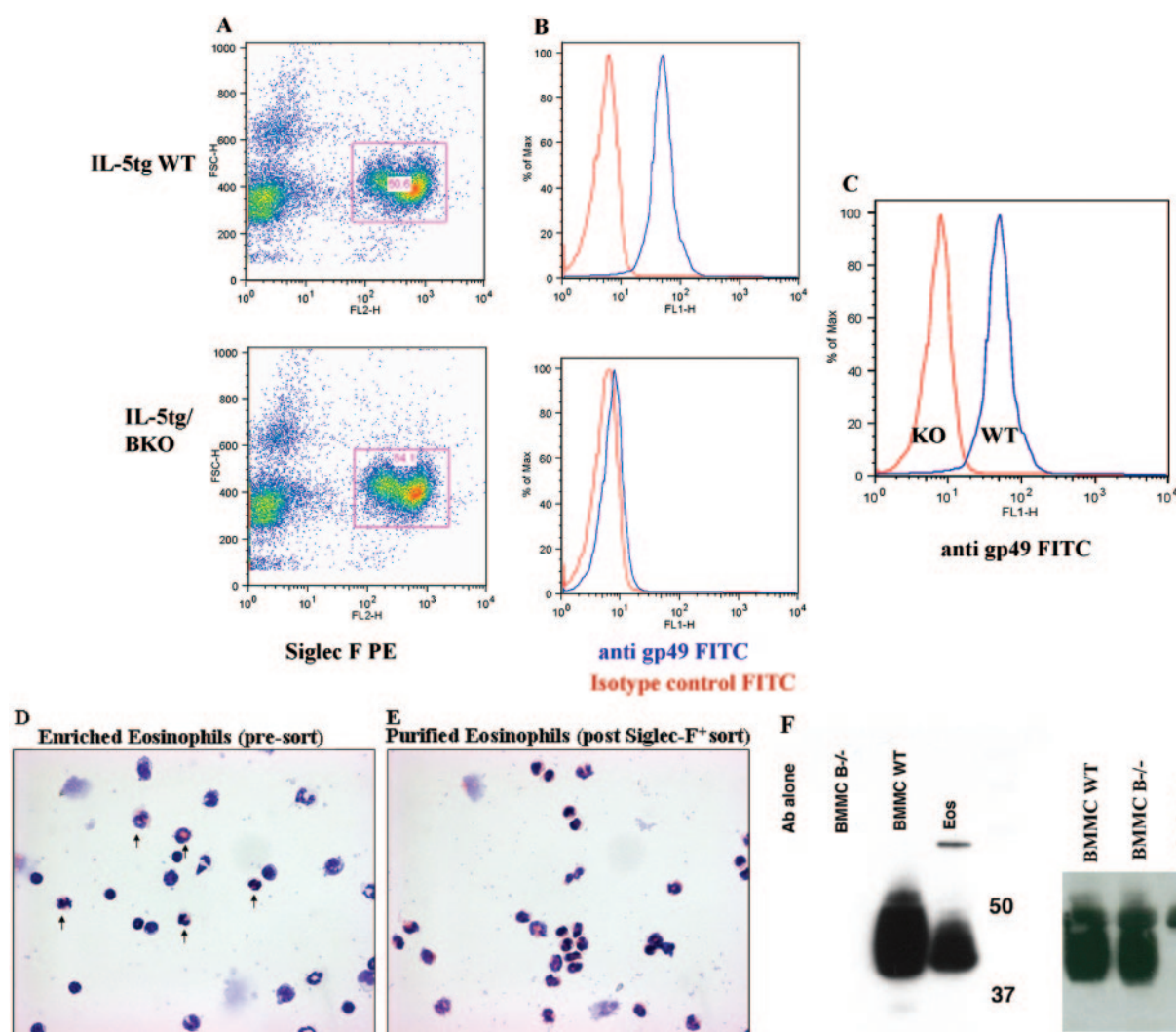


Fig. 5. Expression of gp49B on peripheral blood eosinophils. IL-5 Tg mice were crossed with gp49BKO mice to produce naïve mice with large numbers of gp49B-negative eosinophils. (A–C) Flow cytometric analysis of eosinophils enriched from whole blood of IL-5 Tg gp49B^{+/+} (WT) and IL-5 Tg × gp49BKO mice. (A) Siglec F was used as a marker for murine eosinophils. The Siglec-F^{hi} cells consisted of ~90% eosinophils. (B and C) The Siglec-F⁺ cells express gp49 (blue) on the cell surface of WT mice but not KO mice compared with an isotype control (red). Cells were pooled from four mice, and this is a representative of three independent experiments. Cytospin picture of WT C57BL/6 Siglec-F⁺ cells (D) presort and (E) postsort. Note the predominance of granulocytes with bi-lobed nuclei (eosinophils) in the postsort cytospin compared with cells examined in the presort analysis. (F) Western blot immunoprecipitated samples of equivalent protein concentrations from WT and gp49BKO BMMC and eosinophils from WT mice. BMMC from gp49BKO mice were used as controls for antibody specificity.

body [Fig. 5B, lower panel, gp49⁺ cells (blue), vs. isotype control (red)]. Conversely, when peripheral blood eosinophils from IL-5 Tg mice were analyzed for gp49 expression, they were uniformly positive for gp49 compared with staining with an isotype antibody [Fig. 5B, upper panel, IL-5 Tg gp49⁺ cells (blue), vs. isotype control (red)]. In addition to using isotype controls to verify gp49 receptor expression, the positive staining of gp49 on IL-5 Tg cells was confirmed by comparing staining intensity for anti-gp49 antibodies in WT mice with the gp49BKO mice as a physiologic control [Fig. 5C, WT gp49⁺ cells (blue), vs. gp49BKO control cells (red)], as all the antibodies (primary, secondary, etc.) used for staining cells from WT and gp49BKO mice were the same. The expression of Siglec-F on the surface of WT C57BL/6 mouse peripheral blood eosinophils was verified by postsort analysis of Siglec F⁺ cells by H&E staining of this isolated cell preparation on cytopsin slides of presorted, eosinophil-enriched blood (Fig. 5D) and Siglec-F⁺ eosinophils post-FACS sort (Fig. 5E). Histological analysis of these cytopsin slides did not reveal any significant changes in percentage of systemic neutrophils or eosinophils in the gp49BKO animals compared with WT controls. Therefore, gp49B may act as a negative regulator of neutrophil and eosinophil activation but may not affect cellular production.

As the gp49 antibody used in FACS does not distinguish between gp49B and the closely related, noninhibitory family member gp49A, Western blot was performed using a gp49B-specific antibody to verify that SiglecF⁺-sorted WT eosinophils were gp49B⁺ (Fig. 5F). BMMC from WT mice were used as the positive control for anti-gp49B antibody specificity in the Western blot, as mast cells have been shown previously to express the gp49B receptor [9–14]. BMMC from gp49BKO mice were used as negative controls, as these cells do not express the gp49B receptor. These data demonstrate that gp49B is present on the surface of naïve Siglec-F⁺ eosinophils in peripheral blood. A second immunoblot was run in parallel to that used in Figure 5F and was blotted with anti gp49A sera. BMMC from WT and gp49BKO mice have gp49A expression, but even under conditions of overexposure, eosinophils from gp49B or WT express no detectable gp49A (Fig. 5F).

Eosinophils in lung homogenates of WT RW-treated mice express gp49B

Mice have increased numbers of eosinophils, neutrophils, lymphocytes, and mucus-producing goblet cells in their lungs after allergen challenge but few mast cells [48]. Although Siglec-F has been used to identify naïve eosinophils in peripheral murine blood [47], it is unknown whether activated eosinophils in the tissue express Siglec-F. To determine if Siglec-F is expressed on eosinophils, which have migrated into the tissue following RW sensitization and challenge, whole lung homogenates were analyzed by flow cytometry. In a representative experiment, ~21% of the cells were side-scatter (SSC)^{hi} and Siglec-F⁺ (Fig. 6A). In Figure 6B, the dot-plot represents cells from whole lung homogenates following RBC lysis (red), and the blue overlay represents cells gated on Siglec-F^{hi}. This dot-plot shows that the SSC^{hi} and Gr-1^{med} cells are predominantly Siglec-F⁺ eosinophils. Siglec-F⁺ lung cells (eosinophils) were also positive for gp49 expression (Fig. 6C, blue), as compared with staining with the isotype antibody control (Fig.

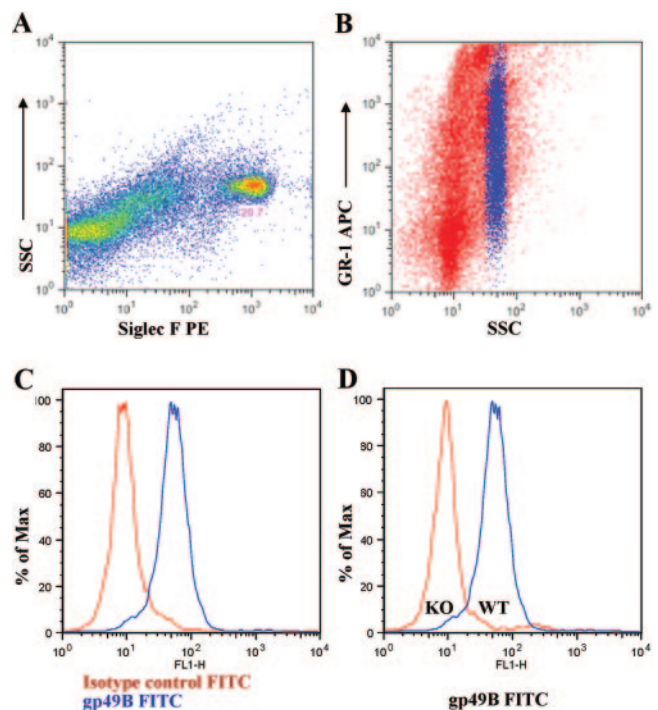


Fig. 6. Flow cytometric analysis of eosinophils from whole lung homogenates from RW-treated WT and gp49BKO mice. Liberase digestion, RBC lysis, and staining for Siglec-F PE, GR-1 APC, and gp49B (and isotype control) FITC were performed on whole lungs extracted from WT and gp49BKO mice post-RW sensitization and challenge. (A) Total Siglec-F-positive cell population from WT lungs. (B) Siglec F⁺ lung cells are GR-1^{med}, SSC^{hi}. (C) gp49B (and isotype) staining on lung cells from WT mice on Siglec F⁺ cells. (D) Overlay of WT and KO gp49B staining on Siglec F⁺ lung cells demonstrating the lack of gp49 staining in lung cells from gp49BKO mice. These data are a representative of three independent experiments.

6C, red). To test for antibody specificity and to determine whether the observed staining was a result of expression of gp49B, eosinophils from RW-treated gp49BKO and WT mice were analyzed in parallel. All of gp49 staining was lost on gp49BKO eosinophils from whole lung homogenates (Fig. 6D, red) compared with cells from the WT mice (Fig. 6D, blue). These data demonstrate that gp49B is present at the surface of Siglec-F⁺ eosinophils, which have migrated to the lung following allergen sensitization and challenge.

DISCUSSION

The role of gp49B in inflammatory responses at two different mucosal sites—the conjunctiva and the lung [3]—was assessed using a model of RW sensitization and challenge. This study reveals that the inhibitory receptor gp49B regulates eosinophils as well as neutrophils and mast cells, which are involved in the allergic inflammatory cascade, and plays a major role in dampening allergic inflammation. The conjunctival-challenge model was used as an easily accessible model of mucosal tissue to assess the dynamic response of mast cells, neutrophils, and eosinophils to an allergen [36]. Neutrophil and eosinophil infiltration and mast cell degranulation in the conjunctiva of gp49BKO mice were increased following sensitization and

challenge with RW, demonstrating that gp49B may negatively regulate eosinophil recruitment to the conjunctiva in response to an allergen.

The regulatory role of gp49B in modulating an allergic response to RW was also examined in the lungs. Allergic pulmonary inflammation and cytokine production were examined in the BALF of gp49BKO mice. IL-13 levels in the BALF of the gp49BKO mice as compared with WT mice were enhanced significantly after RW challenge. IL-13 is produced by a number of allergic effector cells including Th2 cells and eosinophils [41, 43] and has been found to enhance mucus production, AHR, and the increased production of Th2-driven chemokines responsible for the induction of allergic disease [39]. Attempts to evaluate a functional role for enhanced IL-13 production in gp49BKO mice during methylcholine challenge for evaluation of AHR proved to be difficult. The AHR in gp49BKO mice was increased beyond consistent detection limits in RW-treated mice, which had been infected previously with *A. suum*, as these mice died upon challenge with low-dose methylcholine challenge (6 mg/ml; data not shown). This same dose of methylcholine resulted in a significant increase in AHR in *A. suum*-infected, RW-challenged WT mice as well; however, the methylcholine was not lethal for WT mice. This suggests that the gp49B receptor may play a significant role in the regulation of allergic asthma.

Assessment of antigen-specific cytokine secretion in *in vitro* splenocyte assays isolated from RW-exposed WT and gp49BKO mice revealed no significant difference in the Th2-driven levels of IL-5, which is essential for eosinophil differentiation [49] or IL-13, shown to be involved in eosinophilic inflammation, mucus secretion in the lung, and AHR [41, 42]. The majority of the spleen is comprised of lymphocytes, which do not express gp49B. To determine if T cells at the site of allergen challenge in the lungs of gp49BKO mice were differentially activated compared with WT controls after RW challenge, we purified the CD4⁺ and CD8⁺ T cells from the lungs of RW-treated gp49BKO mice and RW-treated WT control animals. Flow cytometry analysis did not demonstrate a significant difference in the surface expression of the activation marker CD25 on T cells from gp49BKO or WT control mice after RW sensitization and challenge (data not shown). This *ex vivo* analysis suggests that there was no significant difference in T cell activation in the two groups. These data are supported by the fact that systemic skewing of Th2-related IgG1 and IgE antibodies was comparable in WT and gp49BKO mice following exposure and challenge to RW. The inhibitory activity of gp49B may therefore regulate allergic disease through direct inhibition of allergic effector cells such as mast cells, neutrophils, and eosinophils rather than by inhibiting the lymphocytes essential for the initiation of the allergic cascade.

The production of Th2 cytokines can be controlled by two separate pathways: the Jak/STAT pathway [50] and the ERK pathway [51]. Sprouty-related enabled/vasodilator-stimulated phosphoprotein homology 1 domain-containing protein 1 (Spred-1) has been identified as a negative regulator of the ERK pathway [52]. Inoue et al. [53] demonstrated recently that Spred-1 KO mice display significantly increased IL-13 and eotaxin levels following allergen sensitization and challenge. These data partially differed from ours in the gp49BKO model,

in that we did not observe a significant increase in eotaxin levels (data not shown) following RW sensitization and challenge. Eosinophils can be regulated by the expression of eotaxin, a chemokine important for eosinophil migration into sites of inflammation [54]. The observed increase in eosinophil numbers and lack of significant increase in eotaxin levels suggest that there is an inherent eosinophil defect, which is not regulated by the ERK pathway or Spred-1.

To increase the overall eosinophil numbers in response to a physiologic inflammatory stimulus, a parasitic nematode infection model with *A. suum* was used in combination with RW sensitization and challenge (*A. suum*+RW study). In this model, high levels of parasite-driven IL-5 increase the fraction of eosinophils in the peripheral blood from 1–3% to 60–70% [36]. A significant increase in lung inflammation with a predominance of eosinophils was observed in gp49BKO mice infected with *A. suum* and treated with RW. This finding suggests that gp49B may regulate eosinophils directly and that gp49B may be responsible for regulating eosinophil migration in Th2-driven disease models.

The enhanced eosinophilic inflammation and subsequent lung pathology seen in the *in vivo* studies using gp49BKO mice suggest an important role for this receptor in the inhibition of hyperactive eosinophils, which if unregulated, can have severe consequences on the host response to allergens. Through binding to its cognate ligand $\alpha_3\beta_3$ [19], gp49B may down-modulate transmigration through the endothelium, which would reduce the number of infiltrating cells into sites of inflammation.

Ex vivo studies were performed to determine if the gp49B inhibitory receptor is expressed on eosinophils in the blood and lungs. Siglec-F was used as a specific marker on eosinophils by FACS analysis. Siglec-F⁺ eosinophils isolated from peripheral blood and from RW-treated lungs stained positively for gp49. As a control, eosinophils isolated from gp49BKO mice were negative for gp49 staining. These data demonstrate the presence of an inhibitory receptor on the surface of naïve and activated eosinophils.

FACS analysis data were verified by performing a Western blot on BMMC proteins, which have been demonstrated previously to be gp49B⁺, and eosinophils from WT mice. Although the immunoprecipitation antibody is specific for gp49A and gp49B, the blotting antibodies used in this Western blot discriminate between the cytoplasmic tails of gp49A and gp49B [16]. These data demonstrate gp49B protein expression in BMMC and eosinophils of WT mice. The more intense gp49B⁺ band in the WT BMMC lane over what was observed in the WT eosinophils suggests that BMMC have enhanced expression of gp49B, as the Western blot proteins were normalized for protein amounts not cell size. The gp49B-specific blotting antibody did not detect gp49B expression on BMMC from gp49BKO mice, thus verifying the specificity of the antibody for the inhibitory molecule gp49B in comparison with the related, noninhibitory molecule gp49A.

These data demonstrate that gp49B plays a major role in regulating allergic conjunctivitis and pulmonary hypereosinophilia. In addition, gp49B is present on naïve and activated peripheral and tissue-bound eosinophils. The expression of the gp49B receptor may have a direct impact on the development of different categories of allergic inflammatory diseases in

which eosinophils play a role. A candidate human receptor with similar function to murine gp49B, CD85k [55] appears to be expressed at low but consistent levels on normal human eosinophils, as assessed by flow cytometry and Taqman (data not shown). As this inhibitory receptor is now known to be present and functional on the surface of a number of major effector cells involved in allergic inflammation, including eosinophils, it could represent a novel target for drug therapy.

ACKNOWLEDGMENTS

This research was supported by the Intramural Research Program of the NIH/NIAID and the U.S. Department of Agriculture. We thank Larry Faucette, Julie Kim, and Dr. Jerrold Ward for their technical assistance and also thank Dr. Kim Dyer for her careful review of the manuscript.

REFERENCES

1. Keane-Myers, A. (2001) The pathogenesis of allergic conjunctivitis. *Curr. Allergy Asthma Rep.* **1**, 550–557.
2. Wills-Karp, M. (1999) Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu. Rev. Immunol.* **17**, 255–281.
3. Busse, W. W., Lemanske, R. F. (2001) Asthma. *N. Engl. J. Med.* **344**, 350–362.
4. Chiappara, G., Gagliardo, R., Siena, A., Bonsignore, M. R., Bousquet, J., Bonsignore, G., Vignola, A. M. (2001) Airway remodeling in the pathogenesis of asthma. *Curr. Opin. Allergy Clin. Immunol.* **1**, 85–93.
5. Cohn, L., Elias, J. A., Chupp, G. L. (2004) Asthma: mechanisms of disease persistence and progression. *Annu. Rev. Immunol.* **22**, 789–815.
6. Huber, H. L., Koessler, K. K. (1922) The pathology of bronchial asthma. *Arch. Intern. Med.* **30**, 689–760.
7. Cambier, J. C. (1997) Inhibitory receptors abound? *Proc. Natl. Acad. Sci. USA* **94**, 5993–5995.
8. Veillette, A., Latour, S., Davidson, D. (2002) Negative regulation of immunoreceptor signaling. *Annu. Rev. Immunol.* **20**, 669–707.
9. Katz, H. R., Austen, K. F. (1997) A newly recognized pathway for the negative regulation of mast cell-dependent hypersensitivity and inflammation mediated by an endogenous cell surface receptor of the gp49 family. *J. Immunol.* **158**, 5065–5070.
10. Rojo, S., Burshtyn, D. N., Long, E. O., Wagtmann, N. (1997) Type I transmembrane receptor with inhibitory function in mouse mast cells and NK cells. *J. Immunol.* **158**, 9–12.
11. McCormick, M. J., Castells, M. C., Austen, K. F., Katz, H. R. (1999) The gp49A gene has extensive sequence conservation with the gp49B gene and provides gp49A protein, a unique member of a large family of activating and inhibitory receptor of the immunoglobulin superfamily. *Immunogenetics* **50**, 286–294.
12. Daheshia, M., Friend, D. S., Grusby, M. J., Austen, K. F., Katz, H. R. (2001) Increased severity of local and systemic anaphylactic reactions in gp49B1-deficient mice. *J. Exp. Med.* **194**, 227–234.
13. Katz, H. R. (2002) Inhibition of anaphylactic inflammation by the gp49B1 receptor on mast cells. *Mol. Immunol.* **38**, 1301–1305.
14. Wang, L. L., Mehta, I. K., LeBlanc, P. A., Yokoyama, W. M. (1997) Mouse natural killer cells express gp49B1, a structural homologue of human killer inhibitory receptors. *J. Immunol.* **158**, 13–17.
15. Zhou, J. S., Friend, D. S., Feldweg, A. M., Daheshia, M., Li, L., Austen, K. F., Katz, H. R. (2003) Prevention of lipopolysaccharide-induced microangiopathy by gp49B1: evidence for an important role for gp49B1 expression on neutrophils. *J. Exp. Med.* **198**, 1243–1251.
16. Rojo, S., Stebbins, C. C., Peterson, M. E., Dombrowicz, D., Wagtmann, N., Long, E. O. (2000) Natural killer cells and mast cell from gp49B null mutant mice are functional. *Mol. Cell. Biol.* **20**, 7178–7182.
17. Castells, M. C., Wu, X., Arm, J. P., Austen, K. F., Katz, H. R. (1994) Cloning of the gp49B gene of the immunoglobulin superfamily and demonstration that one of its two products is an early-expressed mast cell surface protein originally described as gp49. *J. Biol. Chem.* **269**, 8393–8401.
18. Katz, H. R., Vivier, E., Castells, M. C., McCormick, M. J., Chambers, J. M., Austen, K. F. (1996) Mouse mast cell gp49B1 contains two immunoreceptor tyrosine-based inhibition motifs and suppresses mast cell activation when colligated with the high-affinity Fc receptor for IgE. *Proc. Natl. Acad. Sci. USA* **93**, 10809–10814.
19. Castells, M. C., Klickstein, L. B., Hassani, K., Cumplido, J. A., Lacouture, M. E., Austen, K. F., Katz, H. R. (2001) gp49B1- $\alpha_3\beta_3$ interaction inhibits antigen-induced mast cell activation. *Nat. Immunol.* **2**, 436–442.
20. Magone, M. T., Chan, C. C., Rizzo, L. V., Kozhich, A. T., Whitcup, S. M. (1998) A novel murine model of allergic conjunctivitis. *Clin. Immunol. Immunopathol.* **87**, 75–84.
21. Ennis, M. (2003) Neutrophils in asthma pathophysiology. *Curr. Allergy Asthma Rep.* **3**, 159–165.
22. Rothenberg, M. E. (1998) Eosinophilia. *N. Engl. J. Med.* **338**, 1592–1600.
23. Conroy, D. M., Williams, T. J. (2001) Eotaxin and the attraction of eosinophils to the asthmatic lung. *Respir. Res.* **2**, 150–156.
24. Parwaresch, M. R., Walle, A. J., Arndt, D. (1976) The peripheral kinetics of human radiolabeled eosinophils. *Virchows Arch. B Cell Pathol.* **1**, 57–66.
25. Beeson, P. B., Bass, D. A. (1977) *The Eosinophil*. Philadelphia, PA, USA, W.B. Saunders, 6.
26. Rothenberg, M. E., Owen Jr., W. F., Silberstein, D. S., Soberman, R. J., Austen, K. F., Stevens, R. L. (1987) Eosinophils cocultured with endothelial cells have increased survival and functional properties. *Science* **237**, 645–647.
27. 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.
28. 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 allergic airways remodeling. *Science* **305**, 1776–1779.
29. Klion, A. D., Law, M. A., Riemenschneider, W., McMaster, M. L., Brown, M. R., Horne, M., Karp, B., Robinson, M., Sachdev, V., Tucker, E., Turner, M., Nutman, T. B. (2004) Familial eosinophilia: a benign disorder? *Blood* **103**, 4050–4055.
30. Tomassini, M., Tsicopoulos, A., Tai, P. C., Gruart, V., Tonnel, A. B., Prin, L., Capron, A., Capron, M. (1991) Release of granule proteins by eosinophils from allergic and nonallergic patients with eosinophilia on immunoglobulin-dependent activation. *J. Allergy Clin. Immunol.* **88**, 365–375.
31. Gounni, A. S., Lamkhioed, B., Ochiai, K., Tanaka, Y., Delaporte, E., Capron, A., Kinet, J. P., Capron, M. (1994) High-affinity IgE receptor on eosinophils is involved in defense against parasites. *Nature* **367**, 183–186.
32. Kita, H., Abu-Ghazaleh, R. I., Sur, S., Gleich, G. J. (1995) Eosinophil major basic protein induces degranulation and IL-8 production by human eosinophils. *J. Immunol.* **154**, 4749–4758.
33. Rajakulasingam, K., Till, S., Ying, S., Humbert, M., Barkans, J., Sullivan, M., Meng, Q., Corrigan, C. J., Bungre, J., Grant, J. A., Kay, A. B., Durham, S. R. (1998) Increased expression of high affinity IgE receptor- α chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. *Am. J. Respir. Crit. Care Med.* **158**, 233–240.
34. Lee, N. A., McGarry, M. P., Larson, K. A., Horton, M. A., Kristensen, A. B., Lee, J. J. (1997) Expression of IL-5 in thymocytes/T cells leads to the development of a massive eosinophilia, extramedullary eosinophilopoiesis, and unique histopathologies. *J. Immunol.* **158**, 1332–1344.
35. Dawson, H. D., Beshah, E., Nishi, S., Solano-Aguilar, G., Morimoto, M., Zhao, A., Madden, K. B., Ledbetter, J. K., Dubey, J. P., Shea-Donohue, T., Lunney, J. K., Urban Jr., J. F. (2005) Localized multi-gene expression patterns support an evolving Th1/Th2 paradigm in response to infections with *Toxoplasma gondii* and *Ascaris suum*. *Infect. Immun.* **73**, 1116–1128.
36. Schopf, L., Luccioli, S., Bundoc, V., Justice, P., Chan, C. C., Wetzel, B. J., Norris, H. H., Urban Jr., J. F., Keane-Myers, A. (2005) Differential modulation of allergic eye disease by chronic and acute *Ascaris* infection. *Invest. Ophthalmol. Vis. Sci.* **46**, 2772–2780.
37. Miyazaki, D., Nakamura, T., Toda, M., Cheung-Chau, K. W., Richardson, R. M., Ono, S. J. (2005) Macrophage inflammatory protein-1 α as a costimulatory signal for mast cell-mediated immediate hypersensitivity reactions. *J. Clin. Invest.* **115**, 434–442.
38. Al-Shami, A., Spolski, R., Kelly, J., Keane-Myers, A., Leonard, W. J. (2005) A role for TSLP in the development of inflammation in an asthma model. *J. Exp. Med.* **202**, 829–839.
39. Borchers, M. T., Ansay, T., DeSalle, R., Daugherty, B. L., Shen, H., Metzger, M., Lee, N. A., Lee, J. J. (2002) In vitro assessment of chemokine

- receptor-ligand interactions mediating mouse eosinophil migration. *J. Leukoc. Biol.* **71**, 1033–1041.
40. Abdala-Valencia, H., Earwood, J., Bansal, S., Jansen, M., Babcock, G., Garvy, B., Wills-Karp, M., Cook-Mills, J. M. (2007) Nonhematopoietic NADPH oxidase regulation of lung eosinophilia and airway hyperresponsiveness in experimentally induced asthma. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **292**, L1111–L1125.
 41. Wills-Karp, M. (2004) Interleukin-13 in asthma pathogenesis. *Immunol. Rev.* **202**, 175–190.
 42. Wynn, T. A. (2003) IL-13 effector functions. *Annu. Rev. Immunol.* **21**, 425–456.
 43. Yang, M., Hogan, S. P., Mahalingam, S., Pope, S. M., Zimmermann, N., Fulkerson, P., Dent, L. A., Young, I. G., Matthaei, K. I., Rothberg, M. E., Foster, P. S. (2003) Eotaxin and IL-5 cooperate in the lung to regulate IL-13 production and airway eosinophilia and hyperactivity. *J. Allergy Clin. Immunol.* **112**, 935–943.
 44. Schmid-Grendelmeier, P., Altznauer, F., Fischer, B., Bizer, C., Straumann, A., Menz, G., Blaser, K., Wuthrich, B., Simon, H. U. (2002) Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J. Immunol.* **169**, 1021–1027.
 45. Reiman, R. M., Thompson, R. W., Feng, C. G., Hari, D., Knight, R., Cheever, A. W., Rosenberg, H. F., Wynn, T. A. (2006) Interleukin 5 (IL-5) augments the progression of liver fibrosis by regulating IL-13 activity. *Infect. Immun.* **74**, 1471–1479.
 46. Chu, C. C., Max, E. E., Paul, W. E. (1993) DNA rearrangement can account for in vitro switching to IgG1. *J. Exp. Med.* **178**, 1381–1390.
 47. 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.
 48. Epstein, M. M. (2004) Do mouse models of allergic asthma mimic clinical disease? *Int. Arch. Allergy Immunol.* **133**, 84–100.
 49. Sanderson, C. J. (1992) Interleukin-5, eosinophils, and disease. *Blood* **79**, 3101–3109.
 50. Miike, S., Nakao, A., Hiraguri, M., Kurasawa, K., Saito, Y., Iwamoto, I. (1999) Involvement of JAK2, but not PI 3-kinase/Akt and MAP kinase pathway, in anti-apoptotic signals of GM-CSF in human eosinophils. *J. Leukoc. Biol.* **65**, 700–706.
 51. Pazdrak, K., Schreiber, D., Forsythe, P., Justement, L., Alam, R. (1995) The intracellular signal transduction mechanism of interleukin-5 in eosinophils: the involvement of lyn tyrosine kinase and the Ras-Raf-1-MEK-microtubule-associated protein kinase pathway. *J. Exp. Med.* **181**, 1827–1834.
 52. Wakioka, T., Sasaki, A., Kato, R., Shouda, T., Matsumoto, A., Miyoshi, K., Tsuneoka, M., Komiya, S., Baron, R., Yoshimura, A. (2001) Spred is a Sprouty-related suppressor of Ras signaling. *Nature* **412**, 647–651.
 53. Inoue, H., Kato, R., Fukuyama, S., Nonami, A., Taniguchi, K., Matsumoto, K., Nakano, T., Tsuda, M., Matsumura, M., Kubo, M., Ishikawa, F., Moon, B. G., Takatsu, K., Nakanishi, Y., Yoshimura, A. (2005) Spred-1 negatively regulates allergen-induced airway eosinophilia and hyperresponsiveness. *J. Exp. Med.* **201**, 73–82.
 54. Williams, T. J., Jose, P. J. (2000) Role of eotaxin and related CC chemokines in allergy and asthma. *Chem. Immunol.* **78**, 166–177.
 55. Brown, D., Trowsdale, J., Allen, R. (2004) The LILR family: modulators of innate and adaptive immune pathways in health and disease. *Tissue Antigens* **64**, 215–225.
 - Akbari, O., Stock, P., Meyer, E., Kronenberg, M., Sidobre, S., Nakayama, T., Taniguchi, M., Brusby, M. J., DeKruyff, R. H., Umetsu, D. T. (2003) Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat. Med.* **9**, 582–588.