Published in: Inflammation Research (2009)
Status: Postprint (Author's version)

Mouse models of asthma: a comparison between C57BL/6 and BALB/c strains regarding bronchial responsiveness, inflammation, and cytokine production

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Abstract

Objective Animal models of asthma mimic major features of human disease. Since the genetic background of experimental animals might affect hyperresponsiveness and inflammation, we studied its potential influence and the mechanisms leading to differences in strains.

Methods We applied a mouse model of allergic asthma to BALB/c and C57BL/6 mice.

Results BALB/c mice displayed greater levels of airway reactivity to methacholine than C57BL/6 mice. Moreover, BALB/c mice exhibited higher numbers of mast cells in lung tissue when compared to C57BL/6. On the contrary, eosinophil and neutrophil counts in bronchoalveolar lavage fluid (BALF) as well as peribronchial eosinophilia were greater in C57BL/6. IL (Interleukin)-4, IL-5, IL-13, and CCL11 levels measured in whole-lung extracts were higher in BALB/c, while, in sharp contrast, CCL11 and CCL5 levels were higher in BALF of C57BL/6 mice.

Conclusions We observed phenotypic differences between C57BL/6 and BALB/c mice in an asthma model with different distributions of pro-inflammatory cytokines and inflammatory cells.

Keywords : Animal models · Asthma · Cytokines · Chemokines · Inflammation

INTRODUCTION

Asthma is a complex inflammatory disease clinically characterized by airway hyperresponsiveness, inflammatory cell infiltration in bronchoalveolar lavage fluid (BALF) and bronchial walls, and airway structural changes.

Experimental animal models can afford important information on several aspects of asthma. To date, mice are increasingly used in these models for many reasons (e.g., worldwide availability, relatively low costs, ELISA availability) and because genetic maps of the murine genome are available allowing the conception of different molecular tools and the use of RNA interference (SiRNAs) and anti-sense sequences. Moreover, much information is available concerning murine immunology, and the use of engineered mice with altered expression or deletion of relevant gene products is of particular interest to demonstrate the validity of a molecular target or to explore the importance of a single mediator [1-3]. Many experimental issues (duration of allergen exposure, route of administration of antigen, age, sex, strain, etc.) need to be taken into account when developing such models and interpreting findings.

In humans, no significant correlation was found between the degree of airway responsiveness and sputum inflammatory cells, which are two major features of asthma [4-6]. Concurrently, different strains of mice exhibit various degrees of airway responsiveness and inflammation despite identical sensitization and airway challenge with an allergen [7, 8]. Some differences regarding allergen-induced lung inflammation have been described between C57BL/6 mice and BALB/c mice [8]. The role of T-helper cells in determining the airway inflammation and bronchial hyperresponsiveness in a mouse model is dependent on the genetic background [9]. It has also been reported that lung remodeling is strain-dependent [10]. When designing experiments, it appears crucial to choose the adequate mouse strain when addressing a particular aspect of asthma or explore a specific pathway to

address the effectiveness of pharmacological compounds.

A better knowledge of the mechanisms determining these phenotypes is essential for a comprehensive study of asthma pathogenesis and to select the most adequate and reproducible model to study the distinct features of asthma and to assess the activity of a compound.

In this study, we focussed on BALB/c and C57BL/6 mouse strains, which are the two most commonly used in animal models of asthma. BALB/c mice are thought to represent a relatively hyperresponsive strain (Th_2 prone) as compared to C57BL/6 mice, which are generally considered as low Th_2 responders since they display low airway responsiveness in response to an allergen. Here, we compare bronchial responsiveness, characteristics of airway inflammation, and expression of major Th_2 and cytokines generating eosinophilia in bronchoalveolar lavage and lung tissue of BALB/c and C57BL/6 mice in order to assess the mechanisms that might explain the differences between these two strains.

MATERIALS AND METHODS

Sensitization and allergen exposure protocol

Care and use of experimental animals (C57/BL6 and BALB/c mice) were conducted following the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research (USA), and the experimental protocol was approved by the animal ethics committee (University of Liège) under no. 2005/437. BALB/c and C57/BL6/129 male mice of 6-8 weeks were sensitized on days 1 and 8 by intraperitoneal injection of 10 µg ovalbumin (OVA) (Sigma-Aldrich, Schnelldorf, Germany) emulsified in aluminium hydroxide (AlumInject; Perbio, Erembodegem, Belgium). From day 21 to 27, mice were exposed daily for 30 min either to phosphate saline buffer (PBS) (n = 10 per strain) or allergen (OVA 1%) (n = 32 per strain). These solutions were administered by exposure in standard Plexiglas boxes (30 × 20 × 15 cm) to an aerosol generated by an ultrasonic nebulizer (Devilbiss 2000, DeVilbiss Healthcare; Somerset, PA). Mouse sacrifice was performed on day 28 after measuring the bronchial responsiveness. The experiments showed in this paper summarize different experiences with cohorts of 10-32 mice per experimental condition.

Bronchial airway responsiveness measurement

Bronchial airway responsiveness was measured using the whole-body plethysmography and the FlexiVent System. Barometric plethysmography, as described by Hamelmann et al. [11], was used to measure airway responsiveness to methacholine (MCh) in each group of mice 24 h after the final allergen exposure [11]. Enhanced pause (Penh) was used as the main index of airway responsiveness. Penh was measured for 3 min in baseline conditions. Mice were then exposed to the inhalation of PBS and subsequent increasing doses of MCh (3, 6, 12, 24, and 48 g/l) during 1 min. Every aerosol was separated by a 15-min recovery period in order to allow airway Penh to go back to baseline level. Data presented in this report correspond to the mean of the first 3-min record performed after each exposure to methacholine or placebo. Penh results were expressed as absolute values. The second method used to measure bronchial reactivity is the FlexiVent System allowing the airway resistance to be measured directly. Mice were anesthetized by intraperitoneal injection (200 µl) of a mixture of ketamine (10 mg/ml, Merial, Brussels, Belgium) and xylazine (1 mg/ml, VMD, Arendonk, Belgium). A tracheotomy was performed by inserting a 20-gauge polyethylene catheter into the trachea and ligating it around the catheter to avoid leaks and disconnections. Mice were ventilated with a FlexiVent small-animal ventilator (SCIREQ, Montreal, Canada) as described by Hantos et al. [12]. Following baseline lung-function measurements, mice were exposed to a saline aerosol (PBS) followed by aerosols containing increasing doses (3, 6, 9, and 12 g/1) of methacholine (ICN Biomedicals, Asse Relegem, Belgium). Aerosols were generated by ultrasonic nebulizer (SYST'AM, LS 2000, Dupont Medical, Rhode-Saint-Genèse, Belgium). Each aerosol was delivered for 2 min, and lung-function measurements as described above were assessed at 1-min intervals following each aerosol. Mean airway resistance after methacholine exposure was the major parameter measured during the challenge.

Bronchoalveolar lavage fluid (BALF)

Mice were sacrificed, and a bronchoalveolar lavage was immediately performed using 4 × 1 ml PBS-EDTA 0.05 mM (Calbiochem, Darmstadt, Germany) as previously described [13, 14]. Cells were recovered by gentle manual aspiration. After centrifugation (1,200 rpm for 10 min, at 4°C), supernatant was collected and frozen at -80°C for protein assessment. Total cell counts were obtained using a hemocytometer. Differential cell counts based on morphologic criteria were performed on cyto-centrifuged preparations after staining with Diff-Quick

(Dade, Belgium).

Pulmonary histology and tissue processing

After BAL, left main bronchus was clamped and left lung was excised. The right lung was infused with 4% paraformaldehyde and embedded in paraffin. Five-micrometer sections were cut off from paraffin and stained with haematoxylin-eosin. The extent of peribronchial inflammatory-cell infiltration around the bronchi in lung tissue was estimated by a score calculated by quantification of peribronchial inflammatory cells (eosinophils, lymphocytes, macrophages, etc.), as previously described [14]. Briefly, a value of 0 was adjudged when no inflammatory cells were detectable around the bronchi. A value of 1 was given when there were occasionally inflammatory cells, a value of 2 when most bronchi were surrounded by a thin layer (one to five cells) of inflammatory cells, and a value of 3 when most bronchi were surrounded by a thick layer (more than five cells) of inflammatory cells. Since five to seven randomly selected tissue sections per mouse were scored, peribronchial inflammation scores are expressed as a mean value per animal and can be compared between the groups used in this study.

Congo Red staining was used specifically to detect eosinophils in lung tissue. Eosinophilic infiltration in airway walls was quantified by manual count and reported to the perimeter of the epithelial basement membrane yielding an eosinophilic inflammatory count expressed as number of cells/mm of epithelial basement membrane (ImageJ program, http://rsb.info.nih.gov/nih-image/). Mast cells were detected in lung tissue using toluidine-blue staining, and the number of mast cells was counted in paraffin sections. Alpha-smooth muscle actin (α-SMA) was detected by immunohistochemistry with mouse primary antibody anti-α-SMA-FITC (Sigma-Aldrich, Schnelldorf, Germany) allowing the detection of the smooth-muscle-cell layer as previously described [15]. Left lung was crushed using a Mikro-Dismembrator (Braun Biotech International, Melsungen, Germany). For protein extraction, crushed lung tissue was incubated overnight at 4°C in a solution containing 2 M urea, 1 M NaCl, and 50 mM Tris (pH 7.5) and subsequently cen-trifuged for 15 min at 16,000×g.

Measurements of IgE and cytokines by ELISA

Before performing BALF, blood was removed from the heart of mice using a syringe for measurement of OVA-specific serum IgE. First, microtiter plates were coated with OVA and then serum samples were added, followed by a biotinylated polyclonal rabbit anti-mouse IgE (1/8,000) (S. Florquin, ULB, Brussels, Belgium). The binding of this antibody was detected with peroxidase-conjugated anti-rabbit IgG (affinity purified Abs; Calbiochem). A serum pool from OVA-sensitized animals was used as internal laboratory standard; 1 unit was arbitrarily defined as 1/100 dilution of this pool. OVA-specific IgG_1 and IgG_{2a} were detected in serum using peroxidase-labeled goat anti-IgG_1 and anti-IgG_{2a} Abs (affinity purified Abs; Southern Biotechnology Associates, Birmingham, AL).

IL (Interleukin)-4, IL-5, IL-10, IL-13, IL-17, IFN-γ, CCL5 (RANTES or regulated on activation, normal T-cell expressed, and secreted), and CCL11 (eotaxin-1) levels in lung protein extracts and in supernatants from BALF were assessed using commercial ELISAs (R&D systems, Abingdon, UK).

Statistical analysis

Results of BAL cell count, pulmonary histology, and cytokine levels were expressed as mean ± SEM, and the comparison between the groups was performed using Kruskall-Wallis test with Dunn's multiple comparisons test. P values <0.05 were considered as significant. Spearman test was used to perform the correlation study. Kruskall-Wallis and Spearman tests were performed using Graphpad Instat version 3.00 (Graphpad Software, San Diego, CA, USA, www.graphpad.com.).

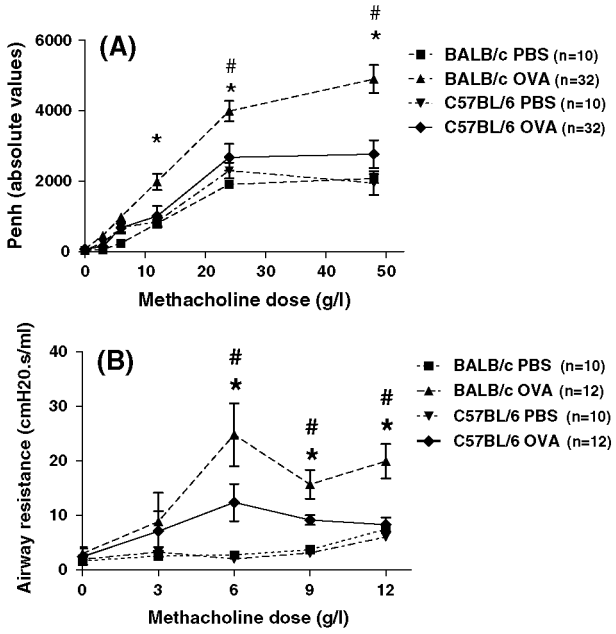
RESULTS

Hyperreactivity after methacholine challenge

Airway responses following the exposure to increasing doses of methacholine (MCh) were measured for each mouse 24 h after the final allergen or PBS exposure using two different methods, the whole-body plethysmography and the FlexiVent System. As assessed by plethysmography, enhanced pause (Penh) was significantly increased in BALB/c mice exposed to allergen when compared to sham-exposed BALB/c ($P <$ 0.05). In C57BL/6 mice, no significant difference in Penh values was observed between OVA or PBS-exposed mice at any dose of MCh. Bronchial responsiveness measured by Penh was increased in BALB/c mice after allergen exposure [MCh doses of 24 and 48 g/l ($P < 0.05$)] (Fig. 1a). The absolute values of maximum Penh

recorded during the challenge were also significantly higher in BALB/c mice exposed to allergen as compared to allergen-exposed C57/BL6 mice (2,071.45 ± 297.64 vs. 1,003.634 ± 124, respectively). In this study, a direct measurement of bronchial resistance was also performed by using the FlexiVent System. This protocol avoids nasal respiration and allows a direct measurement of bronchial responses to complex perturbations in OVA- or placebo-exposed mice. Allergen-induced airway responsiveness was significantly higher in BALB/c mice as compared to C57BL/6 mice for different doses of methacholine: 6, 9, and 12 g/l ($P < 0.05$) (Fig. 1b).

Fig. 1 *Measurement of enhanced pause (Penh) by whole-body plethysmography* (a) and bronchial resistance by FlexiVent System (b). a The y-axis represents the Penh absolute value. Increasing doses of methacholine were administered by aerosols. BALB/c mice exposed to OVA ($n = 32$) showed a significant increase in airway hyperreactivity to methacholine (MCh) when compared to BALB/c mice exposed to PBS inhalation ($n = 10$) (*$P < 0.05$). Penh values were significantly increased in BALB/c mice at doses of 24 and 48 g/l when compared to C57BL/6 mice exposed to the same allergen ($n = 32$) (#$P < 0.05$). b Airway resistance measured by FlexiVent System. Airway resistance was significantly increased after allergen exposure in BALB/c mice ($n = 12$) when compared to BALB/c mice exposed to PBS ($n = 10$) (*$P < 0.05$). BALB/c mice exposed to allergen ($n = 12$) displayed an increase in bronchial resistance when compared to C57BL/6 exposed to OVA inhalation ($n = 12$) for doses of 6, 9, and 12 g/1 methacholine (#$P < 0.05$)



Bronchoalveolar lavage fluid (BALF) cellular composition

Allergen exposure induced a significant increase in eosinophil counts in both strains of mice as compared to respective sham-exposed mice. Nevertheless, eosinophil percentages after allergen exposure were significantly higher in BALF from C57BL/6 as compared to BALB/c ($P < 0.05$) (data not shown). C57BL/6 mice also displayed an increase in neutrophil percentages as compared to BALB/c mice ($P < 0.05$) (data not shown). Total cell counts in BALF of C57BL/6 mice were increased when compared to BALB/c due to a massive increase in granulocyte counts ($P < 0.05$) (Table 1). Epithelial cell counts were significantly lower after allergen inhalation in BALF of BALB/c mice ($P < 0.05$). On the contrary, numbers of these cells were significantly increased in BALF of C57BL/6 mice after allergen exposure ($P < 0.05$). No significant difference was observed for any other cell types (Table 1).

Lung histology

Airways from BALB/c and C57BL/6 mice exposed to PBS aerosols did not show any inflammation. OVA exposure resulted in a significant increase in peribronchial and perivascular inflammation both in BALB/c and C57BL/6 mice when compared to PBS-exposed counterparts ($P < 0.0001$) without any significant difference between the strains (Fig. 2a). Eosinophils infiltrated in the peribronchial area were specifically assessed using Congo Red-stained slides. Significantly increased counts of eosinophils were detected in BALB/c and C57BL/6 mice after allergen exposure without any significant difference between the two genotypes (Fig. 2b). On the

other hand, number of mast cells was significantly increased after exposure to ovalbumin aerosols in C57BL/6 ($P < 0.05$) and in BALB/c mice ($P < 0.0001$) when compared to PBS-exposed counterparts. Interestingly, the number of mast cells was significantly increased in BALB/C mice after allergen exposure when compared to C57BL/6 mice ($P < 0.0001$) (Fig. 2c). As assessed by immunohisto-chemistry, thickness of the airway smooth-muscle-cell layer around the bronchi was increased after allergen exposure in both strains of mice when compared to sham-exposed mice ($P < 0.05$), but no significant difference was observed between BALB/c and C57BL/6 mice (Fig. 2d).

A significant correlation was found between peribronchial inflammation score and eosinophil numbers in BALF of C57BL/6 mice exposed to OVA ($r = 0.58$, $P < 0.0005$) (Fig. 3a) suggesting that eosinophilic infiltration of the airway wall is related to BALF eosinophilia in that strain. In sharp contrast, no such correlation was found for BALB/c mice ($r = 0.025$, $P = 0.9$) (Fig. 3b).

Table 1 *Differential cell counts in BALF*

	BALB/c PBS ($n = 10$)	BALB/c OVA ($n = 32$)	C57BL/6 PBS ($n = 10$)	C57BL/6 OVA ($n = 32$)
Epithelial cells (10^4/ml)	7 ± 1.4	2.8 ± 0.4*	0.8 ± 0.2#	4.4 ± 0.9*
Eosinophils (10^4 /ml)	0.2 ± 0.1	18.9 ±4.1*	0.9 ± 0.8	70.1 ± 8.5*♦
Neutrophils (10^4/ml)	0.08 ± 0.04	0.8 ±0.1*	0.2 ± 0.1	1.7 ± 0.3*♦
Lymphocytes (10^4 /ml)	0.5 ± 0.2	1.8 ± 0.5	0.2 ± 0.1	3.7 ± 1.1
Macrophages (10^4 /ml)	34.7 ± 4.4	16.4 ± 1.4*	19.2 ± 2.9	19.3 ± 3.3
Total cells (10^4/ml)	42.5 ± 5.1	40.7 ± 5	21.3 ± 9.2	99.2 ± 10.6*♦

* $P < 0.05$ when compared to PBS-exposed mice
♦ $P < 0.05$ when compared to BALB/c mice exposed to allergen
\# $P < 0.05$ when compared to BALB/c mice exposed to PBS inhalation

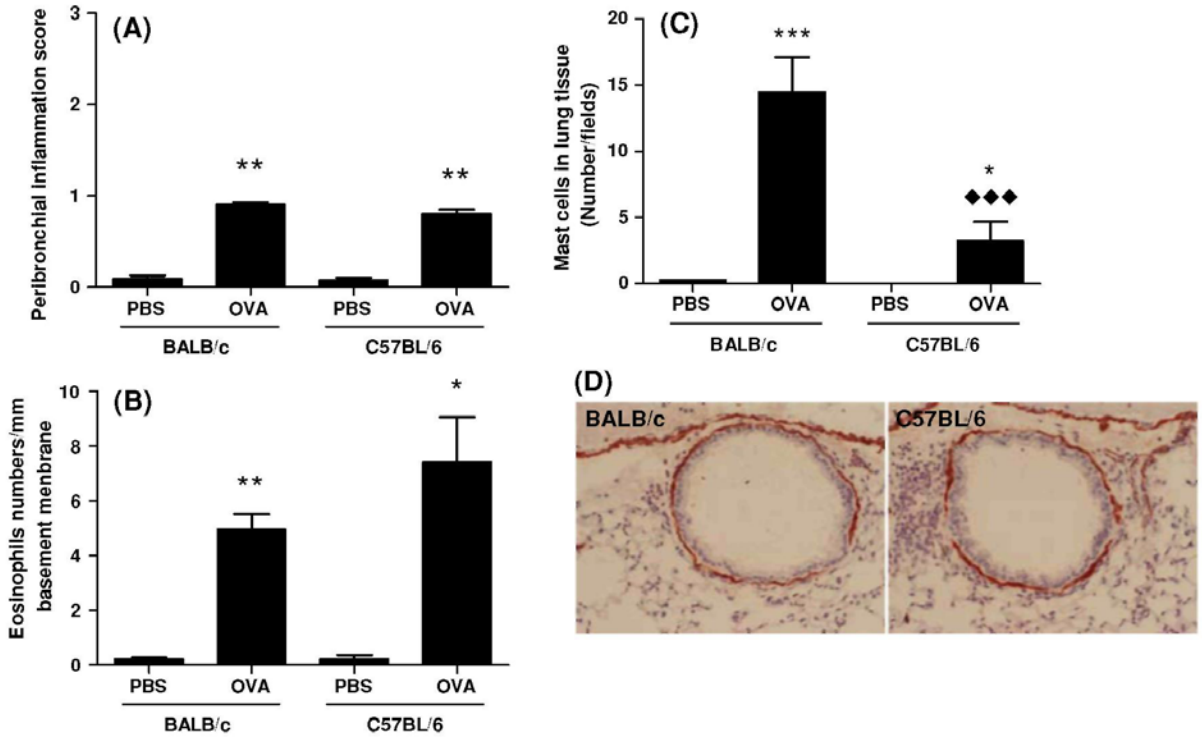
Antibody and cytokine levels by ELISA measurements

Levels of specific IgE, IgG_1, IgG_{2A} antibodies, and relevant cytokines were measured by ELISA in serum (Fig. 4), lung protein extracts, and BALF (Fig. 5). Allergen exposure induced a similar increase in specific anti-OVA serum IgE levels in the sera of the two strains of mice ($P < 0.0005$ for BALB/c mice and $P < 0.0001$ for C57BL/6 mice when compared to PBS-exposed mice) (Fig. 4a). After allergen challenge, levels of IgG_1, a marker of Th_2-prone milieu, were significantly increased in the sera from BALB/c and C57BL/6 mice. Interestingly, we demonstrated that the levels of IgG_1 were drastically higher in serum from BALB/c mice as compared to C57BL/6 ($P < 0.05$) (Fig. 4b). In contrast, levels of IgG_{2A}, a putative marker of Th_1, were not different across all experimental groups (Fig. 4c).

CCL11 (eotaxin-1) levels were measured both in lung protein extracts and in BALF. When considering lung extracts, we found higher CCL11 levels in allergen-exposed BALB/c mice as compared to allergen-exposed C57BL/6 ($P < 0.05$) (Fig. 5a). In sharp contrast, in BALF, CCL11 levels were significantly higher in BALF of C57BL/6 mice as compared to BALB/c mice after OVA exposure ($P < 0.05$) (Fig. 5b). IL-5 levels measured in lung protein extracts had a tendency to increase after allergen exposure in both genotypes, but this increase did not reach statistical significance. Interestingly, a significant difference was observed between BALB/c and C57BL/6 mice at baseline and after OVA exposure ($P < 0.05$) with ΓL-5 levels being higher in BALB/c mice when compared to C57BL/6 mice (Fig. 5c). Levels of CCL5 [regulated upon activation, normal T-cell expressed, and secreted (RANTES)] measured in BALF were significantly increased after allergen exposure when compared to BALB/c or to C57BL/6 sham-exposed mice ($P < 0.01$ and $P < 0.005$, respectively). Nevertheless, the increase in CCL5 levels was two times higher in BALF of C57BL/6 OVA-exposed mice when compared to BALB/c mice after the same allergen exposure ($P < 0.05$) (Fig. 5d). Levels of IL-13 measured by ELISA in lung protein extracts were significantly increased after OVA exposure in both strains when compared to sham-exposed mice ($P < 0.001$). Nevertheless, the extent of this increase was consistently and significantly more important in allergen-exposed BALB/c mice as compared to the corresponding C57BL/6 mice ($P < 0.05$) (Fig. 5e). Levels of IL-4 measured in lung protein extracts were significantly increased after exposure to aerosolized OVA only in BALB/c mice when compared to the C57BL/6 mice ($P < 0.05$) (Fig. 5f). Levels of IL-17 were not different between the two strains of mice when measured in lung protein extracts and were not detectable in BALF (data not shown). Again, no significant difference was observed between the different experimental

groups when considering the levels of Th_1 cytokine IFN-γ (Fig. 6a) and regulatory cytokine IL-10 (Fig. 6b) measured in lung protein extracts. Levels of these two cytokines were not detectable in BALF.

Fig. 2 a Peribronchial inflammation score measured as described in the section "Materials and methods". b Eosinophil counts in the peribronchial area reported to the perimeter of the epithelial basement membrane and expressed as eosinophil number/mm. c Number of mast cells counted in lung tissue after toluidine-blue staining. d Immunohistochemistry showing the smooth-muscle-cell layer in BALB/c (right panel) and C57BL/6 mice (left panel). ***P* < 0.001 when compared to PBS-exposed mice; *** *P* < 0.0001 when compared to PBS-exposed mice; **P* < 0.05 when compared to PBS-exposed mice; ♦♦♦ *P* < 0.0001 when compared to BALB/c mice exposed to allergen. BALB/c exposed to PBS: *n* = 10; BALB/c exposed to ovalbumin: *n* = 32; C57BL/6 exposed to PBS: *n* = 10; C57BL/6 exposed to ovalbumin: *n* = 32



DISCUSSION

Bronchial hyperresponsiveness and eosinophilic inflammation in lung tissue and BALF are two distinct features of human asthma that can be studied in mouse models of asthma [4, 16]. According to the mouse strains used in the present study, we found marked differences following antigen exposure regarding bronchial responsiveness, BALF cellular composition, mast cells and eosinophil tissue infiltration and cytokine production. After allergen exposure, sensitized BALB/c mice displayed greater levels of airway reactivity to methacholine (MCh) than C57BL/6. BALF eosinophils were higher in C57BL/6 mice as compared to BALB/c mice. In C57BL/6 mice, a correlation was found between eosinophil counts in BALF and peribronchial eosinophilic infiltration measured in lung tissue. Although allergen-induced inflammation in the peribronchial area was similar between the two strains, eosinophilic infiltration of the bronchial wall was more pronounced in C57BL/6 mice. On the contrary, mast cell counts were significantly increased in lung tissue of BALB/c mice. IL-4, IL-13, and CCL11 levels measured in lung protein extracts of BALB/c mice were higher than those of C57BL/6 mice, while, on the contrary, CCL11 and CCL5 levels were higher in BALF of C57BL/6. The main finding of the present study is the demonstration that a differential distribution of cytokines in BALF and lung allows for distinguishing two different mouse strains commonly used to study induced asthmatic reaction.

The finding of important differences in inflammation features and airway responsiveness between the two strains cannot be explained solely by a different response to sensitization since there were no differences regarding allergen-specific IgE levels in serum. As important effects of age, sex, and hormonal variations have been described to have a crucial effect on bronchial hyperresponsiveness [17], we always used male mice that were 6 weeks old at the beginning of the experiment.

Published in: Inflammation Research (2009)
Status: Postprint (Author's version)

Fig. 3 a Correlation between the peribronchial inflammation score and eosinophil counts in BALF of C57BL/6 mice. b Correlation between the peribronchial inflammation score and eosinophil counts in BALF of BALB/c mice. BALB/c exposed to PBS: $n = 10$; BALB/c exposed to ovalbumin: $n = 32$; C57BL/6 exposed to PBS: $n = 10$; C57BL/6 exposed to ovalbumin: $n = 32$

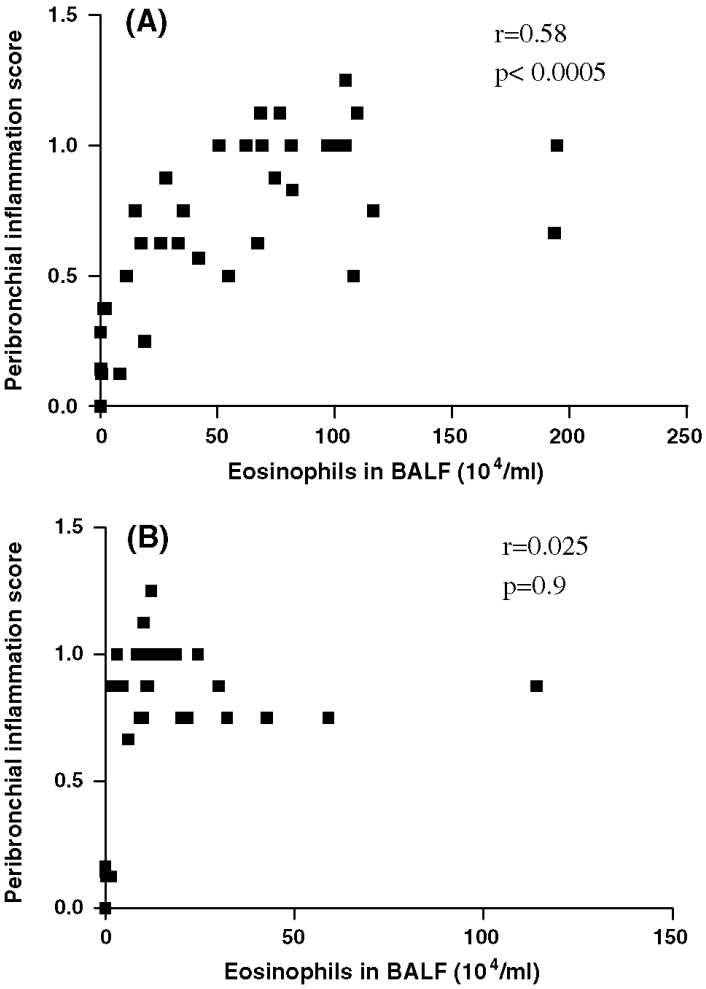
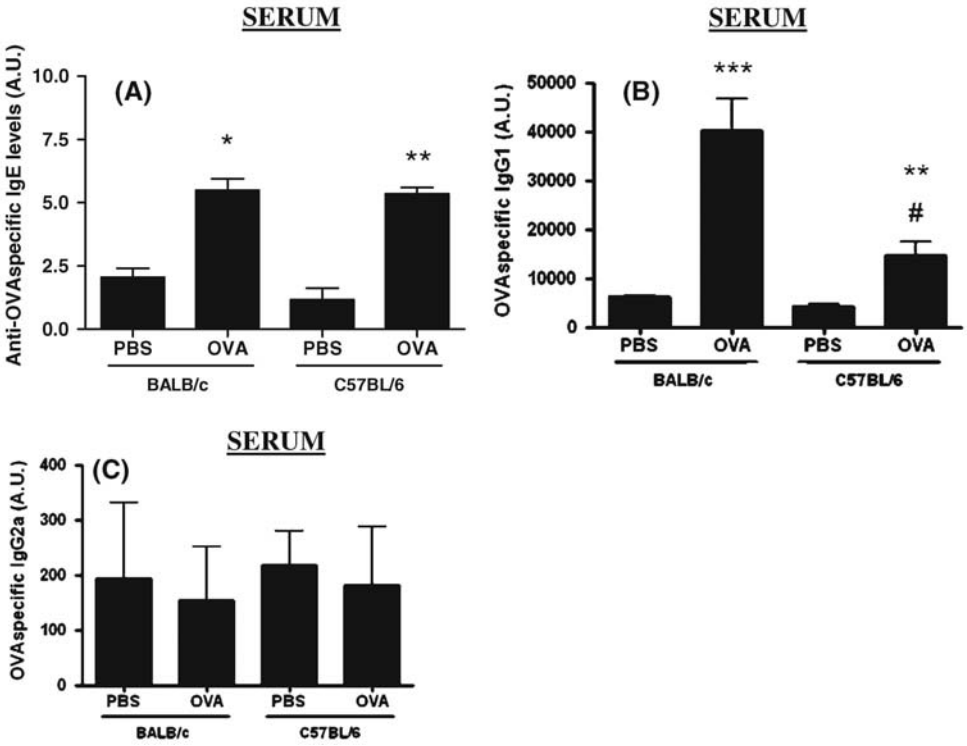


Fig. 4 a Serum levels of specific anti-OVA IgE measured by ELISA. b Serum levels of specific IgG_1 measured by ELISA. c Serum levels of specific IgG_{2A} measured by ELISA. **P* < 0.05 when compared to PBS-exposed mice; ***P* < 0.005 when compared to PBS-exposed mice; ****P* < 0.001 when compared to PBS-exposed mice; #*P* < 0.05 when compared to OVA-exposed mice. BALB/c exposed to PBS *n* = 10; BALB/c exposed to ovalbumin: *n* = 32; C57BL/6 exposed to PBS: *n* = 10; C57BL/6 exposed to ovalbumin: *n* = 32



The differences observed in methacholine responsiveness could be explained by the distinct pattern observed regarding IL-13 production. IL-13 is a pleiotropic cytokine produced mainly by T cells and is critical for the development of airway hyperresponsiveness (AHR) associated with allergen exposure [18]. It has indeed been reported that treatment of mice with anti-IL-13 mAb inhibited AHR [19, 20] and that Il-13-induced airway inflammation might involve a direct effect on airway smooth muscles [21]. Our results strongly suggest that IL-13 production is intrinsically different in the two mouse strains and accounts for the increased bronchial hyperresponsiveness in BALB/c mice. This increased bronchial hyperresponsiveness to methacholine in BALB/c mice could also be explained by increased mast cell numbers found in the lung tissue of those mice since the degranulation of these cells induces a release of mediators such as histamine, leukotrienes, and TNF-α [22]. Those mediators markedly increase vascular permeability, followed by inflammatory cell recruitment, and further release of pro-inflammatory mediators, but they also act directly on smooth muscle cells and consequently increase bronchial hyperreactivity [23-25]. Mast cells are also a major source of IL-4, which is increased in those mice after allergen exposure [26]. Nevertheless, other factors than IL-13 or mediators released by mast cells could take part in the hyperresponsiveness in mice since two distinct quantitative trait loci (QTL) for susceptibility to allergen-induced airway hyperresponsiveness, Abhr1 (allergen-induced bronchial hyperresponsiveness) and Abhr2, have been identified on chromosome 2 in backcross progeny from A/J and C3H/HeJ mice [21], suggesting that some genetic factors are also important in the pathogenesis of this phenomenon.

We report in the present study that allergen-induced eosinophilic infiltration is more pronounced in BALF from C57BL/6. This finding has been reported previously by other authors but remained unexplained [8]. We considered major eosinophil chemoattractants and found that CCL11 levels are higher in BALF of C57/BL6 mice while CCL11 levels are higher in the protein from lung parenchyma of BALB/c mice. Those data strongly suggest that a CCL11 polarized secretion might account for most of the differences between the strains regarding eosinophilia [27]. Ichinose and colleagues also reported some strain variations regarding CCL11 levels in mouse models of asthma. CCL11 is produced by various cells including bronchial epithelium and type I alveolar epithelial cells [28], and these latter could be key actors in a putative polarized secretion of this chemokine. CCL5, a CC chemokine mainly produced by lymphocytes, was increased after allergen challenge. CCL5 levels

were significantly higher in bronchoalveolar lavage from C57BL/6 than BALB/c mice. As CCL5 is a powerful eosinophil chemoattractant, some authors suggest that CCL5 and CCL11 may regulate eosinophil trafficking into the airways of asthmatic in a coordinated manner [29]. Nevertheless, the picture is rendered much more complex if one considers a network of interactions including many cytokines. Indeed, it was reported that IL-13 enhances eotaxin expression [28], and the high levels of IL-13 production in BALB/c mice might account for at least a part of the increase in CCL11 production in lung extracts.

Fig. 5 *a* CCL11 levels measured by ELISA in lung protein extracts, b CCL11 levels measured by ELISA in BALF. c IL-5 levels measured by ELISA in lung protein extracts. d CCL5 levels measured by ELISA in BALF. e IL-13 levels measured by ELISA in lung protein extracts, f IL-4 levels measured by ELISA in lung protein extracts. **P* < 0.05 when compared to PBS-exposed mice; ***P* < 0.001 when compared to PBS-exposed mice; #*P* < 0.05 when compared to OVA-exposed mice. BALB/c exposed to PBS: *n* = 10; BALB/c exposed to ovalbumin: *n* = 32; C57BL/6 exposed to PBS: *n* = 10; C57BL/6 exposed to ovalbumin: *n* = 32

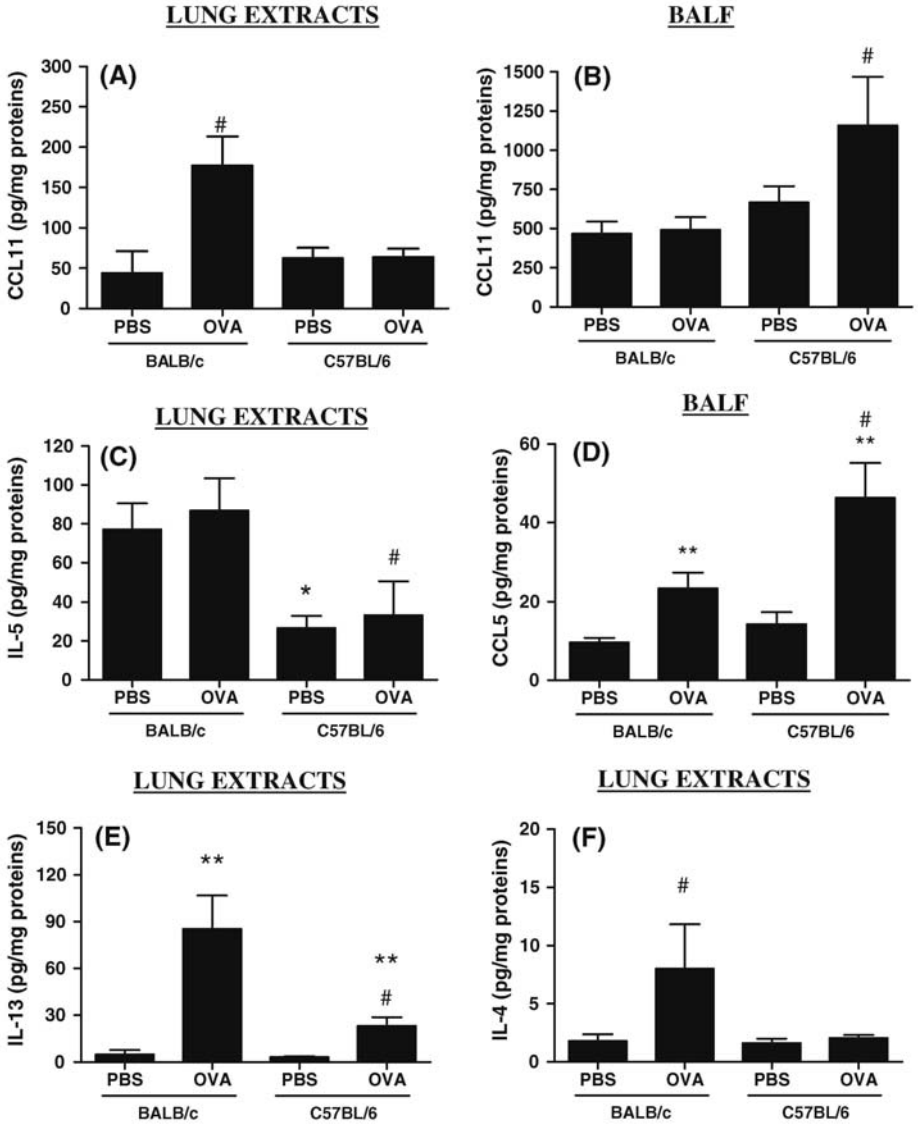
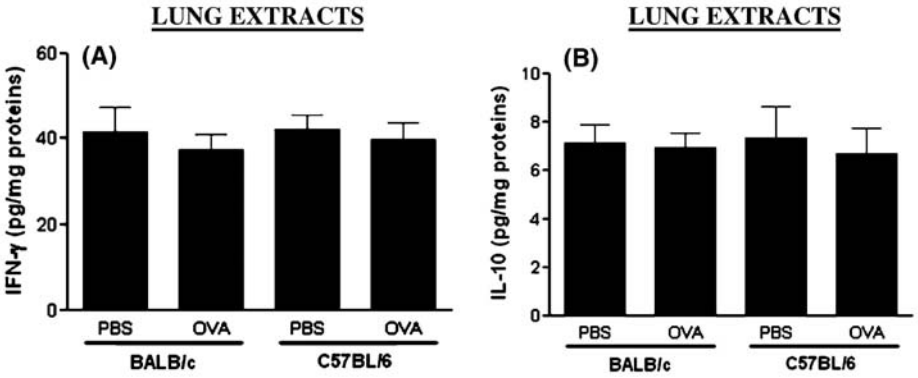


Fig. 6 a IFN-γ levels measured by ELISA in lung protein extracts, b IL-10 levels measured by ELISA in lung protein extracts. BALB/c exposed to PBS: $n = 10$; BALB/c exposed to ovalbumin: $n = 32$; C57BL/6 exposed to PBS: $n = 10$; C57BL/6 exposed to ovalbumin: $n = 32$



Kinetics of inflammation may also account for a part of the differences. Indeed, it was observed that BALF eosinophil numbers peaked after a shorter period in C57BL/6 mice (approximately 1 week) as compared to BALB/c mice (2 weeks) [10, 30]. IL-4 and IL-13 are overproduced in lungs from asthmatics and are thought to be key regulators of the disease. In this study, levels of IL-4 were significantly higher in lung protein extracts of BALB/c mice than C57BL/6 after allergen exposure. IL-4 was reported to be a key factor for B-lymphocyte class switching [31], but in the present study, the difference found in IL-4 production did not affect serum IgE levels. IL-4 expression pattern was also reported to be distinct between A/J mice and C3H/HeJ mice with a lack of increase in the latter [32]. It was previously described that BALF from BALB/c mice contains higher levels of IL-4 than BALF from C57BL/6 mice after a 4-week allergen exposure [10]. Nevertheless, in that study, no significant difference was found regarding IL-13 between the two genotypes. We can hypothesize that the time course of allergen exposure (4 weeks) did not allow previous authors to determine that there was an initial increase in IL-13 [10]. Shinagawa and Kojima [10] did not report any increase in bronchial responsiveness to methacholine by using a technique similar to ours. Again, the time course of the protocol used in that study probably did not allow finding a difference that should have been abolished after a 4-week inhalation protocol. Some characteristics of airway remodeling, such as changes in airway wall thickness, collagen deposition around the bronchi, and goblet cell hyperplasia, were reported in mouse models of asthma [10]. As we limited our study to a 7-day inhalation period, any possible strain differences regarding airway remodeling were not assessed.

These data confirmed that BALB/c mice are Th_2-prone and have the ability to develop bronchial hyperresponsiveness. In a 7-day exposure protocol, BALB/c mice displayed some key features of asthma: airway responsiveness and bronchial inflammation along with a hyperproduction of Th_2 cytokines. C57BL/6 mice are hyporesponsive to methacholine but display a strong allergen-induced eosinophilic inflammation, suggesting that they present a mixed phenotype Th_1/Th_2. We show here that features of eosinophilic inflammation are related to polarized cytokine production. These results suggest that BALB/c mice are suited to study early or mild asthma, whereas C57BL/6 mice are better suited to study the inflammatory component of severe asthma.

In conclusion, genetic background of mice is important for the characteristics of inflammatory diseases such as asthma. This parameter must be taken into account in the design and interpretation of experimental protocols intending to demonstrate mechanisms of allergic inflammation or the pharmacological activity of some molecules.

Acknowledgements

This work was supported by grants of the Walloon Region Government (DGTRE), the Fonds National de la Recherche Scientifique (FNRS, Brussels, Belgium), the Fondation Leon Fredericq (University of Liege), the CHU (Liege, Belgium), Action de Recherches Concertées, Communauté Française de Belgique and European Union (FP6), the Interuniversity Attraction Poles Programme—Belgian Science Policy (Brussels, Belgium). D.C. is a research associate of the FNRS. N.R. and G.P. received grants from the Télévie and FRIA (FNRS, Belgium), respectively. The authors are indebted to Fabienne Perin, Christine Fink, and Fabrice Olivier for their

excellent technical assistance.

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