Intranasal Exposure of Mice to House Dust Mite Elicits Allergic Airway Inflammation via a GM-CSF-Mediated Mechanism

Elizabeth C. Cates, Ramzi Fattouh, Jennifer Wattie, Mark D. Inman, Susanna Goncharova, Anthony J. Coyle, José-Carlos Gutierrez-Ramos and Manel Jordana

*J Immunol* 2004; 173:6384-6392; doi: 10.4049/jimmunol.173.10.6384

http://www.jimmunol.org/content/173/10/6384

**Why *The JI***?

- **Rapid Reviews!** 30 days* from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

**References**

This article cites 30 articles, 10 of which you can access for free at: http://www.jimmunol.org/content/173/10/6384.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Intranasal Exposure of Mice to House Dust Mite Elicits Allergic Airway Inflammation via a GM-CSF-Mediated Mechanism

Elizabeth C. Cates,* Ramzi Fattouh,* Jennifer Wattie,† Mark D. Inman,‡ Susanna Goncharova,* Anthony J. Coyle,‡ José-Carlos Gutierrez-Ramos,‡ and Manel Jordana*‡

It is now well established that passive exposure to inhaled OVA leads to a state of immunological tolerance. Therefore, to elicit allergic sensitization, researchers have been compelled to devise alternative strategies, such as the systemic delivery of OVA in the context of powerful adjuvants, which are alien to the way humans are exposed and sensitized to allergens. The objectives of these studies were to investigate immune-inflammatory responses to intranasal delivery of a purified house dust mite (HDM) extract and to evaluate the role of GM-CSF in this process. HDM was delivered to BALB/c mice daily for 10 days. After the last exposure, mice were killed, bronchoalveolar lavage was performed, and samples were obtained. Expression/production of Th2-associated molecules in the lymph nodes, lung, and spleen were evaluated by real-time quantitative PCR and ELISA, respectively. Using this exposure protocol, exposure to HDM alone generated Th2 sensitization based on the expression/production of Th2 effector cells. Moreover, this inflammation was accompanied by airways hyper-responsiveness and a robust memory-driven immune response. Finally, administration of anti-GM-CSF-neutralizing Abs markedly reduced immune-inflammatory responses in both lung and spleen. Thus, intranasal delivery of HDM results in Th2 sensitization and airway eosinophilic inflammation that appear to be mediated, at least in part, by endogenous GM-CSF production. The Journal of Immunology, 2004, 173: 6384–6392.

Experimental modeling of allergic airway inflammation, particularly in mice, has contributed dramatically to our understanding of the pathogenesis of asthma. However, because conventionally used models involve i.p. administration of Ag in conjunction with potent chemical adjuvants (generally aluminum hydroxide) to generate unequivocal allergic airway inflammation, they are hampered by design when it comes to elucidating the subtle immunological elements required to generate allergic sensitization. Importantly, the need to use those artifices when eliciting allergic sensitization and airway inflammation is due in large part to the nature of the Ag selected, OVA. Indeed, not only does passive respiratory exposure to OVA lead to inhalation tolerance (1–4), but continuous exposure to OVA of an already sensitized animal results in a diminution and complete abrogation, not an increase or maintenance, of the airway inflammatory response (5).

To extend our knowledge of asthmatic inflammation, at least within the basic experimental domain, it thus seemed imperative to explore immune-inflammatory responses to real-life Aeroallergens. House dust mites (HDM)† are a significant source of indoor allergens, resulting in a range of atopic symptoms in 10% of individuals (6). Specifically, the species Dermatophagoides pteronyssinus is the most frequently implicated source of mite-related allergens in subjects with respiratory allergy (7). To date, the majority of work, in animal models, relating to allergy to HDM Ags has been performed in immunologically deficient animals in the form of a humanized SCID mouse model (8, 9) or in either of the above-mentioned conventional models (10–13). In this regard, Hsiue et al. (14) showed that multiple aerosol inhalations of crude mite extract were insufficient to cause sensitization in guinea pigs and that, in fact, i.p. injections were required to elicit overt Th2 sensitization. In addition, very little work has been performed in systems using whole mite extracts; most studies used single isolated allergens and/or their epitopes. Although whole mite extracts are complex from an immunological perspective, they are ultimately more representative of real-life Aeroallergen exposure.

In the present study we have investigated the impact of respiratory mucosal exposure to the HDM D. pteronyssinus on the generation of allergic-inflammatory responses. In contrast to Der p 1, which is well known to elicit inhalation tolerance (15; reviewed in Ref. 16), our data demonstrate that daily intranasal exposure to a HDM extract for 10 days without additional adjuvants elicits Th2-type sensitization, robust eosinophilic airway inflammation, and

---

*Department of Pathology and Molecular Medicine, Center for Gene Therapeutics, Division of Respiratory Diseases and Allergy, McMaster University, Hamilton, Ontario, Canada; †Firestone Institute for Respiratory Health, St. Joseph’s Healthcare, Hamilton, Ontario, Canada; and ‡Millennium Pharmaceuticals, Cambridge, MA 02139

Received for publication September 9, 2003. Accepted for publication September 2, 2004.

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

1 This work was supported by the Canadian Institutes of Health Research, the Hamilton Health Sciences Corp., and the St. Joseph’s Hospital Foundation. E.C.C. is the holder of an Ontario Graduate Scholarship. R.F. is supported by a scholarship from the Natural Sciences and Engineering Research Council of Canada.

2 Address correspondence and reprint requests to Dr. Manel Jordana, HSC-4821, Department of Pathology and Molecular Medicine, McMaster University, 1200 Main Street, West Hamilton, Ontario, Canada L8S 3Z5. E-mail address: jordana@mcmaster.ca

3 Abbreviations used in this paper: HDM, house dust mite extract; BAL, bronchoalveolar lavage; DC, dendritic cell; rDerp1, recombinant Der p 1 protein; TSLP, thymic stromal lymphopoietin.
airway hyper-responsiveness in mice. The intranasal administration of a polyclonal GM-CSF-neutralizing Ab concurrent with HDM exposure resulted in a marked attenuation of the airway inflammatory response as well as the production of Th2-associated cytokines. These findings suggest that the presence of GM-CSF in the airway microenvironment critically influences the generation of allergic airway responses to HDM.

Materials and Methods

Animals

Female BALB/c mice (6–8 wk old) were purchased from Charles River Laboratories (Montreal, Canada). The mice were housed in a specific pathogen-free environment under a 12-h light, 12-h dark cycle. All experiments described in this report were approved by the Animal Research Ethics Board of McMaster University.

Protocol of mucosal sensitization

HDM extract (Greer Laboratories, Lenoir, NC) was resuspended in saline (0.9% NaCl Irrigation Solution, Baxter, Toronto, Canada) at a concentration of 2.5 mg/ml. This suspension was delivered to the mice, with a specific pathogen-free environment under a 12-h light, 12-h dark cycle. All experiments described in this report were approved by the Animal Research Ethics Board of McMaster University.

Real-time quantitative PCR (TaqMan)

For TaqMan, primers and FAM-labeled probes were designed for GATA-3 with PrimerExpress version 1.5 software (Applied Biosystems, Foster City, CA) and were used as described by Ritz et al. (19). The IL-4, IL-5, IL-13, and CCR8 primers; FAM-labeled probes; GAPDH primers; and VIC-labeled probe were obtained from Applied Biosystems. PCR was performed using TaqMan Universal PCR Master Mix in the ABI PRISM 6700 Sequence Detection System operated by Sequence Detector version 1.7 software (Applied Biosystems). Gene expression is quantified relative to the expression of GAPDH, with naive samples defined as 1, and all other values plotted relative to them.

Splenocyte culture

Spleens were harvested into tubes containing sterile HBSS (In vitro A Life Technologies, Burlington, Canada). Splenocytes were isolated as previously described (18). Cells (8 × 10⁶/ml) were cultured in complete RPMI 1640 either alone or supplemented with 3.125 µg/ml HDM in flat-bottom, 96-well plates (optimal dose as determined by dose response; BD Biosciences, Franklin Lakes, NJ). After 5 days of culture, supernatants were harvested for cytokine measurements.

Cytokine and Ig measurements

Measurements of in vitro cytokine production were performed in one of two ways. 1) ELISA kits for mouse IL-4, IL-5, and IL-13 were purchased from R&D Systems. These assays have thresholds of detection <2, 1.5, and 7 pg/ml, respectively. 2) Cytokine production was detected using the Luminex 100 Total System (Luminex, Austin, TX) using xMAP multiplexing technology. 5-Plex cytokine kits containing microspheres specific for IFN-γ, IL-5, IL-10, IL-13, and IL-4 were purchased from Upstate Bio-technology (Charlottesville, VA).

Measurement of HDM-specific IgG1

Ninety-six-well Maxi-Sorp plates were coated overnight at 4°C with 100 µl of a 4 µg/ml solution of HDM in carbonate-bicarbonate buffer (pH 9.6; Sigma-Aldrich Canada, Oakville, Canada). Coated wells were blocked with 1% BSA in PBS for 2.5 h at room temperature. The ELISA was performed using four serial dilutions of each serum sample prepared using the reagent diluent (0.3% BSA in PBS and biotinylated goat anti-mouse IgG1 (0.25 µg/ml; Southern Biotechnology Associates, Birmingham AL). The dilutions used were 1/20, 1/200, 1/2000, and 1/20000. Alkaline-phosphatase-streptavidin (Sigma-Al clandestine ADR, Birmingham AL) were coated overnight at 4°C with 100 µl of HDM extract (Greer Laboratories, Lenoir, NC) was resuspended in saline (0.9% NaCl Irrigation Solution, Baxter, Toronto, Canada) at a concentration of 2.5 mg/ml. This suspension was delivered to the mice, with a specific pathogen-free environment under a 12-h light, 12-h dark cycle. All experiments described in this report were approved by the Animal Research Ethics Board of McMaster University.

In vivo recall with HDM

After complete resolution of the initial airways inflammation (3 wk to 1 mo after the HDM exposure), mice were subjected to an in vivo recall challenge with HDM. Mice were re-exposed to 25 µg of HDM, administered intranasally, daily for 3 consecutive days. Initially, experiments were performed in which mice received 25 µg of HDM for 2, 3, and 5 days and were killed 2, 3, or 5 days after each rechallenge period (data not shown). The most robust inflammatory response occurred 5 days after 3 HDM re-exposures. Therefore, this protocol was used in all subsequent experiments.

Anti-GM-CSF neutralizing Ab (anti-GM) treatment

In specific instances, mice were treated with anti-GM (R&D Systems, Minneapolis, MN) to block the effects of endogenous GM-CSF production. In these cases, for the first 3 days of the exposure protocol, mice received 25 µg of anti-GM in a 10-µl volume immediately preceding their exposure to HDM. The dose of anti-GM was reduced to 25 µg in a 10-µl volume for the last 7 days of the sensitization protocol. Polyclonal goat IgG (R&D Systems) was used as a control in these experiments.

Collection and measurement of specimens

Seventy-two hours after the last HDM exposure, mice were killed, bronchoalveolar lavage (BAL) was performed, and differential cell counts were determined according to a previously established protocol (18). In addition, blood was collected by retro-orbital bleeding. Serum was obtained by centrifugation after incubating whole blood for 30 min at 37°C. Finally, lung tissue was fixed in 10% formalin and embedded in paraffin. Three-micron sections were stained with H&E.

Isolation of mRNA from tissues and generation of cDNA

Lungs and thoracic lymph nodes were collected, placed in RNAlater (Ambion, Austin, TX), and stored at -20°C for future RNA extraction. Total RNA was extracted using TriPure isolation reagent (Roche Canada, Laval, Canada). Genomic DNA was eliminated using the DNA-free kit (Ambion). Finally, cDNA was generated using the RETROscript kit (Ambion) using random decamers as primers.

Flow cytometric analysis

Flow cytometric analysis was performed on lung cells isolated as previously described (18). The cells were then stained with a panel of fluorescently conjugated Abs (BD Pharmingen, San Diego, CA). The following Abs were used: anti-CD11c-FITC, anti-MHC II-FITC, anti-B220-FITC, anti-CD4-FITC, anti-CD8-FITC, anti-CD11b-PE, anti-B7-1-PE, anti-CD3-PE, anti-CD69-PE, anti-MHC II-biotin, anti-B7-2-biotin, anti-CD4-biotin, and anti-CD3-biotin. Appropriate isotype controls were also purchased from BD Pharmingen. The anti-TCR-α/β-FITC Ab was provided by Mil- lenium Pharmaceuticals (Cambridge, MA). To minimize nonspecific binding, 4–8 × 10⁶ cells were reincubated with Fc block (CD16/CD32, BD Pharmingen). For each Ab combination, 4–8 × 10⁶ cells were incubated with mAbs at 0–4°C for 30 min; the cells were then washed and treated with second-stage reagents. Streptavidin-PerCP (BD Biosciences, San Jose, CA) was used as a second-stage reagent for detection of biotin- labeled Abs. Titration was performed to determine the optimal concentration for each Ab. Cells were fixed in 1% paraformaldehyde and counted on a FACScan (BD Biosciences, Mountain View, CA). A minimum of 100,000 events were acquired. Analysis was performed using WinMDI software.

Downloaded from http://www.jimmunol.org/ by guest on October 23, 2017
Airway hyper-responsiveness measurements

Airway responsiveness was measured using the flow interrupter technique (20–22) on the basis of the response of total respiratory system resistance to saline and increasing doses (10, 33, 100, and 330 μg) of i.v. methacholine. Briefly, mice were anesthetized using 2,2,2-trifluoroethanol (Avertin; 240 mg/kg, i.p.; Aldrich Chemical, Milwaukee, WI), and the trachea was exposed and cannulated using a blunt 18-gauge needle. The needle was then attached to a ventilator (RV5; Voltek Enterprises, Toronto, Canada) designed to deliver constant inspiratory flow despite the disturbances to the function of the respiratory system that occur during methacholine challenge. Using the pattern of ventilation described by Inman et al. (20), total respiratory system resistance was measured, and the difference in resistance at each dose of methacholine was assessed.
Results

Inflammatory responses in the airway

Intranasal delivery of HDM elicited a robust inflammatory response in the lungs/airways of mice that was measurable in the BAL and was clearly evident histopathologically. Exposure to HDM resulted in an increase in total cells to \( \sim 4 \times 10^6 \) (from \( 7 \times 10^5 \) in naive mice; Fig. 1A). The cellular distribution consisted of 76% mononuclear cells and 22% eosinophils (Fig. 1, B and C). Infiltration of neutrophils was negligible (3%; data not shown). Intranasal delivery of saline did not yield any inflammatory response. In agreement with our findings in the BAL, lungs from mice exposed to HDM showed evidence of severe peribronchial and perivascular inflammation as well as goblet cell hyperplasia (Fig. 1D).

To further define the types of immune effector cells present within this inflammatory infiltrate, we isolated lung mononuclear cells on day 9 of the exposure protocol and performed a flow cytometric analysis (Fig. 1, E and F). HDM exposure led to an expansion of both activated CD8\(^+\) and CD4\(^+\) cells (based on CD69 expression), 23- and 47-fold over that in naive cells, respectively. Th2 effector cells (CD3\(^-\)/CD4\(^+\)/CD11b\(^+\)/T1ST2\(^+\)) were also greatly expanded (45-fold over naive cells; Fig. 1E). The APC population was also examined by flow cytometry (Fig. 1F). We observed a 2-fold increase in macrophages (MHC II\(^+\)/CD11b\(^-\)) and a 4.5-fold increase in dendritic cells (DCs; MHC II\(^+\)/CD11c\(^+\)). The degree of APC activation was examined by assessing the number of APCs expressing the costimulatory molecules B7.1 and B7.2. Our data show that for cells expressing MHC II, there was an \( \sim 5\)-fold increase in both B7.1 and B7.2 expression.

In some groups of mice, the inflammation caused by the initial exposure to HDM was allowed to resolve, and the mice were rechallenged with HDM. The resolution of the initial inflammatory response was determined by killing mice 3 wk to 1 month after the exposure protocol and assessing the nature of the inflammatory infiltrate in the BAL. HDM re-exposure resulted in a recapitulation and, in fact, an augmentation of the initial inflammatory response. This response consisted of \( \sim 6.6 \) million total cells in the BAL fluid 120 h after recall challenge (approximately twice the number of total cells observed in the initial inflammatory response). Eosinophils constituted \( \sim 25\% \) of this population (Fig. 2).

Airway responsiveness measurements

To determine whether the inflammatory response in the lung contributed to airway dysfunction, we evaluated the total respiratory resistance in mice exposed to saline and mice exposed to 10 days of HDM using a flow interrupter technique. HDM exposure resulted in a higher, although not significant, background level of airway resistance compared with that in controls. Mice exposed to HDM were hyper-responsive to increasing doses of methacholine compared with saline-exposed controls. This reached statistical significance at a dose of 33 \( \mu g/kg \) and remained significantly elevated at increased doses of methacholine (Fig. 3).

Evidence of Th2 sensitization and effector activity in HDM-exposed mice

Evidence of Th2 sensitization in thoracic lymph nodes was evaluated using TaqMan to measure the relative expression of the Th2-associated transcription factor GATA-3 and the Th2-affiliated chemokine receptors CCR4 and CCR8. We observed a significant up-regulation of GATA-3 that peaked 24 h after the first HDM administration at a 12-fold increase over that in naive cells (Fig. 4A). We also noted significant up-regulation of CCR4 and CCR8, both of which reached their highest levels 24 h after three HDM exposures (\( \sim 43\) and 85-fold over naive, respectively; Fig. 4, B and C).

Th2 activity in the effector organ (i.e., the lung) was assessed using TaqMan to examine the expression of Th2-associated cytokines. We detected a significant up-regulation in the relative expression of IL-4 and IL-5 in the lungs of mice exposed to HDM in vivo. As shown in Fig. 5, A and B, IL-4 expression showed a

Data analysis

Data are expressed as the mean \( \pm \) SEM. Statistical analysis was performed using SigmaStat version 2.03 (Jandel, San Ramon, CA). Differences were considered significant at \( p < 0.05 \) (by one-way ANOVA unless otherwise indicated).
220-fold increase, and IL-5 expression showed a 14-fold increase over that in naive cells.

Evidence of systemic Th2 sensitization was obtained by detecting significant Th2 cytokine production by splenocytes from HDM-exposed animals upon HDM recall in vitro. Levels of IL-4, IL-5, and IL-13 were significantly elevated to 160, 1,470, and 19,900 pg/ml, respectively (Fig. 5C). In addition, we were able to measure HDM-specific IgG1 and total IgE in the sera of mice exposed to HDM in vivo. Both these Th2-associated Igs were significantly elevated compared with levels in naive mice (Fig. 5D).

GM-CSF-neutralizing Ab treatment diminishes the response

To test the putative role of GM-CSF in this process, we delivered anti-GM to mice immediately before HDM exposure. First, we observed a marked attenuation of the level of inflammation in the BAL fluid. Indeed, total cells were decreased from $4 \times 10^6$ in HDM-exposed mice to $\sim 2 \times 10^5$ in anti-GM-treated, HDM-exposed mice (Fig. 6A). Eosinophilia in these mice was also considerably decreased from $8 \times 10^5$ to $\sim 1.5 \times 10^5$ (corresponding to a decrease in eosinophils from $\sim 21\%$ of total cells to $7\%$). Mice that received control Ab and HDM had similar numbers of cells in their BAL as mice that were exposed to HDM alone.

Systemically, splenocytes from anti-GM-treated, HDM-exposed mice showed a significant decrease in their production of IL-5 and IL-13 when restimulated with HDM in vitro compared with mice that were treated with HDM alone or in conjunction with control Ab in vivo. Compared with mice treated with control Ab, anti-GM treatment resulted in a 63% decrease in IL-5 (Fig. 6B; 1,350 vs 500 pg/ml) and a 49% reduction in IL-13 (Fig. 6C; 14,800 vs 7,500 pg/ml).

Immune-inflammatory responses in mice exposed to rDerp1

As expected, intranasal delivery of only rDerp1 to mice resulted in a BAL cellular profile indistinguishable from that in naive mice. However, if rDerp1 was delivered into an airway microenvironment enriched with GM-CSF, a robust inflammatory response was observed (Fig. 7, A–C). Indeed, mice exposed to GM-CSF and rDerp1 exhibited an increase in total BAL cells (Fig. 7A) to $4.4 \times 10^6$ (from $9.3 \times 10^5$ in naive mice). This population consisted of mononuclear cells (Fig. 7B) and eosinophils (Fig. 7C). Specifically, the number of eosinophils increased to $3.6 \times 10^5$ (with no eosinophils present in the lungs of naive mice). Interestingly, the number of eosinophils in mice exposed to rDerp1 in the context of GM-CSF was very similar to the number in mice exposed to HDM alone. Virtually no eosinophils were observed in mice exposed to GM-CSF in the absence of allergen (17).

Evidence of systemic Th2 sensitization was acquired through the detection of an increase in Th2-associated cytokine production by splenocytes from mice exposed to rDerp1 in the context of
GM-CSF. When these cells were restimulated with rDerp1 in vitro, levels of IL-4 (Fig. 7D), IL-5 (Fig. 7E), and IL-13 (Fig. 7F) were significantly elevated relative to control values.

**Discussion**

Dust mites are a significant source of indoor aeroallergens. Despite this significance, there is relatively little information available regarding airway immune-inflammatory responses to HDM in murine systems. A great deal of information concerning allergic airway inflammation has been compiled in murine experimental systems using OVA as a surrogate allergen. However, OVA, when used without adjuvants, elicits tolerance. Our data show that the administration of this whole mite extract to mice in the absence of exogenous adjuvants leads not to tolerance, but to a robust inflammatory response. Although this extract is known to contain LPS, it is highly unlikely that it is mediating the effects seen in this study. Indeed, LPS is known to be the most powerful stimulator of IL-12, which preferentially drives Th1-type responses.

The inflammatory response to HDM is characterized by an infiltrate comprising a considerable proportion of eosinophils and Th2 effector cells (as indicated by the expression of T1/ST2, a putative marker of Th2 effector cells) (23) as well as by genetic up-regulation of Th2-affiliated cytokines. These findings are corroborated histopathologically by evidence of peribronchial/perivascular inflammation and mucous production and are correlated physiologically with airway hyper-responsiveness. Systemically, this process is associated with increased serum levels of Th2-affiliated IgGs, total IgE, and HDM-specific IgG1. Conclusive evidence for the generation of a Th2 immune response is provided by early genetic up-regulation in the thoracic lymph nodes of Th2-affiliated molecules (GATA-3, CCR4, and CCR8), splenocyte production of Th2-affiliated cytokines upon HDM recall in vitro, and a very robust inflammatory response upon long-term in vivo recall.

We have previously reported an experimental model in which the expression of exogenous GM-CSF, a cytokine clearly relevant...
in asthma (24), in the airway environment facilitated allergic sensiti-
ization and airway eosinophilic inflammation to an otherwise
innocuous Ag, OVA (17). That intranasal exposure to HDM alone
generated such a similar response prompted us to consider the role
of endogenously produced GM-CSF in this process. However, de-
tection of GM-CSF in the BAL or lung of HDM-exposed mice
proved exceedingly difficult. Given GM-CSF’s powerful nature,
we did not expect to measure high levels of this cytokine; more-
over, increased difficulty in its measurement was probably intro-
buted by the proteolytic nature of the extract delivered. In addi-
tion, GM-CSF is known to become embedded in extracellular
matrix (25), which may have prevented the passage of sufficient
(i.e., detectable) amounts into the BAL fluid. Thus, another ap-
proach for testing our hypothesis regarding the involvement of
GM-CSF in the immune-inflammatory response to HDM had to be
developed. GM-CSF is not only indispensable for DC differen-
tiation and maturation from bone marrow progenitors and peripheral
monocytes in vitro, but administration of GM-CSF to the
mouse lung leads to a massive accumulation and activation of
DCs. Hence, we set out to 1) examine changes in the APC com-
partment upon HDM exposure, and 2) determine whether the
HDM-elicited immune-inflammatory response could be affected
by neutralizing GM-CSF in vivo.

We first evaluated the APC compartment in the lungs of mice
that had been exposed to HDM. We observed an increase in both
macrophages and DCs as well as in the activation of APCs based
on the expression of B7.1 and B7.2. We then tested the putative
role of GM-CSF in this process by delivering anti-GM to mice
immediately before HDM exposure. We observed a marked atten-
uation of the level of inflammation in BAL fluid. Indeed, total cells
were decreased by ∼50% in anti-GM-treated, HDM-exposed
mice. Eosinophilia in these mice was also considerably decreased.
Systemically, splenocytes from anti-GM-treated, HDM-exposed
mice showed a significant decrease in their production of IL-5 and
IL-13 when restimulated with HDM in vitro compared with mice
that were treated with HDM alone or in conjunction with control
Ab in vivo. Furthermore, we investigated the impact of repeated
intranasal exposure to rDerp1. Although rDerp1 alone did not elic-
inflammation of the airways, exposure to rDerp1 in the context of
a GM-CSF-enriched microenvironment resulted in eosinophilic
airway inflammation comparable to that observed in mice exposed
to HDM as well as a Th2-polarized cytokine profile upon in vitro
stimulation of splenocytes with rDerp1.

It is well known that the main Ags of HDM, Der p 1, 3, 5, and
9, are cysteine and/or serine proteases (26), and a number of in
vitro studies have demonstrated that these Ags can stimulate air-
way epithelial cells to liberate a number of cytokines, including
GM-CSF, probably through a protease-activated receptor-2-medi-
ated mechanism (27–29). Our data demonstrate for the first time
that GM-CSF plays an important role in the generation of Th2
sensitization and airway eosinophilic inflammation in BALB/c
mice exposed to an HDM extract in vivo. However, it is also likely
that molecules other than GM-CSF play a role in this process.
In this regard, Soumelis et al. (30) have recently demonstrated
that epithelial cells, including lung fibroblasts and bronchial epithe-
ral cells, can produce thymic stromal lymphoprotein (TSLP), a mol-
ecule capable of inducing marked expansion and activation of
DCs. Moreover, epithelial cells may secrete GM-CSF in response
to HDM exposure (31). However, the role of GM-CSF in the ini-
tiation to conclusion of the immune-inflammatory response to
HDM awaits further studies.

Finally, we suggest that the oppor-
tunity to integrate a discrete pathogenic sequence of events, from
initiation to conclusion, illustrates one of the advantages of using

FIGURE 6. Putative role of GM-CSF in the immune-inflammatory process. A, Airway inflammation in the BAL fluid of BALB/c mice exposed
to HDM in the context of anti-GM. Over a period of 10 consecutive days
mice were exposed daily to HDM immediately after 10 μl of either IgG or
anti-GM. Data were obtained 72 h after the last exposure (n = 6–29; *,
significant decreases compared with IgG/HDM: p < 0.013, by Fisher’s
post-hoc test). B and C, In vitro production of HDM-specific IL-5 and
IL-13 by splenocytes. Over a period of 10 consecutive days mice were
exposed daily to HDM after receiving 10 μl of either IgG or anti-GM. Cells
were obtained 72 h after the last exposure to HDM and were cultured in
RPMI 1640 alone (■) or RPMI 1640 plus 3.125 μg HDM/well (□, n =
3–6; *, significant decreases compared with IgG/HDM: p < 0.004, by
Fisher’s post-hoc test).
real-life aeroallergens. We surmise that the data presented in this report provide a meaningful foundation to explore the biochemical and molecular bases of aeroallergen-induced immunopathology.

Acknowledgments
We are indebted to David Alvarez, Joanna deJong, Beata Gajewska, Noranda Nyholt, Stacey Ritz, Tina Walker, and Ryan Wiley for their help.
References


