

Jill R. Johnson, Filip K. Swirski, Beata U. Gajewska, Ryan E. Wiley, Ramzi Fattouh, Stephanie R. Pacitto, Jonathan K. Wong, Martin R. Stämpfli and Manel Jordana

Am J Physiol Lung Cell Mol Physiol 293:730-739, 2007. First published Jun 22, 2007;
doi:10.1152/ajplung.00056.2007

You might find this additional information useful...

This article cites 44 articles, 28 of which you can access free at:

<http://ajplung.physiology.org/cgi/content/full/293/3/L730#BIBL>

This article has been cited by 1 other HighWire hosted article:

Transforming Growth Factor- β Regulates House Dust Mite-induced Allergic Airway Inflammation but Not Airway Remodeling

R. Fattouh, N. G. Midence, K. Arias, J. R. Johnson, T. D. Walker, S. Goncharova, K. P. Souza, R. C. Gregory Jr., S. Lonning, J. Gauldie and M. Jordana

Am. J. Respir. Crit. Care Med., March 15, 2008; 177 (6): 593-603.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Updated information and services including high-resolution figures, can be found at:

<http://ajplung.physiology.org/cgi/content/full/293/3/L730>

Additional material and information about *AJP - Lung Cellular and Molecular Physiology* can be found at:

<http://www.the-aps.org/publications/ajplung>

This information is current as of September 28, 2009 .

Divergent immune responses to house dust mite lead to distinct structural-functional phenotypes

Jill R. Johnson, Filip K. Swirski, Beata U. Gajewska, Ryan E. Wiley, Ramzi Fattouh, Stephanie R. Pacitto, Jonathan K. Wong, Martin R. Stämpfli, and Manel Jordana

Department of Pathology and Molecular Medicine and Division of Respiratory Diseases and Allergy, Centre for Gene Therapeutics, McMaster University, Hamilton, Ontario, Canada

Submitted 12 February 2007; accepted in final form 18 June 2007

Johnson JR, Swirski FK, Gajewska BU, Wiley RE, Fattouh R, Pacitto SR, Wong JK, Stämpfli MR, Jordana M. Divergent immune responses to house dust mite lead to distinct structural-functional phenotypes. *Am J Physiol Lung Cell Mol Physiol* 293: L730–L739, 2007. First published June 22, 2007; doi:10.1152/ajplung.00056.2007.—Asthma is a chronic airway inflammatory disease that encompasses three cardinal processes: T helper (Th) cell type 2 (Th2)-polarized inflammation, bronchial hyperreactivity, and airway wall remodeling. However, the link between the immune-inflammatory phenotype and the structural-functional phenotype remains to be fully defined. The objective of these studies was to evaluate the relationship between the immunologic nature of chronic airway inflammation and the development of abnormal airway structure and function in a mouse model of chronic asthma. Using IL-4-competent and IL-4-deficient mice, we created divergent immune-inflammatory responses to chronic aeroallergen challenge. Immune-inflammatory, structural, and physiological parameters of chronic allergic airway disease were evaluated in both strains of mice. Although both strains developed airway inflammation, the profiles of the immune-inflammatory responses were markedly different: IL-4-competent mice elicited a Th2-polarized response and IL-4-deficient mice developed a Th1-polarized response. Importantly, this chronic Th1-polarized immune response was not associated with airway remodeling or bronchial hyperresponsiveness. Transient reconstitution of IL-4 in IL-4-deficient mice via an airway gene transfer approach led to partial Th2 repolarization and increased bronchial hyperresponsiveness, along with full reconstitution of airway remodeling. These data show that distinct structural-functional phenotypes associated with chronic airway inflammation are strictly dependent on the nature of the immune-inflammatory response.

allergy; inflammation; lung; Th1/Th2 cells

ASTHMA IS A CHRONIC INFLAMMATORY disease of the airways associated with a particular structural and functional airway phenotype. The structural abnormalities, often referred to as remodeling, are thought to contribute to the heightened reactivity of the airways to a variety of stimuli (32). Ostensibly, chronicity evolves as a consequence of recurrent or sustained episodes of inflammation in response to exposure to seasonal or pervasive allergens, respectively. In this context, we recently developed a model of chronic allergic asthma by continuously exposing mice to an extract of house dust mite (HDM; *Dermatophagoides pteronyssinus*), a common environmental aeroallergen (30), for 5 consecutive wk. Under these conditions, we documented persistent airway inflammation, structural changes in the airway wall, and severe bronchial

hyperreactivity. Immunologically, the nature of the immune-inflammatory response had the typical features of a T helper (Th) cell type 2 (Th2)-polarized response (15).

In this study, we asked whether those structural and functional abnormalities were the result of any type of chronic airway inflammation or, alternatively, inflammation with a particular phenotype. We set out to explore whether we could generate immunologically divergent inflammatory responses to HDM. The experimental approach was to chronically expose IL-4-deficient mice to HDM for 5 wk. In vitro studies demonstrated the critical importance of IL-4 in the priming and differentiation of effector Th2 cells, a subset of cells instrumental to the expression of the allergic phenotype (28, 34, 35). In addition, several in vivo studies showed that IL-4 deficiency results in impaired, although not completely abolished, allergic airway inflammatory and physiological responses (1, 4, 5, 9, 12, 13, 20, 22, 29, 33).

Our data show that IL-4-deficient mice were clearly able to mount an inflammatory response. However, this response was associated with the acquisition of features of Th1 immunity. Despite evident inflammation of the airways, IL-4-deficient mice chronically exposed to HDM did not exhibit the structural or functional phenotype typically observed in genetically intact mice. Furthermore, when an airway gene transfer approach was used, transient expression of murine IL-4 in the airways of IL-4-deficient mice during sensitization to HDM led to a partial repolarization of a Th2 immune-inflammatory response that was associated with a partial emergence of bronchial hyperreactivity and a full reconstitution of remodeling abnormalities.

METHODS

Animals. Female wild-type (WT) and IL-4-deficient (IL-4KO) Balb/c mice (6–8 wk old) were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were housed under specific pathogen-free conditions and a 12:12-h light-dark cycle. Cages, food, and bedding were autoclaved, and all mice were handled in a laminar flow hood by gloved, gowned, and masked personnel. All experiments were approved by the Animal Research Ethics Board of McMaster University and followed the guidelines of the Canadian Council on Animal Care.

Antigen administration. Mice were exposed to purified HDM whole body extract (Greer Laboratories, Lenoir, NC) intranasally (25 µg of protein in 10 µl of saline) for 5 consecutive days, followed by 2 days of rest, for 5 consecutive wk. In some experiments, animals exposed to HDM for 5 wk were reexposed to HDM on 5 consecutive days after a 2-mo rest period. No additional adjuvant was given at any

Address for reprint requests and other correspondence: M. Jordana, Dept. of Pathology and Molecular Medicine, Michael G. DeGroote Centre for Learning and Discovery, Rm. 4087, McMaster Univ., 1200 Main St. West, Hamilton, ON, Canada L8N 3Z5 (e-mail: jordanam@mcmaster.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

time. Negative control animals were instilled with sterile saline intranasally 5 days/wk for 5 wk; age-matched unmanipulated naïve mice were used for airway hyperreactivity (AHR) studies. No differences in airway inflammation or structure were observed between saline-treated and naïve animals.

Adenovirus construct and administration. To elicit local expression of murine IL-4, a replication-deficient human type 5 adenoviral (Ad) construct encoding murine IL-4 cDNA in the E1 region of the viral genome (24) was delivered intranasally to isoflurane-anesthetized animals on day -1 (i.e., 24 h before the 1st exposure to HDM). Ad/IL-4 and the empty vector control [replication-deficient adenovirus (RDA)] were administered at 3×10^7 plaque-forming units (pfu) in a total volume of 30 μ l of PBS vehicle. Delivery of Ad/IL-4 alone to naïve mice resulted in 1,000 pg/ml murine IL-4 in the bronchoalveolar lavage (BAL) fluid 4 days after infection.

Collection and measurement of specimens. Blood, BAL fluid, lungs, and spleens were collected. BAL fluid was collected by dissection of the lungs and cannulation of the trachea with polyethylene tubing (Becton Dickinson, Sparks, MD), as previously described (36). Briefly, the lungs were lavaged twice with PBS (0.25 ml followed by 0.2 ml). Approximately 0.3 ml of the instilled fluid was consistently recovered. The total number of cells in BAL fluid was counted using a hemocytometer. After centrifugation, cell pellets were resuspended in PBS, and smears were prepared by cytocentrifugation (Shandon, Pittsburgh, PA) at 300 rpm for 2 min. The Hema-3 stain set (Biochemical Sciences, Swedesboro, NJ) was used to stain all smears. Differential counts of BAL cells were determined from ≥ 500 leukocytes, which were classified as mononuclear cells, neutrophils, or eosinophils by standard hematologic criteria. Additionally, peripheral blood was collected by retroorbital bleeding using heparinized capillary tubes (Fischer Scientific, Pittsburgh, PA). Serum was prepared by incubation of whole blood for 30 min at 37°C.

Splenocyte culture. Spleens were harvested into sterile tubes containing cold (4°C) sterile Hanks' balanced salt solution (HBSS; Life Technologies, Burlington, ON, Canada). Tissue was triturated into HBSS through nylon mesh (Becton Dickinson, Mississauga, ON, Canada) using the plunger from a 5-ml syringe. Red blood cells were lysed with ACK lysis buffer [0.5 M NH_4Cl , 10 mM KHCO_3 , and 0.1 mM Na_2EDTA (pH 7.2–7.4)]. Remaining splenocytes were washed twice with HBSS and then resuspended in RPMI supplemented with 10% FBS (Life Technologies), 1% L-glutamine, 1% penicillin-streptomycin, and 0.1% β -mercaptoethanol. Cells were cultured in medium alone or with 3 μ g of HDM per well at a density of 8×10^5 cells per well in a flat-bottom, 96-well plate (Becton Dickinson). After 5 days of culture, supernatants were harvested for cytokine measurements.

Quantification of cytokines and immunoglobulins. Cytokine production by cultured splenocytes was evaluated using ELISA kits for murine IL-5, IL-13, and IFN- γ (R & D Systems, Minneapolis, MN), each with a threshold of detection of 1.5–5 pg/ml. For detection of HDM-specific IgG1 and IgG2a, Maxi-Sorb plates (NUNC Brand Products) were coated with 5 μ g of HDM in borate buffer (pH 8.3–8.5) overnight at 4°C. Subsequently, coated wells were blocked with 1% BSA in PBS for 2 h at room temperature. After they were washed, serum samples were incubated overnight at 4°C, washed again, and developed with biotin-labeled IgG1 and IgG2a (Southern Biotechnology Associates, Birmingham, AL) overnight at 4°C. Plates were washed and incubated with alkaline phosphatase-streptavidin for 1 h at room temperature. The color reaction was developed with *p*-nitrophenyl phosphate tablets. Units correspond to the maximal dilution that results in an optical density that is greater than the blank plus two standard deviations.

Lung cell isolation, culture, and evaluation of cytokine expression. Lungs were perfused with 10 ml of HBSS through the right ventricle, cut into small (~2-mm-diameter) pieces, and agitated at 37°C for 1 h in 15 ml of collagenase III (Life Technologies) at a concentration of 150 U/ml in HBSS. The plunger from a 5-ml syringe was used to triturate the lung pieces through a 40- μ m nylon cell strainer (BD

Falcon) into HBSS. Mononuclear cells from the resulting suspension were isolated at the interphase between layers of 30% and 60% Percoll after density gradient centrifugation. Cells were cultured in medium alone or with 3 μ g of HDM per well at a density of 5×10^5 cells per well in a flat-bottom, 96-well plate (Becton Dickinson). After 5 days of culture, supernatants were harvested for cytokine measurements using a 5-Plex kit for IL-4, IL-5, IL-10, IL-13, and IFN- γ (Luminex, Austin, TX).

Assessment of AHR. Airway responsiveness was measured on the basis of the response of total respiratory system resistance (Rrs) to increasing intravenous (internal jugular vein) doses of methacholine (MCh), as previously described (7). Briefly, mice were anesthetized with tribromoethanol (287 mg/kg ip) prepared according to a standard protocol (31). The trachea was exposed and cannulated, and a constant inspiratory flow was delivered by mechanical ventilation (model RV5, Voltek Enterprises, Toronto, ON, Canada). Heart rate and oxygen saturation were monitored via infrared pulse oximetry (Biox 3700, Ohmeda, Boulder, CO) using a standard ear probe placed over the proximal portion of the mouse's hindlimb. Paralysis was achieved using pancuronium (0.03 mg/kg iv) to prevent respiratory effort during measurement. Rrs was measured after consecutive intravenous injections of saline followed by 10, 33, 100, and 330 μ g/kg of MCh (ACIC, Brantford, ON, Canada), each delivered as a 0.2-ml bolus. During each MCh dosing, the mouth pressure signal from the ventilator was converted to a digital signal (Dash 16, Metrabyte, Staughton, MA) and recorded at 400 Hz on a personal computer. Rrs was calculated as described previously (7). Evaluation of airway responsiveness was based on the peak Rrs measured in the 30 s after the saline and MCh challenges.

Histology and immunohistochemistry. The lungs were dissected out of the thoracic cavity of each mouse. Lungs were then inflated in 10% formalin at a standard pressure of 20 cmH₂O, fixed in 10% formalin for ≥ 24 h, dehydrated in 70% ethanol, embedded in paraffin, and cut transversely at a thickness of 3 μ m. Sections were stained with hematoxylin and eosin for evaluation of the severity and the nature of leukocyte infiltration in the lung by light microscopy. Additional sections were stained with periodic acid-Schiff to demonstrate epithelial mucous production and with Picro-Sirius red to demonstrate the presence of collagen in the extracellular matrix. All staining procedures were performed according to standard protocols. The presence of smooth muscle in the airway was detected by immunohistochemistry for anti- α -smooth muscle actin (α -SMA). Nonspecific binding was blocked by incubation of the sections in 1% normal swine serum (NSS) diluted in Tris-buffered saline for 15 min at room temperature. Sections were incubated with α -SMA primary antibody (diluted 1:150 in 1% NSS; clone 1A4, Dako) for 1 h at room temperature and then with biotinylated rabbit anti-mouse secondary antibody (diluted 1:300 in 1% NSS; Dako) for 1 h at room temperature. Subsequently, sections were incubated in streptavidin-peroxidase conjugate (diluted 1:600 in 1% NSS; Dako) for 45 min at room temperature, placed in acetate buffer (pH 5.0) for 5 min, and then incubated in freshly prepared chromogen substrate solution for 15 min. Tissues were counterstained in Mayer's hematoxylin for 1 min, washed, and mounted in glycerin gelatin.

Morphometry. Images for morphometric analysis were captured using OpenLab software version 3.0.3 (Improvision, Guelph, ON, Canada) via a Leica camera and microscope attached to a Macintosh computer (Mac OS 9 operating system). Images (6–10 animals per group) were analyzed using a custom computerized image analysis system (Northern Eclipse software, version 6, Empix Imaging, Mississauga, ON, Canada) on a Pentium IV computer (2.4-GHz processor, Windows XP operating system). Morphometric quantification involved calculation of the percentage of tissue area that was positively stained within regions of interest (1 slide per mouse per stain). Sections of the airway that were associated with connective tissue attachments to associated vessels were excluded from the analysis. Sections stained for periodic acid-Schiff were subjected to digital

color inversion and evaluated in a 40- μ m-thick area from the basement membrane extending into the airway lumen with color settings as follows: red (R) = 66–112, green (G) = 110–209, and blue (B) = 169–221. Parameters used for the evaluation of α -SMA and Picro-Sirius red have previously been described (26).

Data analysis. Values are means $\pm$ SE, unless otherwise indicated. Results were interpreted using Student's *t*-test or ANOVA followed by Tukey's post hoc test (where applicable). Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

Cellular profile in the BAL of animals continually exposed to HDM. WT and IL-4KO mice were exposed to HDM extract, without exogenous adjuvant, for 5 consecutive wk. Airway inflammation was observed in both strains, as evidenced by elevated BAL cell content in Fig. 1A. Total cell numbers were approximately twofold higher in WT than in IL-4KO mice after 5 wk of HDM. The nature of the inflammation in WT mice was characterized by significant numbers of eosinophils in the BAL, whereas inflammation in IL-4KO animals was represented predominantly by macrophages (Fig. 1B).

Histopathological evaluation of lung tissue in HDM-exposed mice corroborated the findings in the BAL. Low-magnification analysis demonstrated extensive peribronchial and perivascular inflammation in HDM-exposed animals of both strains, although inflammation appeared more severe in WT

mice (Fig. 1C). Higher magnification revealed marked eosinophilic infiltration in WT animals after 5 wk of HDM exposure (Fig. 1C), whereas no eosinophils were observed in IL-4KO mice.

Local and systemic immune-inflammatory events in response to chronic allergen exposure. Local immune responses were evaluated by assessment of cytokine production by HDM-stimulated lung mononuclear cells isolated from HDM-exposed WT and IL-4KO mice. After in vitro allergen recall, expression of the Th2-associated cytokines IL-5 and IL-13 was elevated in WT animals and expression of the Th1-associated cytokine IFN- γ was elevated in IL-4KO mice (Table 1). In splenocytes cultured in the presence of HDM, levels of the Th2-associated cytokines IL-5 and IL-13 were elevated in WT mice, whereas cytokine expression in IL-4KO mice was characterized by high levels of IFN- γ , although production of this cytokine was not significantly different between strains (Table 1). Serum immunoglobulin levels of HDM-specific (Th2-associated) IgG1 and HDM-specific (Th1-associated) IgG2a were evaluated in WT and IL-4KO mice after 5 wk of HDM. Similar to the pattern of cytokine expression observed at this time point, high titers of the Th2-associated immunoglobulin IgG1 were observed in WT mice, whereas IL-4KO mice produced significant levels of HDM-specific IgG2a (Table 1).

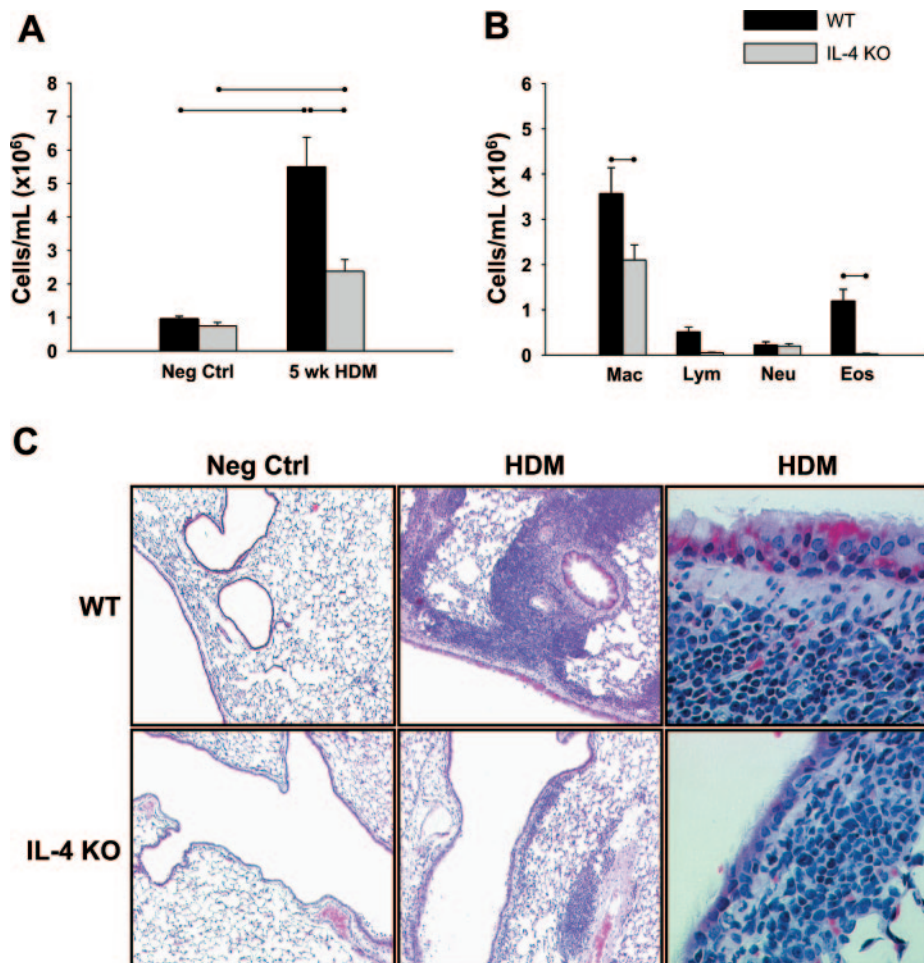


Fig. 1. Divergent inflammatory responses of wild-type (WT) and IL-4-knockout (IL-4KO) mice to continuous exposure to house dust mites (HDM). WT and IL-4KO mice were exposed to intranasal allergen for 5 days/wk, and inflammation was evaluated after 5 consecutive wk of exposure. Total cell recovery (A) and cell differentials (B) in bronchoalveolar lavage (BAL) demonstrate inflammation in lungs of HDM-exposed animals. Hematoxylin-eosin-stained lung sections demonstrate inflammatory infiltrate in HDM-exposed mice compared with negative control (Neg Ctrl) WT and IL-4KO animals (C). Scale bars, 80 μ m (low magnification) and 10 μ m (high magnification). Values are means $\pm$ SE ($n = 7$ –8 from 3 independent experiments). Horizontal bar indicates $P < 0.05$. Statistical analysis was performed using 1-way ANOVA with Tukey's post hoc test (total cell numbers) or Student's *t*-test (differentials). Mac, macrophage; Lym, lymphocyte; Eos, eosinophil.

Table 1. *In vitro* cytokine production by lung mononuclear cells and splenocytes and serum immunoglobulin titers in WT and IL-4KO mice after HDM exposure

	WT	IL-4KO
Lung mononuclear cells		
IL-5, pg/ml	1,673 ± 122	130 ± 5*
IL-13, pg/ml	81 ± 13	6 ± 1*
IFN- γ , pg/ml	15 ± 1	34 ± 2*
Splenocytes		
IL-5, pg/ml	1,370 ± 162	25 ± 14*
IL-13, pg/ml	52,390 ± 15,905	588 ± 343*
IFN- γ , pg/ml	3,317 ± 294	4,904 ± 774
Serum immunoglobulins		
HDM-specific IgG1, U/ml	14,720 ± 4,624	458 ± 202*
HDM-specific IgG2a, U/ml	865 ± 373	4,438 ± 733*

Values are means $\pm$ SE ($n = 4$); data are representative of 2–3 independent experiments. Wild-type (WT) and IL-4-deficient (IL-4KO) mice were exposed to house dust mite (HDM) for 5 wk. * $P < 0.05$ vs. WT.

Evaluation of recall response to HDM in WT and IL-4KO mice. We then investigated the long-term recall response to HDM in both strains of mice. After 5 wk of HDM exposure, administration was discontinued for 7 wk to allow airway

inflammation to subside. Animals were then reexposed to HDM for 5 consecutive days, resulting in increased, and equivalent, cell numbers in the BAL in both strains of mice (Fig. 2A). The nature of this cellular infiltrate recapitulated that observed immediately after 5 wk of exposure to HDM, in that there were virtually no eosinophils in IL-4KO mice (Fig. 2B). Histopathological evaluation of lung tissue recall exposure corroborated the findings in the BAL (Fig. 2C).

Splenocytes were cultured in the presence of HDM, resulting in elevated expression of the Th2-associated cytokines IL-5 and IL-13 by WT mice, whereas cytokine expression in IL-4KO mice was characterized by high levels of IFN- γ (Table 2). Serum immunoglobulin levels were also measured at this time in WT and IL-4KO mice. Similar to the pattern of cytokine expression observed after *in vivo* allergen recall, high titers of IgG1 were observed in WT mice, whereas IL-4KO mice produced significantly elevated levels of IgG2a (Table 2).

Analysis of structural changes in the airway wall. Marked morphological changes in the airway epithelium and subepithelium were apparent in WT mice exposed to HDM, as previously described (15). Lung histology demonstrated increased mucous production (Fig. 3A), collagen deposition (Fig.

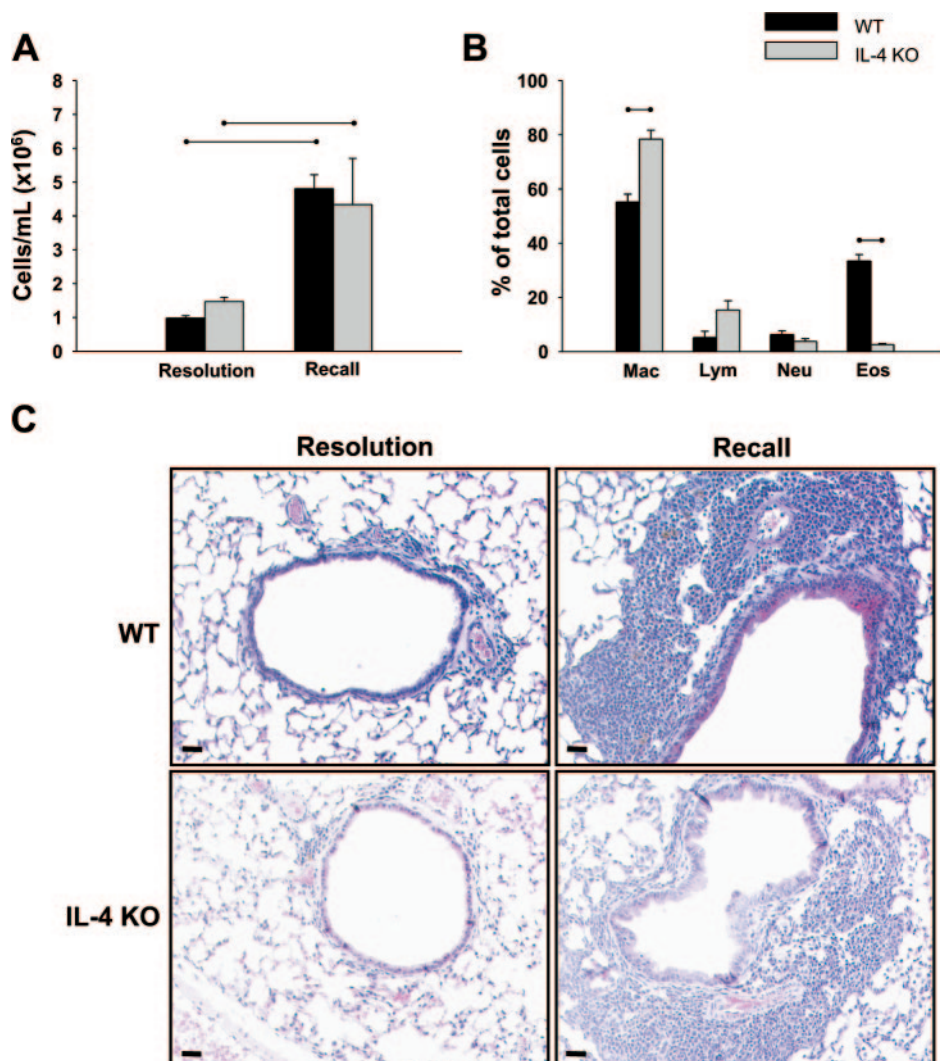


Fig. 2. Divergent inflammatory responses after *in vivo* allergen recall exposure in WT and IL-4KO mice. WT and IL-4KO mice were exposed to HDM for 5 consecutive wk, allowed to rest for 7 wk, and reexposed to HDM for 5 consecutive days. Total cell recovery (A) and cell differentials (B) in BAL demonstrate inflammation after *in vivo* allergen recall in both strains of mice. Hematoxylin-eosin-stained lung sections demonstrate inflammatory infiltrate in mice after *in vivo* allergen recall in WT and IL-4KO strains after 7-wk rest period (C). Scale bars, 40 μ m. Values are means $\pm$ SE ($n = 3$ –8 from 2 independent experiments). Horizontal bar indicates $P < 0.05$. Statistical analysis was performed using 1-way ANOVA with Tukey's post hoc test (total cell numbers) or Student's *t*-test (differentials).

Table 2. *In vitro* cytokine production by splenocytes and serum immunoglobulin titers after *in vivo* HDM recall in WT and IL-4KO mice

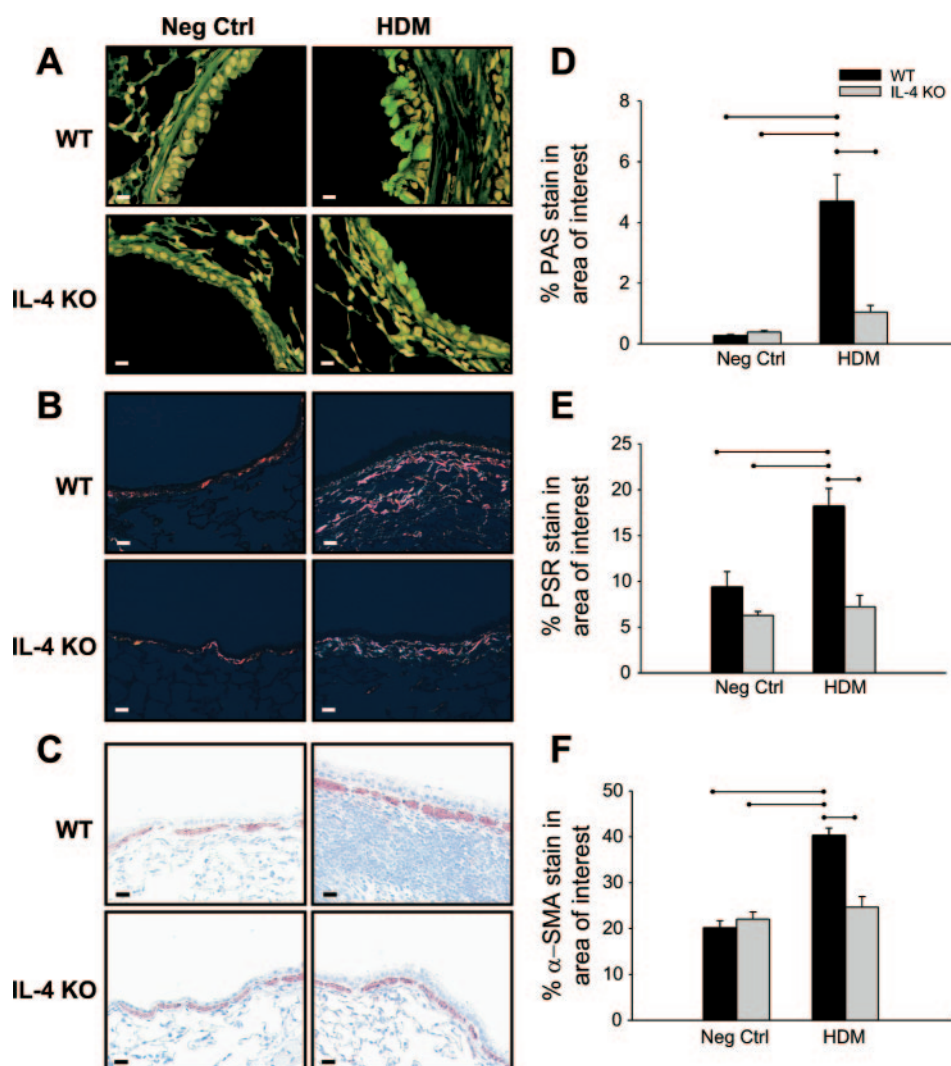
	WT	IL-4KO
Splenocytes		
IL-5, pg/ml	3,160 ± 770	73 ± 73*
IL-13, pg/ml	59,600 ± 6,100	3,500 ± 1,000*
IFN- γ , pg/ml	43 ± 43	460 ± 200*
Immunoglobulins		
HDM-specific IgG1, U/ml	39,260 ± 13,700	5,372 ± 1,900*
HDM-specific IgG2a, U/ml	4,442 ± 540	10,443 ± 300*

Values are means $\pm$ SE ($n = 4-5$); data are representative of 2 independent experiments. * $P < 0.05$ vs. WT.

3B), and peribronchial contractile elements (Fig. 3C) compared with unexposed animals of both strains and HDM-exposed IL-4KO mice. Furthermore, no remodeling changes were apparent in IL-4KO mice after *in vivo* allergen recall (data not shown). Morphometric quantification of the increases demonstrates statistically significant increases in mucous, collagen, and α -SMA staining in the airways of HDM-exposed WT mice compared with all other groups (Fig. 3, D-F).

Reconstitution of IL-4 in the airway before chronic HDM exposure. Preliminary studies showed that 3×10^7 pfu of an adenovirus construct containing the IL-4 transgene resulted in $\sim 1,000$ pg/ml of murine IL-4 in the BAL fluid of control Balb/c mice (data not shown). Administration of this virus, but not the empty vector, to IL-4KO mice 1 day before the initiation of HDM exposure was associated with increased lung inflammation (Fig. 4A) and a partial reconstitution in eosinophilia (Fig. 4B). Furthermore, reconstitution with IL-4 in the context of chronic HDM exposure resulted in substantially increased IL-5 (Fig. 4C) and IL-13 (Fig. 4D) production by HDM-stimulated lung mononuclear cells compared with animals given control virus (RDA). As shown in Fig. 5, A-C, transient expression of IL-4 in IL-4KO mice exposed to HDM led to increases in mucous production, collagen deposition, and contractile elements. The extent of collagen and α -SMA staining was equivalent to that observed in WT mice exposed to HDM (Fig. 5, E and F). Administration of the control RDA vector was not associated with any significant structural changes in the airway wall. Importantly, administration of Ad/IL-4 to unexposed mice did not result in remodeling changes, and delivery of this vector to animals in which airway

Fig. 3. Impact of continuous antigen exposure on airway structural changes. WT and IL-4KO mice were exposed to intranasal allergen for 5 days/wk, and airway remodeling was evaluated after 5 consecutive wk of exposure. A: mucous production in airway epithelia of periodic acid-Schiff (PAS)-stained lung sections of control and HDM-exposed WT and IL-4KO mice. Images were subjected to digital color inversion to highlight mucous-producing cells (green). B: subepithelial accumulation of collagen in Picro-Sirius red-stained (PSR) lung sections of control and HDM-exposed WT and IL-4KO mice visualized under polarized light. C: contractile elements in airway wall of control and HDM-exposed WT and IL-4KO mice shown by immunohistochemistry for α -smooth muscle actin (α -SMA). Scale bars, 20 μ m. Airway structural changes were quantified by morphometry. Epithelial mucous production (D) and subepithelial collagen deposition (E) and contractile elements (F) were evaluated in control and HDM-exposed WT and IL-4KO mice. Values are means $\pm$ SE ($n = 6-11$ from 2 or 3 independent experiments). Horizontal bar indicates $P < 0.05$. Statistical analysis was performed using 1-way ANOVA with Tukey's post hoc test.



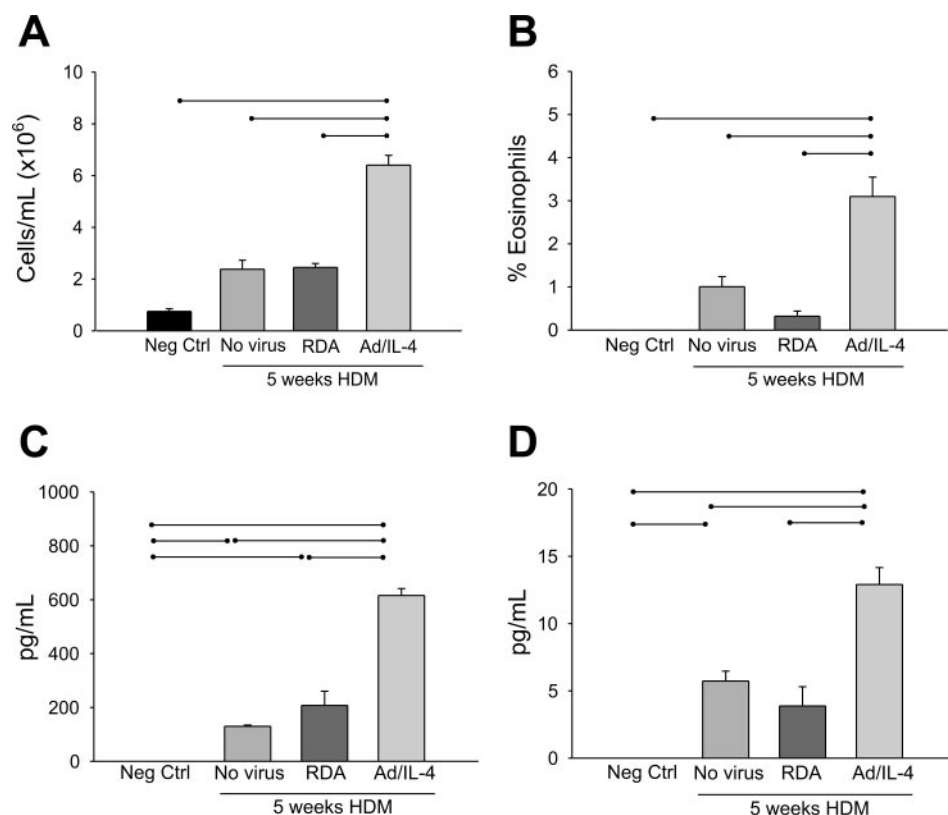


Fig. 4. Impact of intranasal administration of an adenovirus vector expressing murine IL-4 before chronic allergen challenge. IL-4KO mice were treated with no virus, control virus [replication-deficient adenovirus (RDA)], or Ad/IL-4 on day -1 and then exposed for 5 days/wk to HDM. Total cell recovery (A) and eosinophils in the BAL (B) demonstrate inflammation in lungs of HDM-exposed animals. Values are means $\pm$ SE ($n = 7$ –16 from 2 independent experiments). Horizontal bar indicates $P < 0.05$. Statistical analysis was performed using 1-way ANOVA with Tukey's post hoc test. In vitro expression of IL-5 (C) and IL-13 (D) by lung mononuclear cells was evaluated after 5 wk of HDM exposure. Cells were isolated and cultured in HDM-supplemented RPMI growth medium for 120 h, and cytokine levels in supernatants were assessed by ELISA. Data are representative of 2 independent experiments. Values are means $\pm$ SE ($n = 5$). Horizontal bar indicates $P < 0.05$. Statistical analysis was performed using 1-way ANOVA with Tukey's post hoc test.

inflammation was already established (i.e., after 3 wk of HDM exposure) did not elicit significant effects on the airway immune-inflammatory response or the structural-functional phenotype (data not shown).

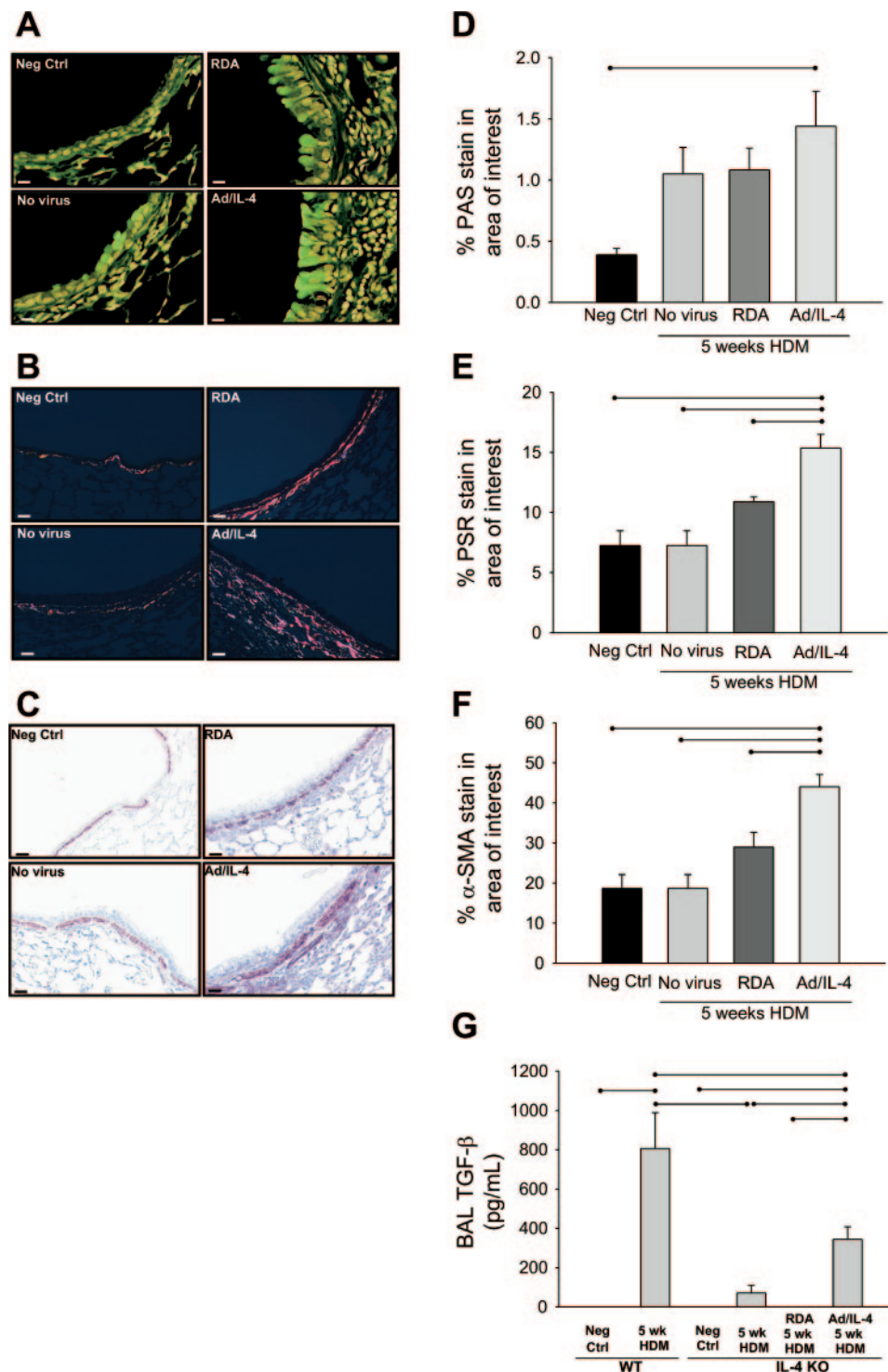
Evaluation of TGF- β expression in the airway. Local expression of transforming growth factor (TGF)- β , a key mediator of airway remodeling, was significantly elevated in WT mice chronically exposed to HDM (Fig. 5G). In contrast, TGF- β expression in IL-4KO mice was not significantly elevated over control levels. Administration of Ad/IL-4 to the airways of IL-4KO mice in the context of chronic HDM exposure resulted in a significant increase in TGF- β production; however, these levels remained lower than those observed in WT animals.

Bronchial hyperresponsiveness to MCh. The key indicators of airway responsiveness, namely, airway reactivity, airway sensitivity, and maximal inducible bronchoconstriction (26), were evaluated in WT and IL-4KO strains in response to intravenous MCh challenge, a technique that predominantly results in central airway constriction (39). Compared with allergen-naïve animals of both strains and HDM-exposed IL-4KO mice, HDM-exposed WT animals demonstrated a significant increase in airway reactivity, as evidenced by the slope of the dose-response curve to MCh (Fig. 6A). Significantly higher maximal inducible bronchoconstriction was also observed in these animals (Fig. 6A). Transient IL-4 expression in IL-4KO mice exposed to HDM led to increased airway reactivity, as evidenced by a steeper MCh dose-response curve (Fig. 6B) and elevated maximal inducible bronchoconstriction (Fig. 6B). However, the extent of these functional changes was clearly less than that observed in WT mice exposed to HDM.

DISCUSSION

Allergic asthma is a disease of a chronic nature elicited by exposure to aeroallergens in genetically predisposed individuals. There is no doubt that airway inflammation is a hallmark of the disease and, similarly, that Th2-polarized immunity underlies this process. Chronic asthma is also associated with a particular structural phenotype, commonly referred to as airway remodeling, which is thought to contribute to airway dysfunction (2, 3). However, it remains to be fully elucidated whether this structural-functional phenotype is dependent on chronic Th2-polarized allergic airway inflammation or any kind of chronic airway inflammation. Our strategy involved the establishment of divergent types of immune-inflammatory responses to the same aeroallergen. We elected to use the most pervasive indoor aeroallergen, HDM, which was delivered exclusively through the respiratory route, with no chemical adjuvants, for a prolonged period of time. In this manner, we intended to recapitulate how humans are exposed to aeroallergens and to reflect the chronic nature of clinical asthma. Furthermore, to observe the development of the immune-inflammatory response to allergen in the presence or absence of an intact Th2 signaling axis, we assessed the immunologic, structural, and physiological consequences of chronic allergen exposure in mice deficient in IL-4. In light of the evidence demonstrating the importance of IL-4 in the initiation of Th2-polarized immune responses, we speculated that chronic aeroallergen exposure in the genetic absence of IL-4 would not prevent the generation of airway inflammation but would, instead, alter its immune polarization.

Fig. 5. Intranasal administration of an adenovirus vector expressing murine IL-4 results in airway remodeling. IL-4KO mice were treated with Ad/IL-4 or empty vector (RDA) on day -1 before 5 consecutive wk of HDM exposure. A: mucous production in airway epithelia of periodic acid-Schiff-stained lungs (A) from IL-4KO mice treated with RDA or Ad/IL-4 on day -1 before 5 wk of HDM exposure. High-magnification images were subjected to digital color inversion to highlight mucous-producing cells (green). Picro-Sirius red staining (B) under polarized light demonstrates subepithelial collagen accumulation, whereas immunohistochemistry for α -SMA (C) indicates contractile elements in airway wall. Scale bars, 20 μ m. Airway structural changes were quantified by morphometry. Epithelial mucous accumulation (D) and deposition of subepithelial collagen (E) and contractile elements (F) were evaluated in lung sections stained for Periodic acid-Schiff, Picro-Sirius red, and α -SMA, respectively. Values are means $\pm$ SE ($n = 4-8$ from 2 independent experiments). Horizontal bar indicates $P < 0.05$. Statistical analysis was performed using 1-way ANOVA with Tukey's post hoc test. G: BAL transforming growth factor (TGF)- β levels in control mice and after 5 wk of HDM exposure. Cytokine levels in BAL supernatants were assessed by ELISA. Data are representative of 2 independent experiments. Values are means $\pm$ SE ($n = 4-8$). Horizontal bar indicates $P < 0.05$. Statistical analysis was performed using 1-way ANOVA with Tukey's post hoc test.



Previous studies examining immune responses to the surrogate aeroallergen ovalbumin (OVA) in the absence of IL-4 have reported a variety of findings, ranging from nonresponsiveness to a fully functional, albeit slightly dampened, Th2 response (1, 4, 5, 9, 12, 13, 20, 22, 29, 33). This diversity of outcomes is likely a reflection of the experimental conditions used in these studies, including the fact that the majority of these studies utilized protocols of rather acute exposure. The use of OVA as the inciting antigen in these studies has a

number of significant implications. 1) OVA is an immunologically innocuous protein that requires concurrent administration of a chemical adjuvant (typically alum or aluminum hydroxide), generally through the peritoneal cavity, to elicit allergic sensitization. 2) Chronic or recurrent administration of OVA results in a diminution, in fact a full abrogation, of the eosinophilic response (38), suggesting the emergence of unknown and, perhaps, confounding regulatory mechanisms. 3) OVA is, from a biochemical perspective, fundamentally dis-

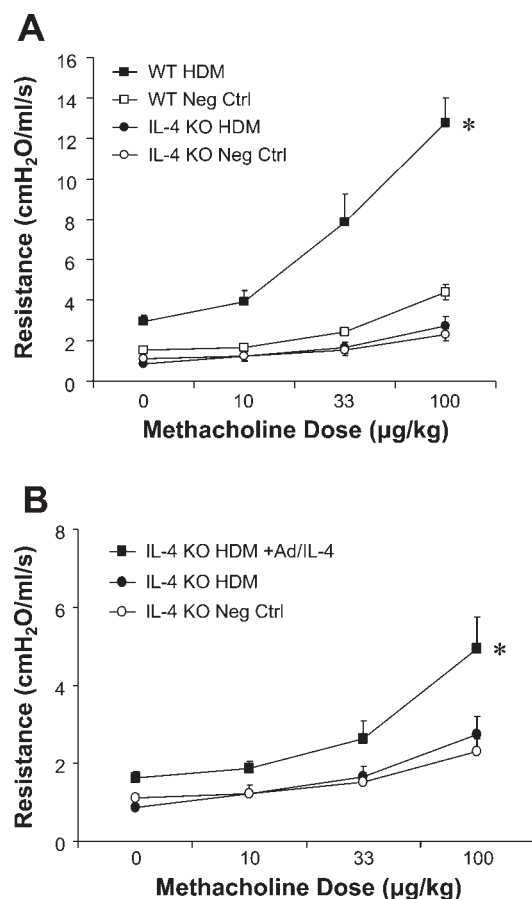


Fig. 6. Impact of chronic HDM exposure on airway functional responses. A: airway hyperreactivity to methacholine (A) in control WT mice, control IL-4KO mice, WT animals exposed to 5 wk of HDM, and IL-4KO animals exposed to 5 wk of HDM. Dose-response relationship between respiratory resistance and increasing intravenous doses of methacholine was evaluated in individual mice. Values are means $\pm$ SE ($n = 8-10$). * $P < 0.05$ vs. control. Statistical analysis was performed for each dose of methacholine by 1-way ANOVA with Tukey's post hoc test. B: airway hyperreactivity to methacholine (B) in control IL-4KO mice, HDM-exposed IL-4KO mice, and mice treated with Ad/IL-4 on day -1 before 5 wk of HDM exposure. Dose-response relationship between respiratory resistance and increasing intravenous doses of methacholine was evaluated in individual mice. HDM was administered for 5 days/wk for 5 consecutive wk. Values are means $\pm$ SE ($n = 6-7$). * $P < 0.05$ vs. IL-4KO control. Statistical analysis was performed for each dose of methacholine using 1-way ANOVA with Tukey's post hoc test.

tinct from HDM, which has robust proteolytic (11, 19), as well as chitinase (27, 40, 44), activities, in addition to other potentially immunogenic protein (10, 37) and nonprotein components (6). In this context, the studies reported here are the first to examine the consequences of persistent exposure to a clinically relevant aeroallergen administered solely through the respiratory tract in the presence or absence of the most important Th2-polarizing cytokine. Under these conditions, respiratory administration of HDM extract has allowed us to investigate the development of the immune response to allergen without the polarizing influence of exogenous adjuvants and, more importantly, to determine the structural and physiological consequences of divergent phenotypes of chronic allergen-driven airway inflammation.

In agreement with our previous data (15), chronic exposure to HDM in WT mice led to robust airway inflammation, which

was characterized by considerable eosinophilia. Chronic exposure of IL-4KO mice to HDM also resulted in considerable inflammation of the airways; this response, however, was virtually devoid of eosinophils. We therefore proceeded to investigate whether this change in cellular profile was the consequence of a divergent immune response. Our data show, as expected, that allergen-stimulated cells from genetically intact mice produced high levels of IL-5 and IL-13. Moreover, the preeminent HDM-specific immunoglobulin in these mice was IgG1, a Th2-associated isotype. In stark contrast, allergen-stimulated cells from IL-4KO mice produced high levels of IFN- γ , which were associated with HDM-specific serum IgG2a as the prevalent immunoglobulin, demonstrating the development of a Th1-polarized immune response to HDM exposure in IL-4KO mice.

To investigate whether these immune responses were transient or maintained, we examined the long-range recall response to HDM. After 5 wk of HDM exposure, the inflammatory response was allowed to resolve, and mice were reexposed to HDM. HDM exposure in IL-4KO mice elicited a robust airway inflammatory response that remained essentially devoid of eosinophils. Similarly, even greater divergence in the immune polarity of the cytokine and immunoglobulin responses between the two strains was detected at this time. Taken together, these findings demonstrate that the genetic absence of IL-4 does not preclude the elaboration of an immune response on chronic exposure to HDM but, importantly, facilitates a response that is divergent, as attested by the emergence of HDM-specific cellular and effector Th1 immunity.

We next investigated the impact of these two divergent immune-inflammatory responses on the airway structural-functional phenotype. Our data showed no evidence of airway remodeling in those mice that developed a Th1-polarized immune-inflammatory response to HDM exposure, as assessed by epithelial mucous production, subepithelial collagen deposition, and staining for peribronchial contractile elements. Notably, the absence of remodeling in these mice was accompanied by negligible AHR, in agreement with previously reported studies in IL-4-deficient mice subjected to chronic respiratory OVA exposure (25).

We then asked whether localized expression of IL-4 in the airway of mice genetically deficient in IL-4 would prompt the emergence of features typical of a Th2-polarized response. The approach that we chose, adenovirus-mediated gene delivery, has inherent limitations, inasmuch as transgene expression is transient (7–10 days) and cannot be effectively repeated. Yet, IL-4 overexpression in the lung during sensitization to HDM led to apparent changes in a number of aspects of the ensuing response. Indeed, we observed an emergence of Th2 inflammatory hallmarks as manifested by airway eosinophilia, as well as the ability of lung mononuclear cells to produce significant levels of IL-5 and IL-13 on in vitro challenge with HDM. Compared with the response observed in intact mice, the extent of eosinophilia was mild; it is possible that prolonged expression of IL-4 in the airway throughout the 5-wk exposure protocol would have resulted in a more robust Th2 immune response. However, this mild but persistent Th2 process led to airway remodeling changes nearly identical to those observed in IL-4-competent mice. In contrast, the reconstitution of the functional response (i.e., AHR) was only partial, since the level of AHR in HDM-exposed IL-4KO mice recon-

stituted with Ad/IL-4 was significantly greater than that detected in HDM-exposed IL-4KO mice, although clearly lower than that observed in HDM-exposed WT mice. In our view, these data suggest that inflammation and remodeling contribute to AHR, although the precise contribution of these two processes, which occur concurrently, to airway dysfunction remains to be determined. The timing of transgene delivery appeared to be critical, since overexpression of IL-4 during the last 2 wk of allergen exposure did not significantly alter the immune, inflammatory, structural, or functional responses to HDM. This observation suggests that once the type of immune response has been established, deviation may not be possible.

It is believed that TGF- β plays a preeminent role in airway remodeling (17, 18, 21). We provide evidence of increased levels of TGF- β in the BAL of genetically intact mice chronically exposed to HDM. In contrast, the levels of TGF- β in the BAL of IL-4KO mice were negligible, which suggests that direct interactions between the aeroallergen and resident lung cells such as monocytes, fibroblasts, and epithelial cells are unlikely to trigger a TGF- β response. Furthermore, our data also demonstrate that the elaboration of allergen-driven Th1-polarized chronic inflammation, which does not elicit a TGF- β response, is not associated with airway remodeling, suggesting a key role for TGF- β and, perhaps, other downstream growth factors in this process. The evidence indicating that overexpression of IL-4 in the airway of mice genetically deficient in IL-4 led to the emergence of features of Th2 immunity and significant increases in TGF- β levels in the BAL supports the argument that airway remodeling elicited by chronic exposure to allergen is dependent on the elaboration of a Th2-polarized immune-inflammatory response.

The studies we report here do not address the relative contributions of Th2-associated cells and mediators to airway remodeling. Recent studies in eosinophil-deficient mice have suggested that these cells can significantly contribute to airway remodeling (14, 23); however, this proposition remains controversial (8, 25, 41, 43). Moreover, the presence of eosinophils depends on the elaboration of a Th2 response. Importantly, eosinophils and Th2 lymphocytes, as well as other cell types important in allergic inflammation, such as mast cells, can produce a plethora of mediators, including TGF- β and other growth factors, that can contribute to airway remodeling (16, 42). The findings reported here establish that a structural-functional phenotype akin to that reported in asthma is directly connected to a Th2-polarized immune-inflammatory process. Future studies with genetic or, particularly, immunologic deletion of specific cells or molecules are needed to understand the consequences of prolonged exposure to HDM; however, findings from these studies cannot likely be extrapolated to responses elicited by other biochemically distinct aeroallergens, such as ragweed.

In conclusion, the present study illustrates a paradigm of host-environment interactions. Chronic exposure to the pervasive aeroallergen HDM elicits, under the experimental conditions used here, an airway immune-inflammatory response; however, the nature of this response is drastically influenced by the genetic background of the host. Importantly, the nature of this immune-inflammatory response fundamentally determines its structural and functional consequences. Indeed, a single genetic change, the deletion of the gene for IL-4, can divert the

response from Th2 polarization, which precludes the development of airway remodeling and bronchial hyperactivity.

ACKNOWLEDGMENTS

The expert technical assistance of Dr. Mark Inman and Jennifer Wattie for the evaluation of airway hyperactivity measurements is greatly appreciated. The authors thank Susanna Goncharova, Tina Walker, Mary Jo Smith, and Mary Bruni for technical support and Mary Kiriakopoulos for administrative assistance.

GRANTS

Financial support for these studies was provided by the Canadian Institutes of Health Research, the Canadian Lung Association, and the Ontario Thoracic Society. J. R. Johnson holds a Canadian Institutes of Health Research Doctoral Fellowship and M. Jordana holds a Canada Research Chair on "The Immunobiology of Respiratory Diseases and Allergy."

REFERENCES

1. Akimoto T, Numata F, Tamura M, Takata Y, Higashida N, Takashi T, Takeda K, Akira S. Abrogation of bronchial eosinophilic inflammation and airway hyperactivity in signal transducers and activators of transcription (STAT) 6-deficient mice. *J Exp Med* 187: 1537–1542, 1998.
2. Boulet LP, Turcotte H, Laviolette M, Naud F, Bernier MC, Martel S, Chakir J. Airway hyperresponsiveness, inflammation, and subepithelial collagen deposition in recently diagnosed versus long-standing mild asthma. Influence of inhaled corticosteroids. *Am J Respir Crit Care Med* 162: 1308–1313, 2000.
3. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola Asthma AM. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 161: 1720–1745, 2000.
4. Brusselle GG, Kips JC, Tavernier JH, van der Heyden JG, Cuvelier CA, Pauwels RA, Bluethmann H. Attenuation of allergic inflammation in IL-4 deficient mice. *Clin Exp Allergy* 24: 73–80, 1994.
5. Coyle AJ, Le Gros G, Bertrand C, Tsuyuki S, Heusser CH, Kopf M, Anderson GP. Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Respir Cell Mol Biol* 13: 54–59, 1995.
6. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, Toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 196: 1645–1651, 2002.
7. Ewart SL, Mitzner W, DiSilvestre DA, Meyers DA, Levitt RC. Airway hyperresponsiveness to acetylcholine: segregation analysis and evidence for linkage to murine chromosome 6. *Am J Respir Cell Mol Biol* 14: 487–495, 1996.
8. Foster PS, Ming Y, Matthei KI, Young IG, Temelkovski J, Kumar RK. Dissociation of inflammatory and epithelial responses in a murine model of chronic asthma. *Lab Invest* 80: 655–662, 2000.
9. Herrick CA, MacLeod H, Glusac E, Tigelaar RE, Bottomly K. Th2 responses induced by epicutaneous or inhalational protein exposure are differentially dependent on IL-4. *J Clin Invest* 105: 765–775, 2000.
10. Hewitt CR, Brown AP, Hart BJ, Pritchard DI. A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. *J Exp Med* 182: 1537–1544, 1995.
11. Hewitt CR, Horton H, Jones RM, Pritchard DI. Heterogeneous proteolytic specificity and activity of the house dust mite proteinase allergen Der P1. *Clin Exp Allergy* 27: 201–207, 1997.
12. Hogan SP, Matthei KI, Young JM, Koskinen A, Young IG, Foster PS. A novel T cell-regulated mechanism modulating allergen-induced airways hyperactivity in BALB/c mice independently of IL-4 and IL-5. *J Immunol* 161: 1501–1509, 1998.
13. Hogan SP, Mould A, Kikutani H, Ramsay AJ, Foster PS. Aeroallergen-induced eosinophilic inflammation, lung damage, and airways hyperactivity in mice can occur independently of IL-4 and allergen-specific immunoglobulins. *J Clin Invest* 99: 1329–1339, 1997.
14. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, Ghiran S, Gerard NP, Yu C, Orkin SH, Gerard C. A critical role for eosinophils in allergic airways remodeling. *Science* 305: 1776–1779, 2004.
15. Johnson JR, Wiley RE, Fattouh R, Swirski FK, Gajewska BU, Coyle AJ, Gutierrez-Ramos JC, Ellis R, Inman MD, Jordana M. Continuous

- exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 169: 378–385, 2004.
16. Kehrl JH, Wakefield LM, Roberts AB, Jakowlew S, Alvarez-Mon M, Derynck R, Sporn MB, Fauci AS. Production of transforming growth factor- β by human T lymphocytes and its potential role in the regulation of T cell growth. *J Exp Med* 163: 1037–1050, 1986.
 17. Kelly MM, Leigh R, Bonniaud P, Ellis R, Wattie J, Smith MJ, Martin G, Panju M, Inman MD, Gauldie J. Epithelial expression of profibrotic mediators in a model of allergen-induced airway remodeling. *Am J Respir Cell Mol Biol* 32: 99–107, 2005.
 18. Kenyon NJ, Ward RW, McGrew G, Last JA. TGF- β 1 causes airway fibrosis and increased collagen I and III mRNA in mice. *Thorax* 58: 772–777, 2003.
 19. King C, Simpson RJ, Moritz RL, Reed GE, Thompson PJ, Stewart GA. The isolation and characterization of a novel collagenolytic serine protease allergen (Der P9) from the dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 98: 739–747, 1996.
 20. Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Kohler G. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* 362: 245–248, 1993.
 21. Kumar RK, Herbert C, Foster PS. Expression of growth factors by airway epithelial cells in a model of chronic asthma: regulation and relationship to subepithelial fibrosis. *Clin Exp Allergy* 34: 567–575, 2004.
 22. Kuperman D, Schofield B, Wills-Karp M, Grusby MJ. Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production. *J Exp Med* 187: 939–948, 1998.
 23. Lee JJ, Dimina D, Macias MP, Ochkur SI, McGarry MP, O'Neill KR, Protheroe C, Pero R, Nguyen T, Cormier SA, Lenkiewicz E, Colbert D, Rinaldi L, Ackerman SJ, Irvin CG, Lee NA. Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 305: 1773–1776, 2004.
 24. Lei XF, Ohkawara Y, Stampfli MR, Mastruzzo C, Marr RA, Snider D, Xing Z, Jordana M. Disruption of antigen-induced inflammatory responses in CD40 ligand knockout mice. *J Clin Invest* 101: 1342–1353, 1998.
 25. Leigh R, Ellis R, Wattie JN, Hirota JA, Matthaei KI, Foster PS, O'Byrne PM, Inman MD. Type 2 cytokines in the pathogenesis of sustained airway dysfunction and airway remodeling in mice. *Am J Respir Crit Care Med* 169: 860–867, 2004.
 26. Leigh R, Ellis R, Wattie J, Southam DS, De Hoogh M, Gauldie J, O'Byrne PM, Inman MD. Dysfunction and remodeling of the mouse airway persist after resolution of acute allergen-induced airway inflammation. *Am J Respir Cell Mol Biol* 27: 526–535, 2002.
 27. McCall C, Hunter S, Stedman K, Weber E, Hillier A, Bozic C, Rivoire B, Olivry T. Characterization and cloning of a major high molecular weight house dust mite allergen (Der F15) for dogs. *Vet Immunol Immunopathol* 78: 231–247, 2001.
 28. Nakamura T, Kamogawa Y, Bottomly K, Flavell RA. Polarization of IL-4- and IFN- γ -producing CD4⁺ T cells following activation of naive CD4⁺ T cells. *J Immunol* 158: 1085–1094, 1997.
 29. Noben-Trauth N, Shultz LD, Brombacher F, Urban JF Jr, Gu H, Paul WE. An interleukin 4 (IL-4)-independent pathway for CD4⁺ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proc Natl Acad Sci USA* 94: 10838–10843, 1997.
 30. O'Brien RM, Thomas WR, Wootton AM. T cell responses to the purified major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 89: 1021–1031, 1992.
 31. Papaioannou VE, Fox JG. Efficacy of tribromoethanol anesthesia in mice. *Lab Anim Sci* 43: 189–192, 1993.
 32. Ramos-Barbon D, Ludwig MS, Martin JG. Airway remodeling: lessons from animal models. *Clin Rev Allergy Immunol* 27: 3–21, 2004.
 33. Ritz SA, Cundall MJ, Gajewska BU, Alvarez D, Gutierrez-Ramos JC, Coyle AJ, McKenzie AN, Stampfli MR, Jordana M. Granulocyte macrophage colony-stimulating factor-driven respiratory mucosal sensitization induces Th2 differentiation and function independently of interleukin-4. *Am J Respir Cell Mol Biol* 27: 428–435, 2002.
 34. Seder RA, Paul WE. Acquisition of lymphokine-producing phenotype by CD4⁺ T cells. *Annu Rev Immunol* 12: 635–673, 1994.
 35. Seder RA, Paul WE, Davis MM, Fazekas de St Groth B. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4⁺ T cells from T cell receptor transgenic mice. *J Exp Med* 176: 1091–1098, 1992.
 36. Stampfli MR, Wiley RE, Neigh GS, Gajewska BU, Lei XF, Snider DP, Xing Z, Jordana M. GM-CSF transgene expression in the airway allows aerosolized ovalbumin to induce allergic sensitization in mice. *J Clin Invest* 102: 1704–1714, 1998.
 37. Sun G, Stacey MA, Schmidt M, Mori L, Mattoli S. Interaction of mite allergens Der P3 and Der P9 with protease-activated receptor-2 expressed by lung epithelial cells. *J Immunol* 167: 1014–1021, 2001.
 38. Swirski FK, Sajic D, Robbins CS, Gajewska BU, Jordana M, Stampfli MR. Chronic exposure to innocuous antigen in sensitized mice leads to suppressed airway eosinophilia that is reversed by granulocyte macrophage colony-stimulating factor. *J Immunol* 169: 3499–3506, 2002.
 39. Wagers SS, Haverkamp HC, Bates JH, Norton RJ, Thompson-Figueroa JA, Sullivan MJ, Irvin CG. Intrinsic and antigen-induced airway hyperresponsiveness are the result of diverse physiological mechanisms. *J Appl Physiol* 102: 221–230, 2007.
 40. Weber E, Hunter S, Stedman K, Dreitz S, Olivry T, Hillier A, McCall C. Identification, characterization, and cloning of a complementary DNA encoding a 60-kd house dust mite allergen (Der F18) for human beings and dogs. *J Allergy Clin Immunol* 112: 79–86, 2003.
 41. Wills-Karp M, Karp CL. Biomedicine. Eosinophils in asthma: remodeling a tangled tale. *Science* 305: 1726–1729, 2004.
 42. Wong DT, Weller PF, Galli SJ, Elovic A, Rand TH, Gallagher GT, Chiang T, Chou MY, Matossian K, McBride J, et al. Human eosinophils express transforming growth factor- α . *J Exp Med* 172: 673–681, 1990.
 43. Yang M, Hogan SP, Henry PJ, Matthaei KI, McKenzie AN, Young IG, Rothenberg ME, Foster PS. Interleukin-13 mediates airways hyper-reactivity through the IL-4 receptor- α chain and STAT-6 independently of IL-5 and eotaxin. *Am J Respir Cell Mol Biol* 25: 522–530, 2001.
 44. Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, Hamid Q, Elias JA. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* 304: 1678–1682, 2004.