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Murine Model for Non-IgE-Mediated Asthma

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Abstract—There is increasing evidence that inflammatory mechanisms other than atopy or eosinophilic inflammation may be involved in the pathogenesis of asthma. The mechanisms associated with non-atopic (non-IgE) or neutrophil-mediated asthma are poorly investigated. Non-atopic airway inflammation and hyperresponsiveness was induced in mice by skin sensitization with dinitrofluorobenzene (DNFB) followed by intra-airway challenge with dinitrobenzene sulfonic acid (DNS). Acute bronchoconstriction and mast cell activation were observed shortly after challenge. Increased levels of the major mast cell mediator, TNF-α, were found in the bronchoalveolar lavage fluid of DNFB-sensitized. Mast cells play a key role in the early release of TNF-α since mast-cell-deficient WBB6F$_1$-*W/W*^{V} mice did not show an increase in TNF-α release after DNFB-sensitization and DNS challenge compared to their ++ littermates. Features of the late-phase pulmonary reaction included mononuclear and neutrophilic cell infiltration, pulmonary edema, *in vitro* tracheal hyperreactivity and *in vivo* airway hyperresponsiveness. These characteristics bear marked similarity with those observed in non-atopic asthmatic patients. Therefore, this model can be used to further study the mechanisms potentially responsible for the development of non-IgE-mediated asthma.

KEY WORDS: mouse; asthma; hyperreactivity; mast cell; neutrophil.

INTRODUCTION

Asthma patients can roughly be divided into two groups, atopic and non-atopic. It has become a paradigm that the majority of patients have atopic asthma, characterized by an elevation of total and allergen-specific IgE in serum, positive skin prick test to common allergens and eosinophilia (1). A recent review of epidemiological reports by Pearce *et al*. (2) suggests that in half of the asthma patients studied, total serum IgE levels are not elevated. Non-atopic asthmatics are skin test negative to common allergens and there is no evidence of increases in allergen-specific or total serum IgE (3, 4). Amin *et al*. (5) recently showed that besides the difference in serum IgE levels, atopic and non-atopic patients had different pathological changes present in their airways despite their similar clinical respiratory symptoms. Atopic patients had increased numbers of eosinophils in their airway tissue, whereas non-atopic asthma is dominated by the infiltration of neutrophils. These findings indicate that other mechanisms than atopy or eosinophilic inflammation may be involved in the development of asthma.

Non-atopic asthma is an increasing problem in the developed world. The mechanisms involved in the induction and ongoing respiratory impairments associated with non-atopic asthma are poorly investigated (2). It has become apparent that non-IgE-mediated cell-mediated reactions can occur in the airways (6–8) of sensitized animals after local challenge with low molecular weight compounds. These reactions in the lung are not mediated by IgE.

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Low molecular weight substances (<5000 Da) are the most common agents causing non-atopic asthma (9). In the mouse, the low molecular weight compounds picrylchloride and toluene diisocyanate (TDI) have been shown to induce pulmonary hypersensitivity reactions (10–12). In the present study, a hypersensitivity reaction was elicited in the airways of mice with the low molecular weight compound dinitrofluorobenzene (DNFB) as the contact sensitizing hapten followed by intranasal challenge with the water-soluble compound dinitrobenzene sulphonic acid (DNS).

MATERIALS AND METHODS

Animals

Male BALB/c mice (6–8 weeks) were obtained from the Central Animal Laboratory (GDL), Utrecht University, Utrecht, The Netherlands. All experiments were conducted in accordance with the Animal Care Committee of the Utrecht University (Utrecht, The Netherlands).

Sensitization and Experimental Procedure

Mice were sensitized on day 0 with either DNFB (0.5% dissolved in acetone: olive oil (4:1)) (Sigma Chemical Co., St. Louis, USA) or vehicle control, both of which were applied epicutaneously to the shaved thorax (50 μL) and all four paws (50 μL). On day 1, DNFB or vehicle control (50 μL) was applied to the thorax alone. DNFB- and vehicle-sensitized mice were intranasally challenged with dinitrobenzene sulphonic acid (DNS, 50 μL, 0.6% in PBS, pH 7.2) on day 5. The sensitization and challenge were performed under light anesthesia (pentobarbitone, 40 mg/kg i.p.).

Mast Cell Activation *In Vivo*

Mouse mast cell protease 1 (mMCP-1) is a protease specific for mouse mast cells, that appears in the blood after mast cell activation. To monitor mast cell activation in time, blood samples of DNFB- and vehicle-sensitized mice were taken 10, 30 min and 3, 6, 24, and 48 h after intranasal DNS challenge as described before. Blood samples were collected and after centrifugation sera were stored at -70°C until use. Levels of mMCP-1 were measured using a commercially available ELISA assay (Moredun Scientific Ltd., Midlothian, UK) as described before (13).

TNF-α in Bronchoalveolar Lavage Fluid

Bronchoalveolar lavages (BAL) were performed in mice 30 min after the challenge as described before (13). At the time of the lavage, mice were killed and the chest cavity was exposed to allow expansion. The trachea was carefully intubated and the catheter was secured with ligatures. Warm saline (37°C,1 mL) was slowly injected in the lungs and withdrawn in 4 $\times$ 1 mL aliquots. The aliquots were pooled and maintained at 4°C. The lavage fluids were centrifuged (580g, 5 min, 4°C) to isolate the BAL cells. The concentration of TNF-α in BAL fluid was measured via a murine commercially available TNF-α ELISA kit (BioSource, Nivelles, Belgium).

Acute Bronchoconstriction

Bronchoconstriction was measured as reported previously (14). In short, 5 min before intranasal DNS challenge, unrestrained conscious mice were placed in a whole body plethysmographic chamber (Buxco Electronics, Inc., Shanon, CT) to analyze the respiratory wave forms and obtain basal line. After 4 min and 30 s, the mice were challenged under light anesthesia (inhalation of halothane 3%) and placed back in the chamber where resistance in each animal was measured over a 15-min period. Airway resistance is expressed as enhanced pause (PenH)

$$\text{PenH} = \text{pause} \times \text{PEP/PIP}$$

where PEP stands for peak expiratory pressure and PIP for peak inspiratory pressure. Pause is defined as (Te $-$ Tr)/Tr, where Te stands for time of expiration and Tr stands for the relaxation time—the time of pressure decay to 36% of the total expiratory pressure signal. During bronchoconstriction, the changes in the box pressure during expiration are more pronounced than during inspiration. Thus bronchoconstriction is reflected by an increase in PenH, a dimensionless value to empirically monitor airway function. After intranasal challenge, for each mouse maximal PenH readings were taken over 1-min time windows at the following time points: 2 min 30 s, 5 min, 10, and 15 min.

Mucosal Permeability

Mucosal permeability was assessed as previously described (15). At 22 h after the challenge, Evans blue dye (1.25%, 50 μL in sterile saline) was injected i.v. into anesthetized mice. The mice were sacrificed 24 h after the challenge and blood samples and BAL samples

were taken as described above. The volumes of the first milliliter of lavage fluid recovered were recorded for each mouse for measurement of Evans blue content and for the calculation of mucosal exudation. No significant differences were found in the volumes of BAL fluids of vehicle- and DNFB-sensitized mice. The extravasation of Evans blue dye-labeled macromolecules from the pulmonary microcirculation to the bronchoalveolar spaces was quantified by measuring the OD of the lavage and plasma samples at a wavelength of 595 nm with a microplate reader. The amount of mucosal leakage in the lavage fluid (μL/lung) was determined by dividing the Evans blue content in the total lavage fluid by the Evans blue content in 1 mL of plasma.

Leukocyte Accumulation in Bronchoalveolar Lavage Fluid

Bronchoalveolar lavages (BAL) were performed in mice 24 and 48 h after the challenge. After sacrificing the animals, the trachea was canulated. Saline (37°C) was slowly injected into the lung and withdrawn in 4 × 1 mL aliquots. The aliquots were pooled and maintained at 4°C. The lavage fluids were centrifuged (580g, 5 min, 4°C) to isolate the BAL cells. Total cells were counted using a haemocytometer and expressed as cells/lung. The BAL cell preparations were analyzed morphologically after centrifugation onto microscopic slides. Air-dried preparations were fixed and stained with hematoxylin and eosin (Sigma Aldrich, St. Louis, MO) to ascertain the leukocyte populations. Results are expressed as leukocytes/lung for neutrophils and mononuclear cells in the airway lumen.

Tracheal Reactivity *In Vitro*

Mice were killed with an overdose of pentobarbitone at 24 and 48 h after intranasal DNS challenge. The trachea, which was resected *in toto*, was carefully cleaned of connective tissue using a binocular microscope as described before (6). A nine-ring piece of trachea (taken from just below the larynx) was then transferred to a 10-mL organ bath containing a modified oxygenated Krebs solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 25 mM $NaHCO_3$, 1 mM $NaHPO_4$, and 11.1 mM glucose). The trachea was directly slipped onto two supports, one coupled to the organ bath and the other coupled to an isometric transducer (Harvard Bioscience, Boston, MA). The solution was aerated (95% O_2:5% CO_2) and maintained at 37°C. Isometric measurements were made using a force displacement transducer and a two-channel recorder (Servogar type SE-120; Plato BV, Diemen, The Netherlands) and measurements were expressed as changes in milligram (mg) force. An optimal preload, determined to be 1 g, was placed on the tissue at the beginning of the experiment. The trachea was allowed to equilibrate for at least 1 h before contractile effects were elicited. During this period, the bath fluid was exchanged every 15 min. At the end of the equilibrium phase, tracheal contractile reactivity was measured by recording cumulative concentration response-curves to carbachol (10^{-8} to 10^{-4} M) (Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands).

Airway Responsiveness *In Vivo*

Airway responsiveness was measured in conscious unrestrained mice 48 h after challenge, by recording respiratory pressure curves in response to inhaled nebulized methacholine using whole-body plethysmography (Buxco Electronics, Inc., Shanon, CT) as described previously (16). As an index of airway responsiveness, increases in PenH were measured. In short, mice were place in a whole-body chamber. After baseline PenH values were obtained for 3 min and averaged, animals were exposed to a saline aerosol and a series of increasing concentrations of methacholine (ranging from 1.6 to 50 mg/mL) aerosols. Aerosols were generated by a Pari LC Star nebulizer for 3 min and after each nebulation readings were recorded for 3 min and averaged. Airway responsiveness was expressed as the PenH per dose methacholine.

Histological Examination

At 24 and 48 h after intranasal hapten challenge, lungs were removed from mice after lethal anesthesia with pentobarbitone. (I) At 24 h after challenge, lungs were removed and filled intratracheally with acetic acid formalin fixing solution (0.8% formalin, 4% acetic acid). Lungs were fixed for at least 24 h in the fixative, dehydrated and embedded in paraplast. Four-micrometer sections were stained with hematoxylin and eosin. (II) Because of optimized histological techniques, the lungs isolated 48 h after challenge were fixed in 4% formaldehyde in PBS and routinely embedded in GMA (glycol methacrylate; Merck, Darmstadt, Germany) (17). Serial sections were cut at 3 μm and stained with hematoxylin in combination with eosin. The parameters were scored according to the method described by Enander *et al.* (18), as indicated in Table 1.

Table 1. Histological Score of Cellular Accumulation in the Lung According to the Method of Enander *et al*. (18)

Accumulation of inflammatory cells	0	1	2	3
Diffusely around bronchioli	No cells	<10 cells layers thick	>10 cells layers thick	Entirely surrounding bronchiolus >10 cells layers thick
Around blood vessels	No cells	≤3 cells layers thick	4–10 cells layers thick	≥10 cells layers thick

Statistical Analyses

mMCP-1 and TNF-α data were analyzed by using a one-tailed unpaired t test. Probability values of $p < 0.05$ were considered significantly different. Data on the cellular accumulation in BAL fluid were processed by a distribution-free Kruskal–Wallis ANOVA (analysis of variance) and the nonparametric Wilcoxon rank test was used to determine the significance of differences of histological scores. The airway dose-response curves to methacholine were statistically analyzed by a general linear model of repeated measurements followed by *post hoc* comparison between groups. Data were log10 transformed before analysis to equalize variances in all groups. *In vitro* hyperreactivity data are expressed as mean and standard error of the mean (SEM). EC50 and Emax values for the carbachol-induced tracheal contractions were calculated by nonlinear least-squares regression analysis of the measured contractions versus carbachol concentration using the sigmoid concentration–response relationship. The data were analyzed by performing a two-way ANOVA. Analyses were performed by the usage of Graphpad prism (version 2.01, San Diego, USA).

RESULTS

Early Phase of the Airway Hypersensitivity Reaction (0–3 h After Challenge)

Mast Cell Activation In Vivo

The appearance of mMCP-1 in the blood is indicative for the activation of mast cells (19). DNFB sensitization followed by intranasal DNS challenge resulted in a biphasic mast cell activation. An early rise was found 10 min, 30 min, and 3 h after challenge in serum of DNFB-sensitized and DNS-challenged mice compared to vehicle-sensitized, DNS-challenged mice (Fig. 1). The most prominent rise in mMCP-1 was observed 30 min after intranasal hapten challenge although at 10 min, 2 h, and 3 h there is also a significant increase in mMCP-1 levels in DNFB-sensitized compared to vehicle-sensitized animals. No significant increase was found 6 h after challenge (Fig. 1).

TNF-α Levels in the Bronchoalveolar Lavage Fluid

Increased TNF-α BAL fluid levels were found 30 min after challenge in DNFB-sensitized Balb/C and +/+ animals compared with vehicle-sensitized BALB/c or +/+ mice (Table 2). However, no difference in TNF-α levels in the BAL fluid was found after challenge in DNFB-sensitized mast-cell-deficient W/W^V mice compared with vehicle-sensitized W/W^V mice (Table 2).

Acute Bronchoconstriction

Intranasal challenge was accompanied by an acute bronchoconstriction in DNFB-sensitized mice compared

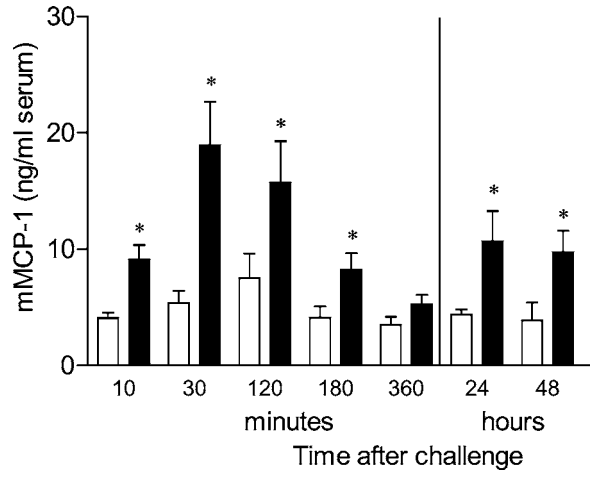

Fig. 1. Mast cell activation is observed in the early and late phase of DNFB-sensitized mice after DNS challenge. mMCP-1 levels in serum of vehicle-sensitized (white bars) and DNFB-sensitized (black bars) mice are assessed at 10, 30 min and 2, 3, 24, and 48 h after challenge (see Materials and Methods section). Results are expressed as mean (ng/mL serum) ± SEM, $n = 6$–12 mice per group. Significant differences between the vehicle-sensitized and the DNFB-sensitized group are denoted by * for $p < 0.05$.

Table 2. Total, Mononuclear and Neutrophil Cell Numbers in BAL Fluid of Vehicle- and DNFB-Sensitized Mice

Sensitization	Total cells (×1000)	Mononuclear cells (×1000)	Neutrophils (×1000)
24 h after challenge			
Vehicle	22.5 (21.0–31.5)	22.3 (20.1–29.4)	1.6 (0.9–2.0)
DNFB	40.5 (37.5–52.5)*	36.1 (33.1–47.3)*	5.0 (3.6–5.7)*
48 h after challenge			
Vehicle	23.3 (16.5–31.5)	22.6 (15.5–31.0)	0.7 (0.5–1.5)
DNFB	40.0 (33.0–46.5)*	35.1 (29.7–43.2)*	3.6 (2.8–5.4)*

Note. Cellular accumulation was studied studied 24 and 48 h after challenge. Results are expressed as median (min–max) ($n = 6$ animals/group). Significant differences between the vehicle-sensitized and the DNFB-sensitized group are denoted by *for $p < 0.05$.

to vehicle-sensitized mice, as monitored by an increase in PenH values. Immediately after challenge PenH values increased, reaching a maximum 5 min after challenge. PenH values gradually returned to basal levels 15 min after the challenge (Fig. 2).

Late Phase of the Hypersensitivity Reaction (24–48 h After Challenge)

Mast Cell Activation In Vivo

In the late phase hypersensitivity reaction (24–48 h), mMCP-1 levels were significantly enhanced in DNFB-sensitized mice compared to control animals (Fig. 1).

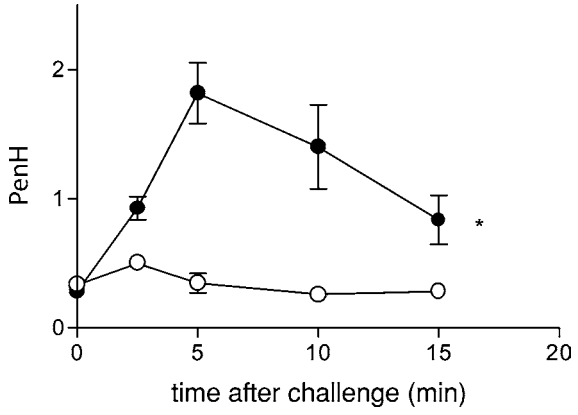

Fig. 2. Acute bronchoconstriction is found in DNFB-sensitized mice after intranasal DNS challenge. Bronchoconstriction is characterized by increases in Penh values, which were recorded in vehicle-sensitized (open circles) and DNFB-sensitized (closed circles) mice 5 min before until 15 min after the challenge. For each mouse max PenH readings were taken over 1-min time windows at 0, 2 min 30 s, 5, 10, and 15 min. * $p < 0.05$, $n = 6$ mice per group.

Changes in Mucosal Permeability

A significant increase in mucosal permeability, as assessed by Evans blue dye accumulation, was found in the BAL fluid of DNFB-sensitized animals 24 h after DNS challenge compared to vehicle-sensitized mice (Fig. 3). No difference in basal mucosal permeability was observed between the two groups before DNS challenge.

Leukocyte Accumulation in Bronchial Alveolar Lavage Fluid

An increase in cellular accumulation into the airway lumen was found in DNFB-sensitized, DNS-challenged mice 24 and 48 h after the challenge. The total cell counts from lung BAL lavages from DNFB-sensitized mice differed significantly from vehicle-sensitized mice at both time points (Table 3). Bronchoalveolar cells in these mice mainly consist of mononuclear cells and neutrophils. Both

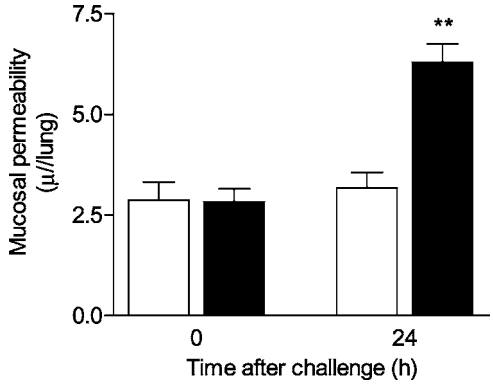

Fig. 3. Mucosal permeability changes (μL plasma/lung) are found 24 h after DNS challenge in DNFB-sensitized (black bars) compared to vehicle-sensitized (white bars) mice. Data are expressed as mean ± SEM for $n = 6$ animals per group. Significant differences between groups are denoted by ** for $p < 0.01$.

Table 3. Cellular Infiltration in the Lung of Vehicle- and DNFB-Sensitized Mice 24 and 48 h After Challenge

Treatment	Accumulation of inflammatory cells	
	Around bronchioli	Around blood vessels
24 h		
Vehicle	0.53 ± 0.08	0.17 ± 0.06
DNFB	0.94 ± 0.04*	0.38 ± 0.02*
48 h		
Vehicle	0.49 ± 0.09	0.17 ± 0.12
DNFB	0.79 ± 0.10*	0.30 ± 0.17*

Note. Lungs were removed and studied for cellular infiltration (see Materials and Methods section and Table 1). Results are expressed as mean ± SEM (4 mice/group). Significant differences between the vehicle-sensitized and the DNFB-sensitized group are denoted by * for $p < 0.05$.

the numbers of mononuclear cells and neutrophils showed to be significantly increased in DNFB-sensitized animals compared to control mice at mentioned time points after hapten challenge (Table 3).

Tracheal Reactivity In Vitro

The intranasal hapten application in DNFB-sensitized mice resulted in a tracheal hyperreactivity reaction to carbachol 24 and 48 h after the challenge compared to vehicle-sensitized animals (Emax 24 h; Veh/DNS: 2020 mg ± 180 mg, DNFB/DNS: 2841 mg ± 201 mg. $n = 6$; $p < 0.05$. Emax 48 h; Veh/DNS: 2095 mg ± 175 mg, DNFB/DNS: 3203 mg ± 133 mg. $n = 6$; $p < 0.05$; Fig. 4).

Airway Responsiveness In Vivo

To confirm the *in vitro* airway hyperreactivity data, *in vivo* we assessed airway responses in conscious, freely moving animals. Airway responsiveness to methacholine was measured 48 h after DNS challenge in vehicle- and DNFB-sensitized mice. DNFB-sensitized mice showed a significant airway hyperresponsiveness compared to vehicle-sensitized mice to concentrations of 25 and 50 mg/mL methacholine ($p < 0.05$; Fig. 5).

Histological Analysis

Within 1 day after intranasal DNS challenge, an accumulation of inflammatory cells around bronchioli and pulmonary blood vessels was induced in animals sensitized with DNFB (Fig. 6C and 6D). On the other hand, in vehicle-sensitized mice, DNS challenge did not result in cellular infiltration (Fig. 6A and 6B). Overall, a significant accumulation of inflammatory cells was seen in DNFB-sensitized mice around the bronchioli and around the blood vessels compared to vehicle-sensitized animals (Table 4, Fig. 6). Differences between sensitized and control mice were shown to be significant at both 24 and 48 h after DNS challenge (Table 4, Fig. 6). No significant differences were observed between the two time points.

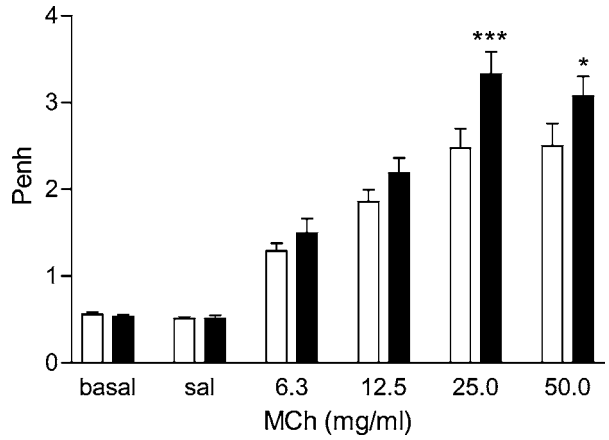

Fig. 4. *In vitro* tracheal hyperreactivity in DNFB-sensitized mice compared to vehicle-sensitized animals. Concentration–response curves to carbachol were measured in DNFB (closed circles) or vehicle (open circles) sensitized mice 24 (A) and 48 h (B) after DNS challenge. Results are expressed as mean ± SEM ($n = 6$). Significant differences between groups are denoted by * or ** for $p < 0.05$ or $p < 0.01$, respectively.

DISCUSSION

In this study, we describe a murine model for non-atopic asthma. A pulmonary hypersensitivity reaction is induced by contact sensitization and intra-airway challenge in mice. This pulmonary reaction is characterized by an early (0–3 h) and late phase hypersensitivity response (24–48 h after challenge). Features of the early pulmonary hypersensitivity reaction include acute bronchoconstriction and mast cell activation. Hapten-specific IgE levels could not be detected in these mice (data not shown). The late phase is characterized by again mast cell activation, pulmonary edema, mononuclear and neutrophilic cell infiltration into the airway lumen around blood vessels and bronchioli, *in vitro* tracheal hyperreactivity, and *in vivo* airway responsiveness. These results support data presented by previous studies performed in small intestine and airways in which edema formation, leukocyte accumulation, and hyperreactivity were present 24 h after challenge (15, 20–22).

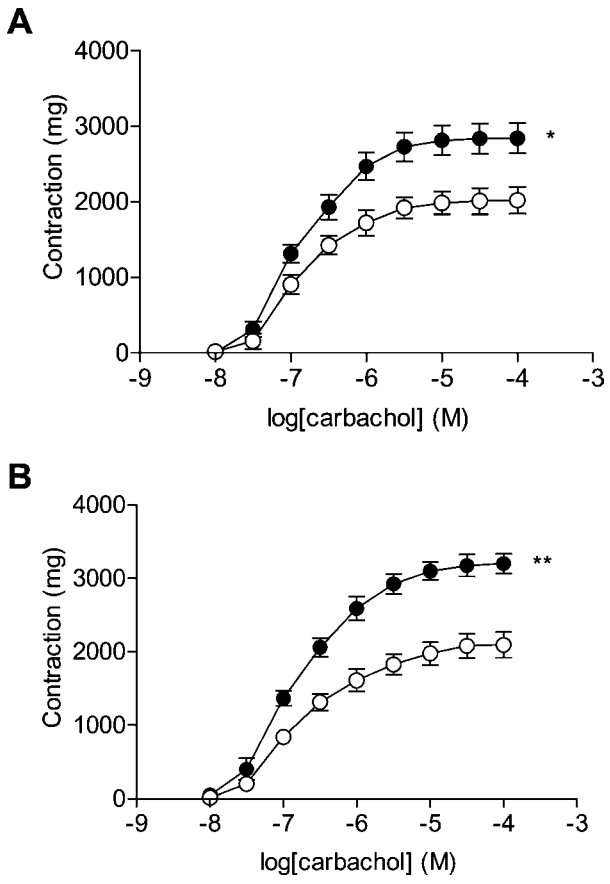

Fig. 5. *In vivo* airway hyperresponsiveness is found in DNFB-sensitized and DNS-challenged mice compared to control animals. Airway responsiveness *in vivo* was measured in conscious, unrestrained mice by whole-body pethysmography before and 48 h after challenge. Concentration–response curves to methacholine were measured in DNFB (closed cirles) or vehicle (open circles) sensitized mice 48 h after challenge. Results are expressed as mean ± SEM ($n = 6$). Significant differences between curves are denoted by * or *** for $p < 0.05$ or $p < 0.001$, respectively.

We have shown that upon DNFB-sensitization and DNS challenge, mast cells were activated, assessed as increased serum mMCP-1 levels. TNF-α is a major mast-cell-derived cytokine and was significantly increased in the BAL fluid of sensitized BALB/c mice shortly after intranasal challenge. TNF- α can be released from several cell types, including activated mast cells, macrophages, and monocytes. Appearance of TNF-α in the BAL fluid directly after the first challenge suggests that TNF-α is released by cells that contain prestored TNF-α. This includes tissue-resident inflammatory cells like mast cells and macrophages. DNFB-sensitized mast cell deficient W/W^V mice showed no increase in TNF-α levels in their BAL fluid directly after challenge compared with their congenic littermates (+/+) or BALB/c mice. Altogether, mast cells play a key role in the release of TNF-α in mice undergoing a pulmonary hypersensitivity reaction.

Table 4. Amount of TNF-α Present in the BAL Fluid of Vehicle- or DNFB-Sensitized Balb/C, $WBB6F_1$-W/W^V Animals, and their Littermates (+/+) 30 min After DNS Challenge

Treatment	Balb/C	++	W/W^v
Vehicle	110.2 ± 47.8	192.1 ± 44.3	120.2 ± 10.4
DNFB	290.0 ±11.9*	307.2 ±21.9*	121.7 ±30.4#

Note. Data are expressed as mean ± SEM for $n = 4$–6 animals per group.
* $p < 0.011$ compared with vehicle-sensitized mice; # $p < 0.01$ compared with DNFB-sensitized BALB/c or DNFB-sensitized $WBB6F_1$-+/+.

Previously, TNF-α has been shown to induce vasodilatation and vascular exudation in the murine hypersensitivity reaction (23). Furthermore, TNF-α could induce an upregulation of adhesion molecules (24) leading to the infiltration of leukocytes, directly or via intermediate mechanisms. The airway inflammation, evaluated by the number of bronchoalveolar lavage cells and the percentage of neutrophils in bronchoalveolar cells, is closely paralleled by the development of airway hyperresponsiveness in the presented model. Although some studies have demonstrated that neutrophil infiltration into the airways is not necessary for an increase in airway responsiveness (25, 26), the hypothesis that neutrophil infiltration alters airway function is, however, supported by a larger number of studies (27–29). We hypothesize that this leukocyte accumulation, dominated by the infiltration of neutrophils, will lead to the induction of *in vivo* airway hyperresponsiveness and *in vitro* tracheal hyperreactivity.

The lymphocyte and the mast cell are two key inflammatory cells in the non-atopic reactions in the mouse airways as well as the skin and the intestine (7, 30, 31). In skin studies, it has been demonstrated that, upon contact sensitization, B lymphocytes are activated to produce hapten-specific immunoglobulin kappa light chains (IgLC) in spleen and lymph nodes (32). *In vitro* studies, using bone marrow-derived mast cells, demonstrated that these cells can degranulate after passive sensitization with IgLC followed by challenge (32). It is hypothesized that the hapten-specific IgLC can arm mast cells (and possibly other cell types). After a second encounter with the hapten, the challenge phase, the hapten will cross-link IgLC bound to the mast cell, causing activation

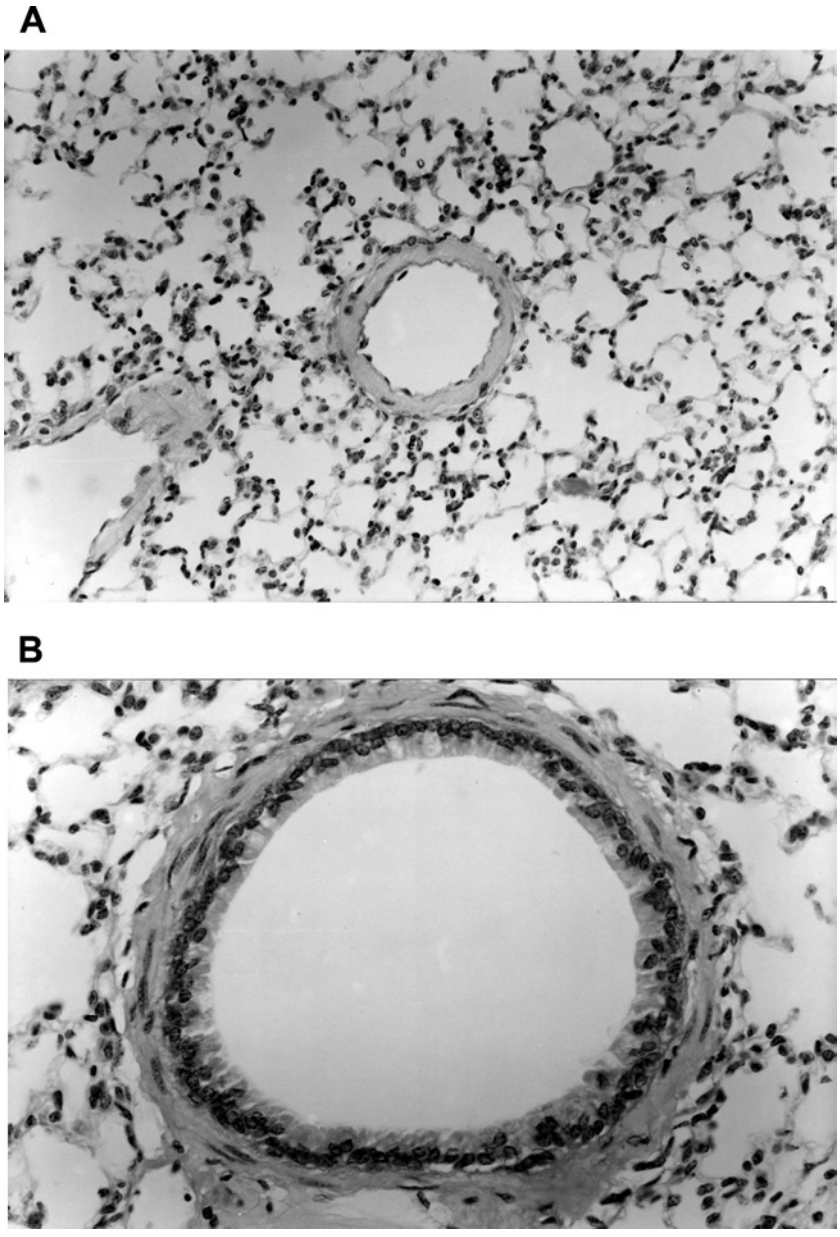

Fig. 6. A marked infiltration of inflammatory cells is observed in DNFB-sensitized and challenged mice compare to control mice. (A) Lung blood vessels (×25) and (B) bronchiolus (×40) of vehicle-sensitized mice challenged with DNS. No cellular infiltrated were found. (C) Lung blood vessels (×25) and (D) bronchiolus (×25) of DNFB-sensitized mice challenged with DNS. All specimens were taken 24 h after challenge.

and subsequent release of mediators. Vasoactive mediators such as TNF-α (23) and serotonin (33) increase the microvascular permeability and induce the infiltration of circulating hapten-specific effector lymphoid cells, generated during the sensitization. These effector T cells can recognize antigen in the context of MHC class II on antigen presenting cells after a second encounter with the hapten. The effector T cells are now triggered to produce cytokines leading to the inflammatory response (see Fig. 7).

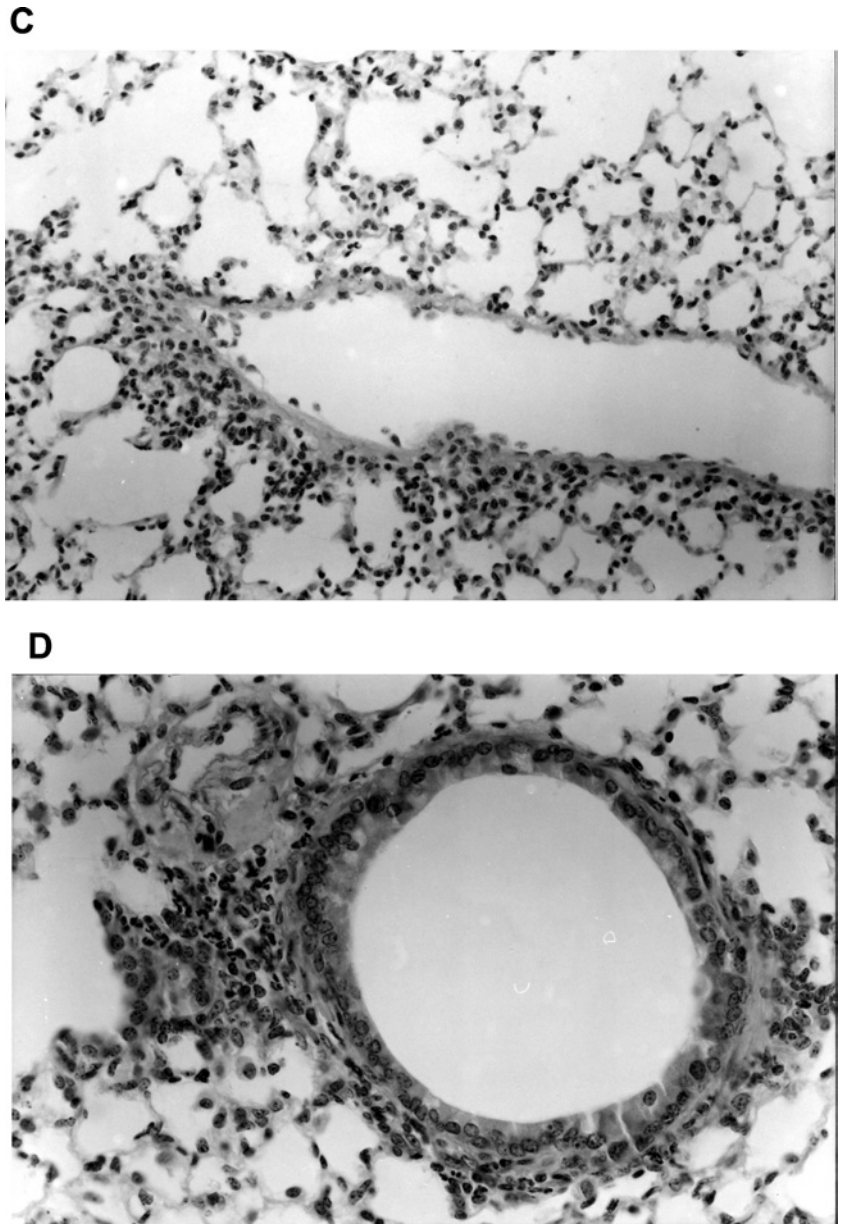

Fig. 6. Continued.

The murine model for asthma described in this report is very relevant since only a weak and inconsistent association between the prevalence of asthma and the prevalence of atopy is found on the basis of available epidemiological evidence. These findings of Pearce *et al*. (2) indicate that the importance of atopy (defined as serum increases in serum IgE and skin prick test positivity) as a cause of asthma may have been overemphasized. Furthermore, it is hypothesized that, other than eosinophilic inflammation (related to atopy), a major proportion of asthma is based on neutrophilic airway inflammation (34). Since most researchers have studied atopic/eosinophilic asthma, it is therefore important to focus on the pathogenesis of non-atopic/neutrophilic asthma.

In summary, our study shows that our murine model for non-atopic asthma is characterized by

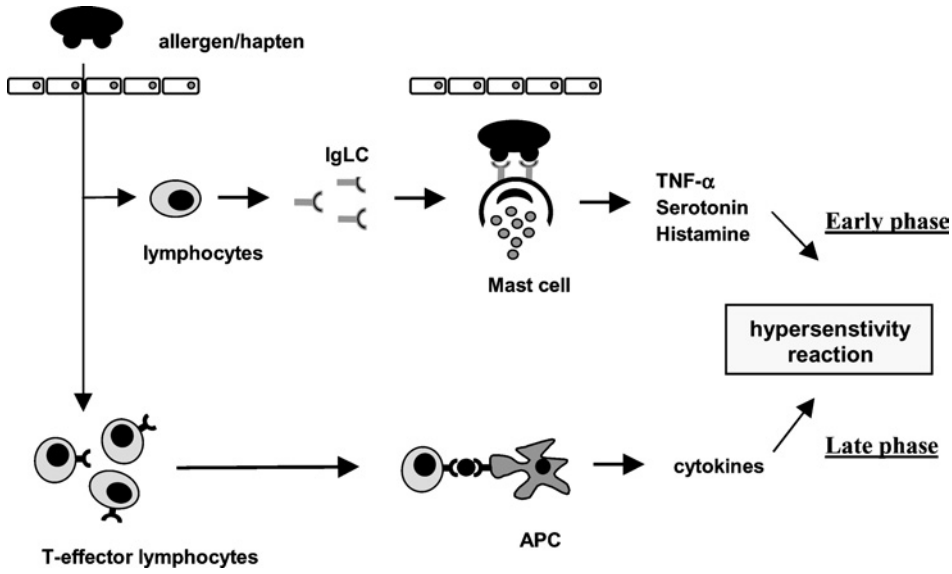

Fig. 7. Schematic representation of the proposed mechanism leading to hypersensitivity reactions in the airways.

bronchoconstriction and mast cell activation in the early phase and mast cell activation, neutrophil accumulation and airway hyperresponsiveness in the late phase reaction. These characteristics bear marked similarity with those observed in non-atopic asthmatic patients. Therefore, this model can be used to further study the mechanisms that may be responsible for the development of non-IgE-mediated asthma.

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