Comparison of acute inflammatory and chronic structural asthma-like responses between C57BL/6 and BALB/c mice

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In this manuscript, we investigated the effects of different durations of allergen exposure in sensitized mice belonging to two different strains. Inflammation, airway hyperresponsiveness and remodelling features were thoroughly characterized, while the influence of genetic background on these traits was considered. In addition, we established a basic model in which some of the asthma-like features (inflammation and airway hyperresponsiveness) could be controlled over time due to specific Ag treatments.

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ABSTRACT

Background: The interactions between airway responsiveness, structural remodelling, and inflammation in allergic asthma remain poorly understood. Prolonged challenge with inhaled allergen is necessary to replicate many of the features of airway wall remodelling in mice. In mice as well as in humans, genetic differences can have a profound influence on allergy, inflammation, airway responsiveness and structural changes.

Methods: The aim of this study was to provide a comparative analysis of allergen-induced airway changes in sensitized BALB/c versus C57BL/6 mice that were exposed to inhaled allergen for 2 ('acute'), 6 or 9 weeks ('chronic'). Inflammation, remodelling and responsiveness were analyzed.

Results: Both strains developed a Th-2 driven airway inflammation with allergen-specific IgE, airway eosinophilia and goblet cell hyperplasia upon 2 weeks of allergen inhalation. This was accompanied by a significant increase in airway smooth muscle mass and hyperresponsiveness in BALB/c but not C57BL/6 mice. Contrarily, airway eosinophilia was more pronounced in the C57BL/6 strain.

Chronic allergen exposure (6 or 9 weeks) resulted in an increase in airway smooth muscle mass as well as sub-epithelial collagen and fibronectin deposition in both strains. The emergence of these structural changes paralleled the disappearance of inflammation in both C57BL/6 and BALB/c mice and loss of hyperresponsiveness in the BALB/c strain. TGF-β_1 was accordingly elevated in both strains.

Conclusion: Airway inflammation, remodelling and hyperresponsiveness are narrowly intertwined processes. Genetic background influences several aspects of the acute allergic phenotype. Chronic allergen exposure induces a marked airway remodelling that parallels a decreased inflammation, largely comparable between both strains.

Key words: airway remodelling, asthma, mouse models, TGF-β, tolerance

LIST OF NON-STANDARD ABBREVIATIONS

AHR = Airway Hyperresponsiveness; OVA = Ovalbumin; PBS = Phosphate-Buffered Saline; BALF = Broncho-alveolar Lavage Fluid; i.p.= intraperitoneal; TGF-β= Transforming Growth Factor beta; HBSS= Hanks' Balanced Salt Solution; PAS= Periodic Acid Schiff; IHC= Immunohistochemistry; DAB= 3, 3'-diaminobenzidine; Penh = Enhanced Pause; ASM= Airway Smooth Muscle;SEM = Standard Error of the Mean; ANOVA = Analysis Of Variance; ECM = Extracellular Matrix

INTRODUCTION

In addition to inflammation-related changes such as cellular infiltration and oedema, asthmatic airways display structural alterations referred to as “airway remodelling” [1;2]. Historically, animal models have proven useful for the elucidation of the immunological mechanisms of the Th-2 driven airway inflammation present in the asthmatic airway. These models of allergic airway inflammation usually rely on short-term (1 day to 2 weeks) inhaled allergen after a systemic sensitization [3]. These models are not adequate to study the features of airway remodelling observed in patients with asthma, as longer periods of allergen exposure are required to mimic these changes [4;5]. The patho-physiology of airway remodelling remains largely unknown, but the structural alterations in the airway are thought to result from (inadequate) attempts of tissue repair in response to inflammation [6]. In addition, it is up to now unclear to what extent this process contributes to the deterioration of asthma symptoms [7] or represents the translation of a repair response that protects against further allergen-induced airway inflammation and bronchoconstriction [8]. The pattern of these structural changes differs between mouse strains [9], suggesting the genetic background might be important. Whether remodelling develops uniformly in all asthma patients is equally unclear [10].

Remarkably, prolonged challenge with inhaled allergen in sensitized mice is often associated with disappearance of eosinophilic airway inflammation and the induction of a state of respiratory tolerance [11-14]. While the immunological mechanisms of this phenomenon are still poorly understood, tolerance appears to develop in most models using chronic allergen challenge [9;14].

Although a wide variety of mouse asthma models exist [3;9;15;16], it remains puzzling how airway inflammation, remodelling and responsiveness relate to each other in these models. Thus, we here aimed to provide a thorough characterisation encompassing an assessment of inflammation and airway (hyper)responsiveness (AHR), as well as of the remodelling features in the two most commonly used mouse strains to study asthma, *in casu* C57BL/6 and BALB/c mice. We provide this comparative analysis in order to gain insight into the relationship between these asthma characteristics in both strains. We found that upon short-term challenge, both strains developed differential responses in inflammation, AHR and airway remodelling, particularly smooth muscle (ASM) proliferation. Upon prolonged exposure, both strains developed more similar structural airway alterations that paralleled a disappearance of eosinophilic inflammation and AHR.

MATERIALS AND METHODS

Animals

C57BL/6 and BALB/c mice (males, 6-8 weeks old) were purchased from Harlan (Zeist, the Netherlands). All experimental procedures were approved by the local ethical committee for animal experiments (Faculty of Medicine and Health Sciences, Ghent University).

Allergen Exposure Protocols

All groups of mice (6-12 mice/group) were sensitized with 10 μg intraperitoneal (i.p.) OVA (Grade III; Sigma, St-Louis, MO) adsorbed to 1 mg $Al(OH)_3$ on day 0 (d0) and d7. From d14 onward, the mice were exposed to aerosolized (Ultraschallvernebler Sirius Nova, Heyer Medizintechnologie, Bad Ems, Germany) OVA (1% wt/v) or PBS 30 min/day, three times a week for 2 ('acute'), 6 or 9 ('chronic') weeks respectively (Figure 1 – *Protocols A, B* and *C*).

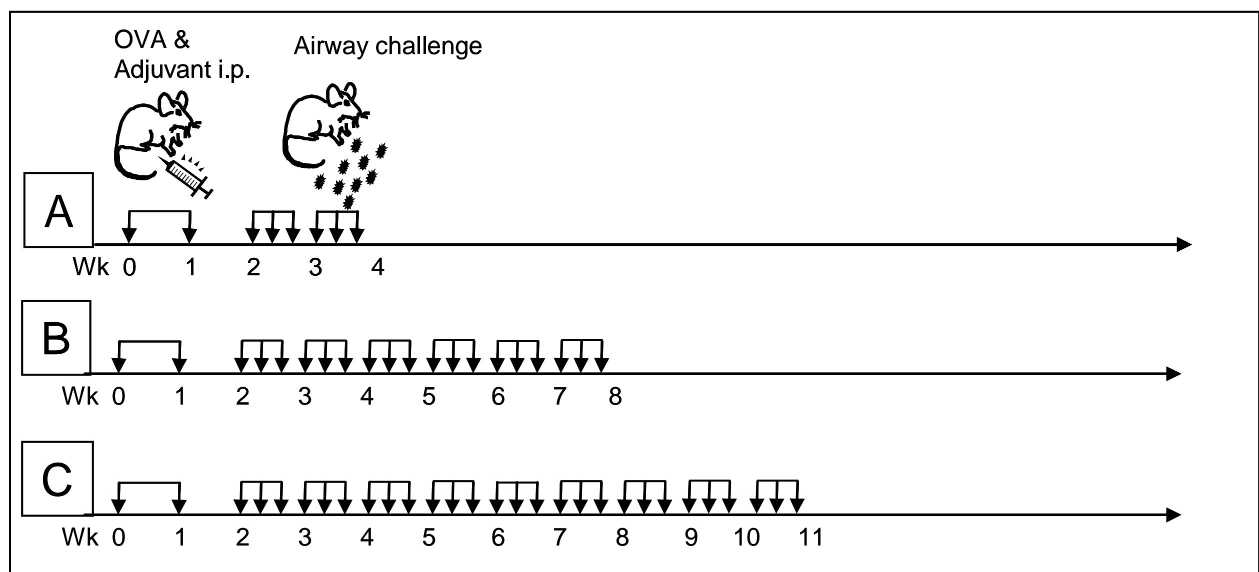


Figure 1: Allergen exposure protocols. Exposure of sensitised C57BL/6 and BALB/c mice to OVA aerosols for 2, 6 and 9 weeks (*Protocols A, B* and *C* respectively)

Bronchoalveolar Lavage Fluid (BALF): cellular analysis

Twenty-four hours after the last aerosol exposure, mice were sacrificed with an i.p. pentobarbital injection (60 mg/kg; Sanofi, Libourne, France). Briefly, BALF was taken by instillation of HBSS via a tracheal cannula. Three lavages with 0.3 ml HBSS followed by three lavages with 1 ml HBSS were performed. The recovered BALF of the first three fractions was centrifuged and the supernatant was used for cytokine detection. The cell pellet

was then added to the rest of the lavage fluid, centrifuged, subjected to red blood cell lysis and resuspended for cell counts on cytospins (May-Grünwald/Giemsa).

Histology

After fixation of the left lung with 4% paraformaldehyde, slices from all left lobes were embedded in paraffin for histological analysis. Sections of 3 µm were stained with Congo Red to highlight eosinophils, Periodic-Acid Schiff (PAS) to highlight goblet cells and with Sirius Red for collagen staining.

Immunohistochemistry (IHC)

IHC for fibronectin was done in an analogous manner as previously described in the rat [17]. Briefly, lung sections of 3 µm were deparaffinized, rehydrated and the non-specific binding sites were blocked with 1% blocking reagent in PBS (Boehringer, Mannheim, Germany). Excess reagent was removed and the sections were incubated for 1h with a mouse anti-mouse fibronectin antibody (Dako A/S, Glostrup, Denmark; dilution factor 1/750). Afterwards, sections were rinsed and incubated with a biotinylated secondary antibody for 30 min. Next, the primary antibody-secondary antibody complex was detected by streptavidin-biotinylated horseradish peroxidase complex. The substrate for the peroxidase was 3, 3'-diaminobenzidine (DAB, Dako), resulting in a brown reaction product for fibronectin quantification. IHC for α-actin smooth muscle contractile elements in the airways (ASM) was performed in an analogous manner, using an anti-mouse α-actin SM antibody (Dako; dilution factor 1/100) and DAB substrate, equally resulting in a brown reaction product.

Quantitative measurements in the airway wall

Quantitative measurements were performed in the airways of each animal with a perimeter of basement membrane (*Pbm*) ranging from 800 to 2000 µm, provided a reasonable cross section was available (ratio of minimal to maximal internal diameter smaller than 1.8). Measurements were performed on the digital representation of the airways using a Zeiss (Oberkochen, Germany) KS400 Image analyser system as described earlier [17]. Morphometrical parameters were marked manually: the area defined by the basement membrane (*Abm*) and the area defined by the total adventitial perimeter (*Ao*). The total bronchial wall area (*WAt*) was calculated (*WAt=Ao-Abm*) and normalized to the square of the Pbm. For the quantification of collagen deposition, the area in the airway wall covered by the Sirius Red stain was determined by the software (*WCt*) and normalized to Pbm. To evaluate the sub-

epithelial fibronectin deposition and the ASM content of the airways, the area covered by the DAB stain (*WFt* and *WαA*) was determined in an analogous manner and also normalized to Pbm. Goblet cells were quantified on PAS stained sections. Results were expressed as number of goblet cells per millimeter basement membrane. Peribronchial infiltration with eosinophils was evaluated in lung sections stained with Congo Red and the total number of eosinophils per mm^2 bronchial wall was determined. All measurements were performed on 5 airways per mouse.

IgE and protein quantification (ELISA)

OVA-IgE in serum was measured with ELISA using coated microtiter plates and biotinylated polyclonal rabbit anti-mouse IgE. TGF-β_1 was determined the BALF using ELISA kits (R&D Systems, Abingdon, UK).

Assessment of airway responsiveness (AHR)

Twenty-four hours after the last aerosol exposure AHR to methacholine was assessed in spontaneously breathing animals using a whole body plethysmograph system (Buxco; Buxco Electronics Inc., Troy, NY). Before performing readings, the system was calibrated by rapid injection of 1 ml air. Pressure differences between the main chamber containing mice and a reference chamber were recorded using the software BioSystem XA (version 157; Buxco). This pressure signal is caused by flow changes in the main box during the respiratory cycle of the animal. The value of 'enhanced pause' (Penh) is used here to monitor airway function [18], since it shows strong correlation with the airway resistance [19]. Penh = [(Te-Tr)/Tr] x (PEP/PIP) where Te is expiratory time (s), Tr is relaxation time or time of the pressure decay to 36% of total box pressure during expiration, PEP is peak expiratory pressure (cm H_2O), and PIP is peak inspiratory pressure (cm H_2O). For each mouse, data of were recorded at baseline, and after exposure to PBS or increasing concentrations of nebulized methacholine (2 – 81 mg/ml), to assess AHR. The solutions were nebulized through an inlet of the main chamber during 1 min. Readings were taken during 5 min after each nebulization. Between readings Penh value returned to baseline. Cumulative dose-response curves were constructed for the changes in Penh after increasing doses of methacholine. The changes in Penh are expressed as percentage increase of maximum Penh value following methacholine challenge compared to the average Penh value after PBS-exposure (calculated by Analyst 1.29; Buxco).

Statistical analysis

Data were analysed with the statistical packet SPSS 15.0 (SPSS Inc.; Chicago, IL). Reported values are expressed as mean ± Standard Error of the Mean (SEM). Mean values of parameters (including quantitative measurements in the airway wall as well as inflammatory cells and mediators) except Penh values were compared between the groups through the Kruskall -Wallis test for multiple comparisons (nonparametric testing). When significant differences were observed, post-hoc comparisons between groups were made, using the Mann-Whitney U-test with Bonferroni corrections. P-values less than 0.05 were considered significant. The dose-response curves of Penh were compared using ANOVA. The concentration of methacholine causing a 250% increase in baseline Penh (PC_{250} Penh) was calculated by log-linear interpolation of the dose-response curve.

RESULTS

1. Airway inflammation in the BALF and bronchial walls

OVA exposure for 2 weeks (*Protocol A*; Figure 1) altered the composition of the BALF leukocytes in both strains (Figure 2) as compared to the PBS exposed mice. Both strains developed a significant influx of mononuclear cells and eosinophils. The percentage of eosinophils in BALF was 21.16 ± 4.41 % in C57BL/6 vs 12.66 ± 4.08 % in BALB/c mice ($p<0.05$ BALB/c vs. C57BL/6).Only C57BL/6 had a discrete but significant concomitant BALF neutrophilia and a significant increase in total BALF cellularity and macrophages. Moreover, both strains developed peribronchial and perivascular eosiniphilic inflammation upon short-term challenge (Figure 3 A/B). C57BL/6 mice, but not BALB/c mice also possessed some degree of interstitial inflammation (Figure 3 C/D).

The numbers eosinophils counted around the airways on Congo Red stained sections were significantly higher in the C57BL/6 strain compared to the BALB/c strain (Table 1).

Upon longer OVA exposure (6 or 9 weeks – *Protocol B* and *C*; Figure 1) the BALF cell composition in OVA-challenged mice of both strains returned to the composition discerned in mice exposed to PBS (Figure 2). At those time points, only a slightly elevated number of eosinophils remained present in the bronchial walls of both mouse strains (Table 1).

TABLE I. Peribronchial eosinophils in epithelium in BALB/c and C57BL/6 mice

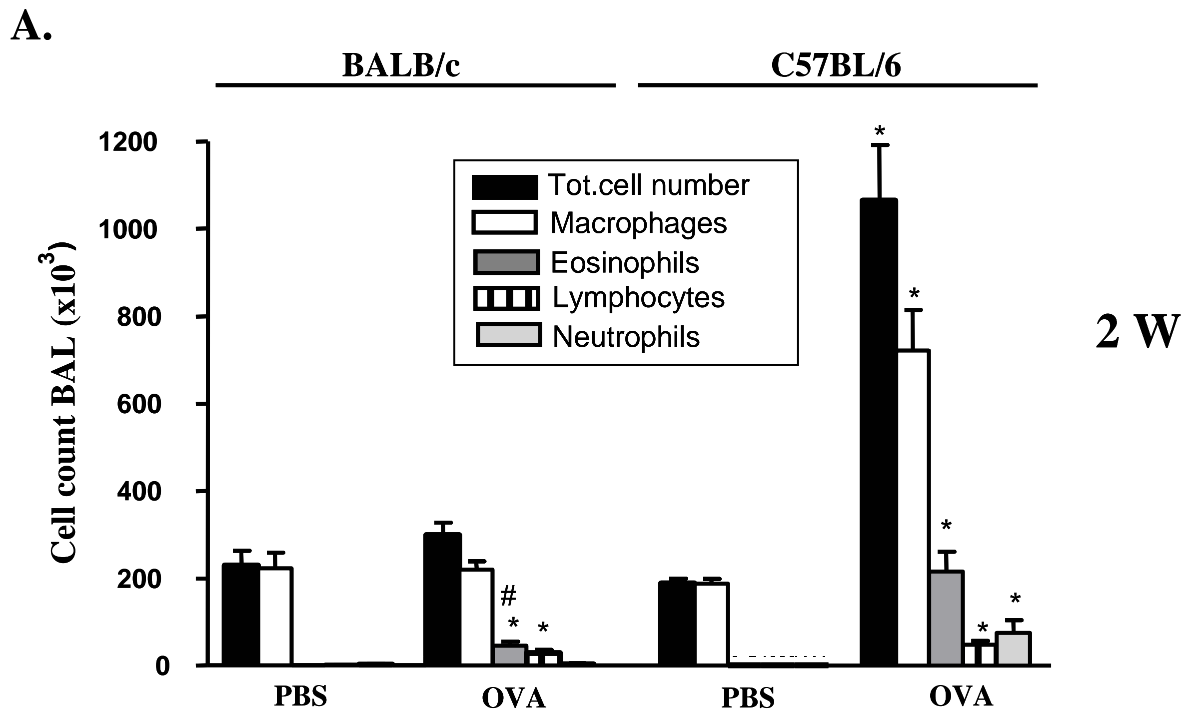
Exposure time	Treatment	Eosinophils ($x10^2$ cells/mm² airway wall)	
		BALB/c	C57BL/6
2 weeks	PBS	1.49±0.17	1.05±0.17
	OVA	5.06±0.30$^{*\#}$	6.61±0.46*
6 weeks	PBS	0.93±0.10	0.57±0.09¶
	OVA	1.51±0.13*¶	1.11±0.09*¶
9 weeks	PBS	0.79±0.11¶	0.69±0.10
	OVA	1.03±0.08*¶	0.79±0.08¶

* $p<0.05$: OVA versus PBS

¶ $p<0.05$: 6 and 9 weeks versus 2 weeks

# $p<0.05$: BALB/c versus C57BL/6

A.



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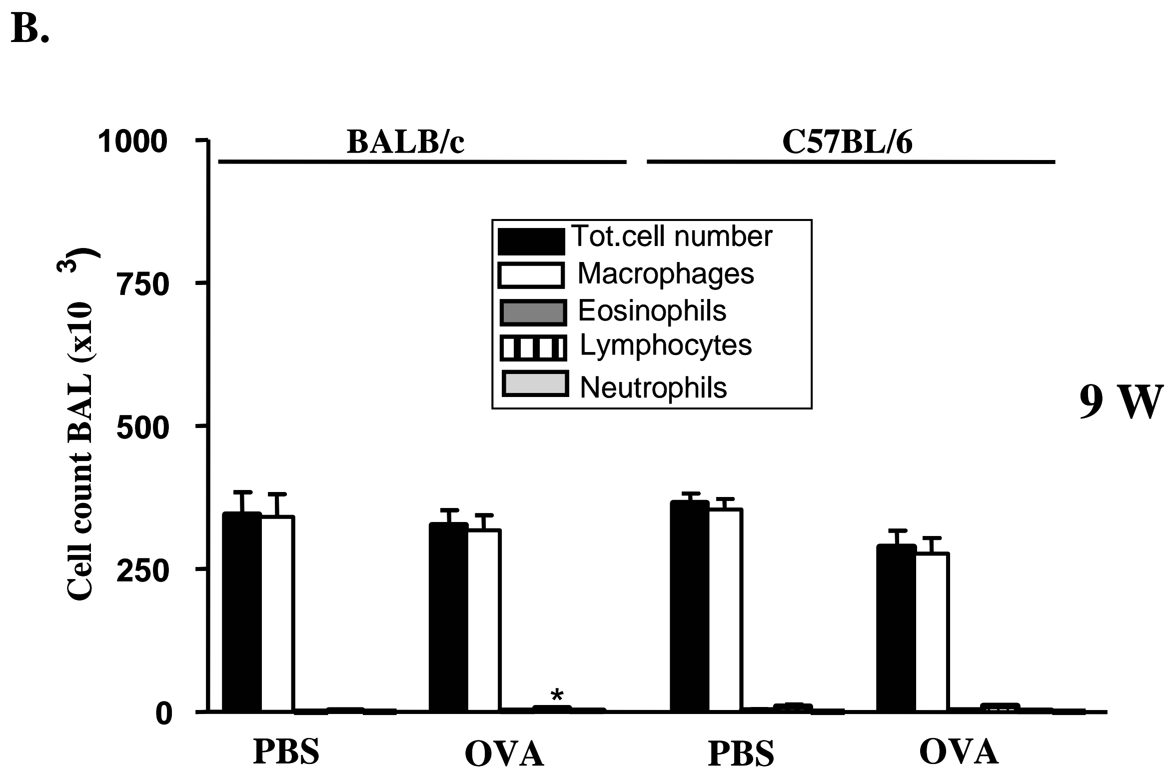


Figure 2: BALF total cell count and differentiation in OVA sensitised C57BL/6 and BALB/c mice exposed to OVA or PBS aerosols for 2 weeks (A) and 9 weeks (B). The BALF total cell counts after 6 weeks of OVA or PBS exposure are not included, as the results are similar to the 9 weeks experiment in both strains.

* $p<0.05$ OVA vs PBS; # $p<0.05$: BALB/c versus C57BL/6

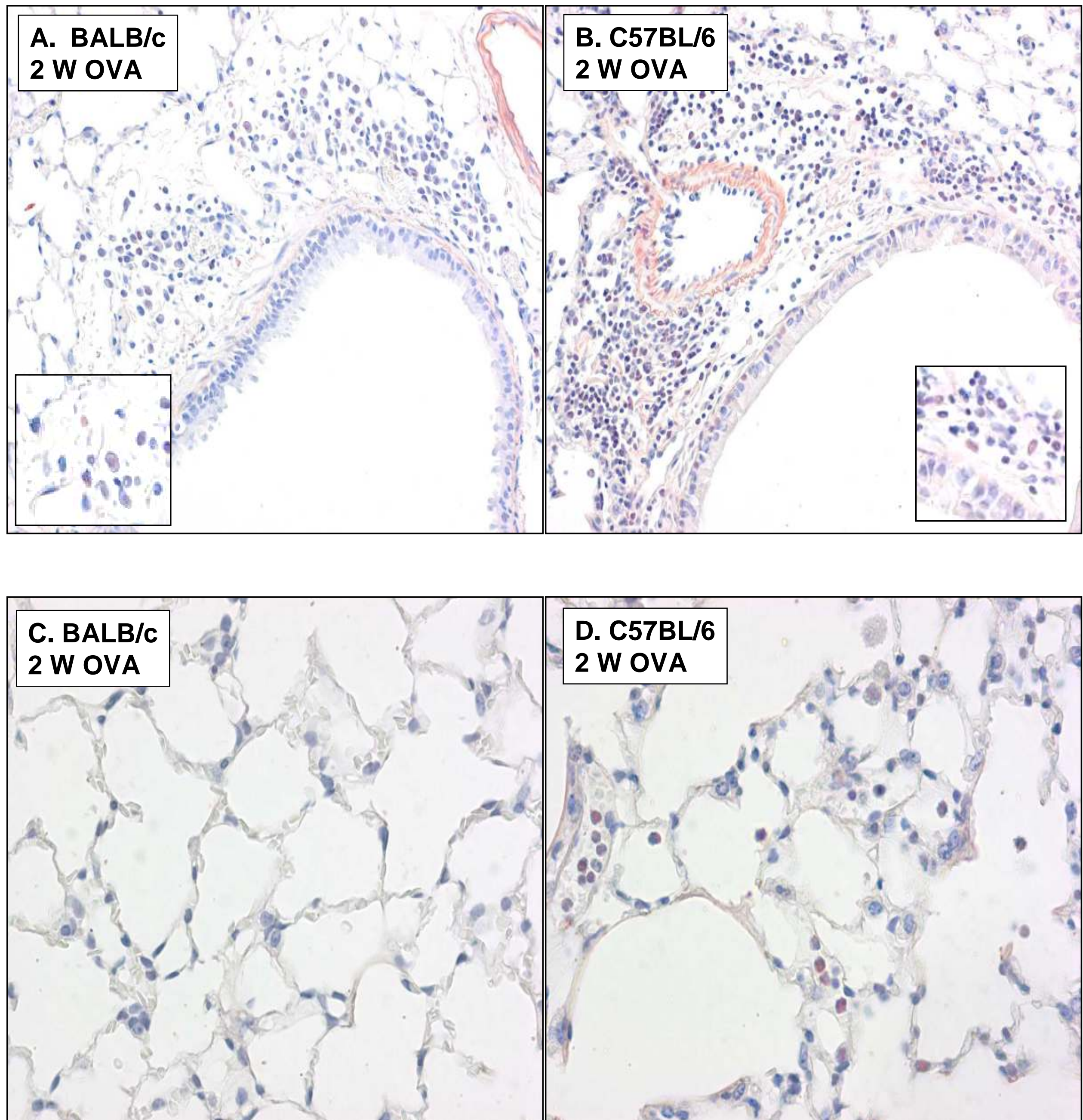


Figure 3: Airway histology (Congo Red stainings) of OVA- sensitized and -challenged BALB/c (A) and C57BL/6 (B) mice (2 weeks OVA exposure; magnification size = x 200). (C) and (D) show Congo Red stained pictures of the lung parenchyma of OVA- sensitized and – challenged (2 weeks) with interstitial inflammation in C57BL/6 (C) and absence of interstitial inflammation in BALB/c (D) (magnification size = x 400).

2. Serum OVA-IgE

Whereas the PBS challenged C57BL/6 mice had very low OVA-IgE levels at all time points, the BALB/c counterparts developed increasing IgE levels attributable uniquely to the i.p. sensitization (Table 2). In the OVA challenged mice of both strains, OVA-IgE increased already after 2 weeks as compared to the PBS challenged counterparts. Upon chronic exposure, both strains hold elevated IgE, however, the titres were markedly higher in the BALB/c mice as compared to the C57BL/6 mice ($p<0.05$ BALB/c vs. C57BL/6).

TABLE II. OVA-specific IgE levels in the serum after sensitization and exposure of BALB/c and C57BL/6 mice

Exposure time	Treatment	OVA- specific IgE (U/ml)	
		BALB/c	C57BL/6
2 weeks	PBS	7.60±1.02	2.22±0.55
	OVA	41.75±5.28 *	33.50±7.19 *
6 weeks	PBS	29.11±2.54 ¶ #	6.38±1.80
	OVA	96.30±10.67 * ¶ #	49.18±9.00 *
9 weeks	PBS	23.00±1.90 ¶ #	3.43±1.14
	OVA	102.20±10.63 * ¶ #	21.83±4.01 *

* $p<0.05$: OVA versus PBS

¶ $p<0.05$: 6 weeks and 9 weeks versus 2 weeks

# $p<0.05$: BALB/c versus C57BL/6

3. Epithelial Remodelling: Goblet Cells

Exposure to OVA resulted, comparably in both strains, in a significant increase in goblet cells from 2 weeks onward (Figure 4A/5A). This increase persisted after 6 and 9 weeks (Figure 4A). However, in both allergen-challenged C57BL/6 and BALB/c mice, the goblet cells did not increase any further upon prolonged challenge.

4. Airway Smooth Muscle (ASM)

The amount of airway smooth muscle did not change over time in the PBS-exposed mice, either in BALB/c or in C57BL/6 mice (Figure 4B and 5B). Exposure to OVA allergen for 2 weeks resulted in a significant increase in ASM staining only in BALB/c mice. However, longer allergen exposure periods induced an increase in ASM in both strains.

5. Morphometry and deposition of ECM proteins

Morphometry

The total wall area (*WAt*) or airway wall thickness of large-sized airways did not differ in both strains exposed to either 2, 6 or 9 weeks of PBS (Figure 6A). Exposure to OVA allergen for 2 weeks resulted in a significant increase in *WAt* only in the BALB/c mice when compared to sham-exposed mice. However, longer allergen exposures induced a significant and persistent increase in *WAt* in both strains.

Fibronectin& Collagen

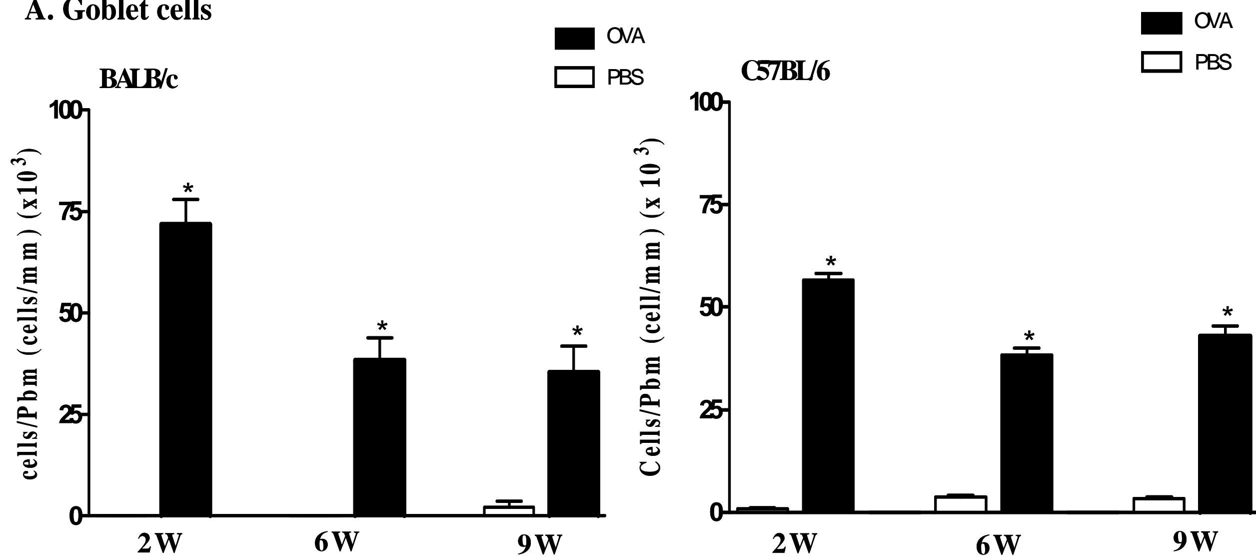
Fibronectin and collagen deposition were similarly increased in both genotypes after 6 and 9 weeks, respectively (Figure 5C and 6B and Figure 5D and 6C).

6. Airway (hyper-) responsiveness (AHR)

BALB/c but not C57BL/6 mice developed a significant leftward shift in the dose-response curve to inhaled methacholine after 2 weeks OVA exposure (ANOVA; $p<0.005$ versus PBS; Figure 7 A/D). After a longer exposure period (6 or 9 weeks), no difference in the dose-response curve was observed for both strains (Figure 7 B-C/E-F). The C57BL/6 mice were less responsive to methacholine than BALB/c mice. At 2 weeks the concentration of methacholine that caused a 250% increase in Penh from baseline in allergen-exposed C57BL/6 was 17.40 ± 5.92 mg/ml methacholine versus 4.69 ± 1.81mg/ml methacholine in BALB/c mice ($p<0.05$ BALB/c vs. C57BL/6).

7. BALF proteins

TGF-β_1 levels in BALF were persistently increased after 2 (*Protocol A*) or 9 (*Protocol C*) weeks allergen exposure in both strains compared to control, PBS- exposed, mice in both strains (Figure 8).



B. Airway Smooth Muscle (ASM)

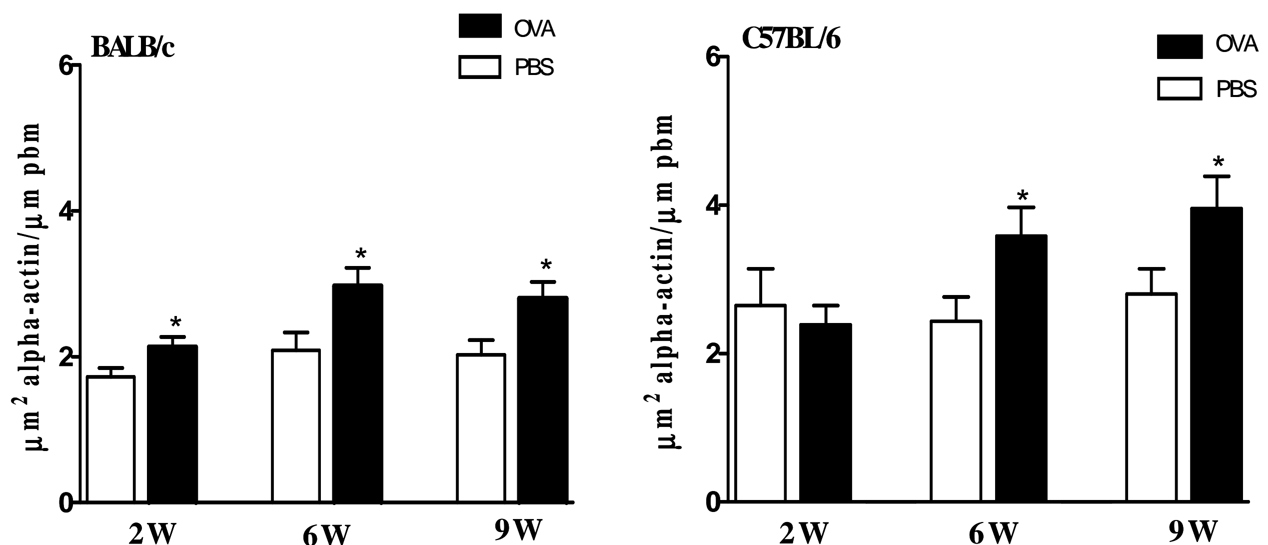
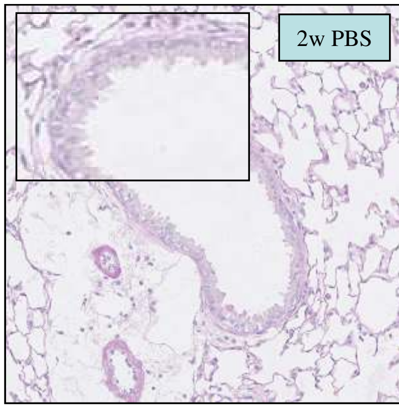
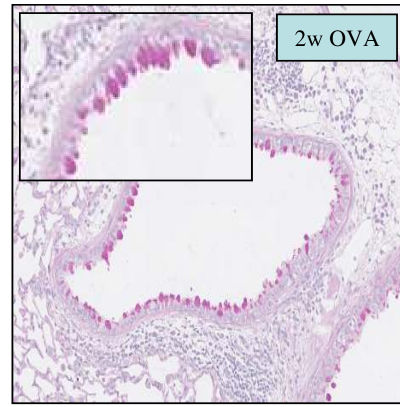


Figure 4: Measurement of indicators of airway remodelling with increasing duration of OVA challenge in C57BL/6 and BALB/c mice (solid black bars = OVA group, open bars = PBS group; n= 6-12 mice/group).
(A) Goblet cells (PAS) (B) Airway Smooth Muscle layer (IHC)
* $p<0.05$ OVA vs PBS

Figure 5 (next page): Histology pictures of the airways of C57BL/6 mice challenged with OVA or PBS aerosols for 2 or 9 weeks (magnification pictures = x 100). An inset with higher magnitude is included. All stainings were performed in the BALB/c strain as well, leading to analogous results at these time points.
(A) PAS staining to highlight goblet cells in C57BL/6 mice challenged with OVA or PBS for 2 weeks (B) IHC with DAB substrate to highlight the increase in ASM contractile elements (brown colour) in BALB/c mice challenged with OVA or PBS for 9 weeks (C) IHC with DAB substrate to highlight fibronectin deposition (brown colour) in C57BL/6 mice challenged with OVA or PBS for 9 weeks (D) Sirius Red staining to highlight collagen deposition in C57BL/6 mice challenged with OVA or PBS for 9 weeks





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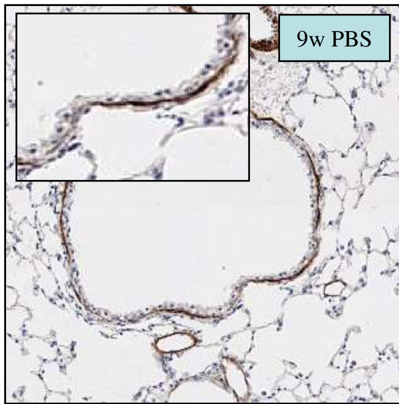

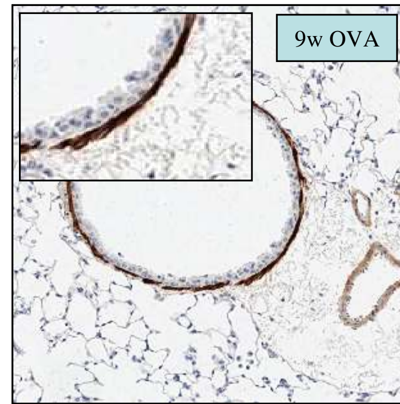

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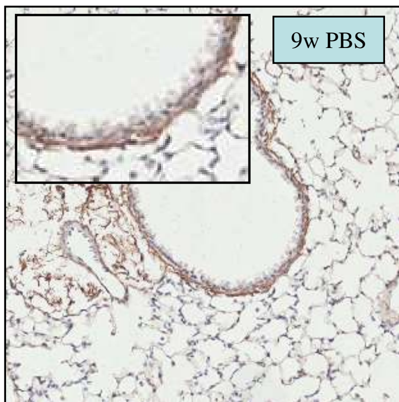

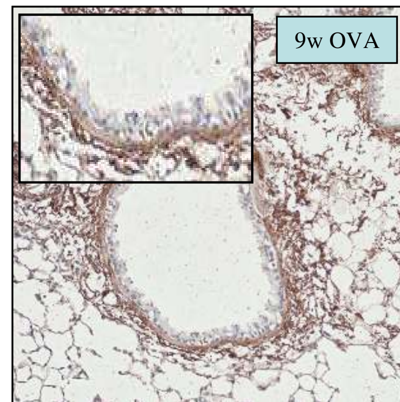

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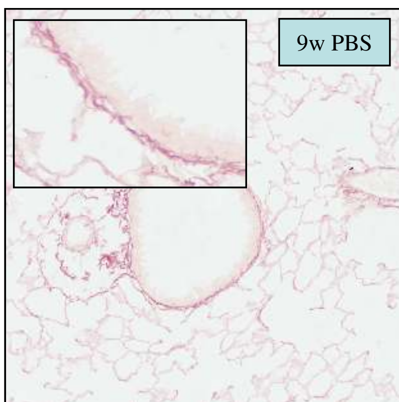
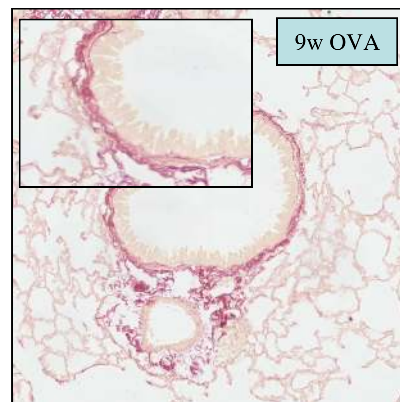
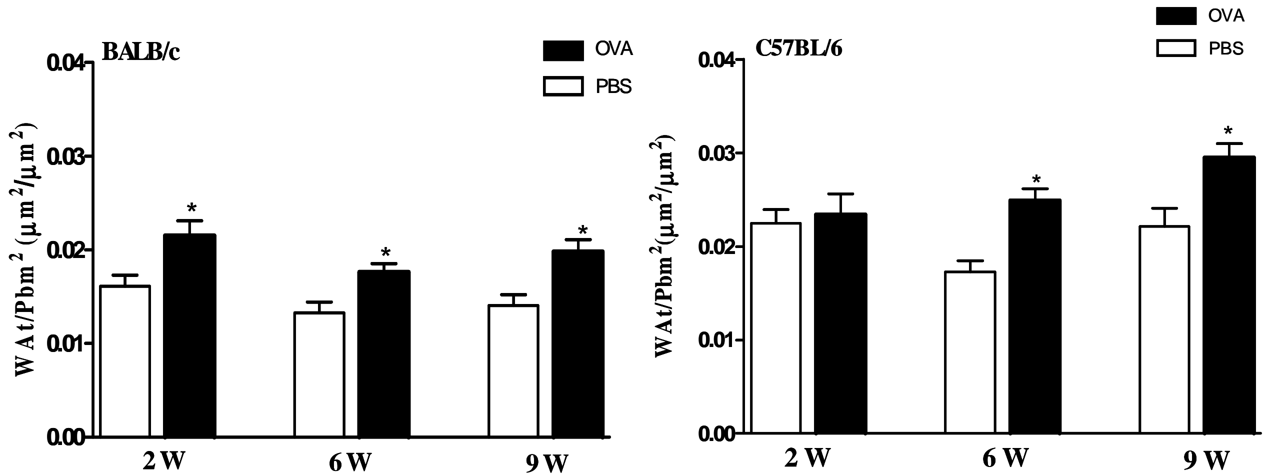
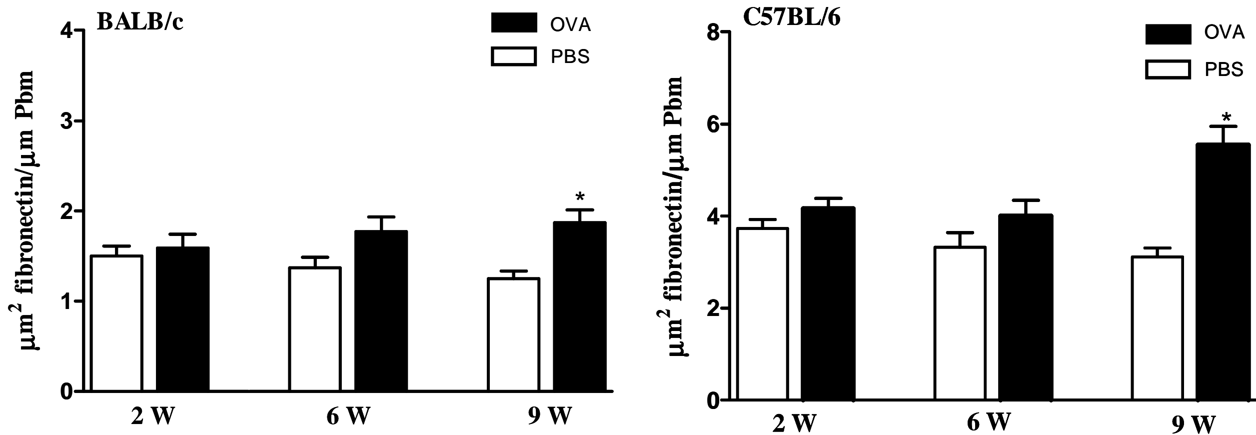




A. Airway wall thickness



B. Fibronectin deposition



C. Collagen deposition

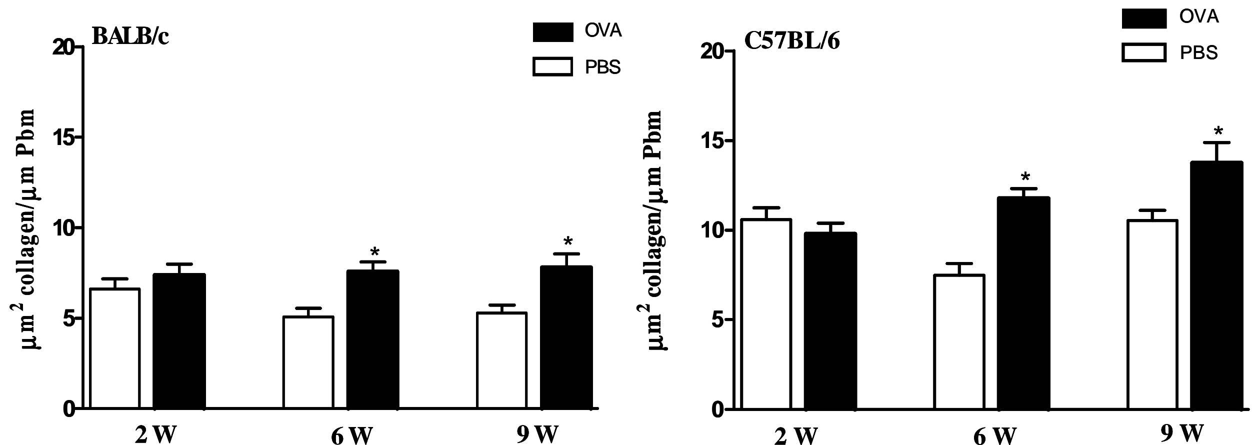


Figure 6: Measurement of indicators of airway remodelling with increasing duration of OVA challenge in C57BL/6 and BALB/c mice (solid black bars = OVA group, open bars = PBS group; n= 6-12 mice/group). (A) Airway wall thickening (Congo Red) (B) Fibronectin deposition (IHC) (C) Collagen deposition (Sirius Red) * $p<0.05$ OVA vs PBS

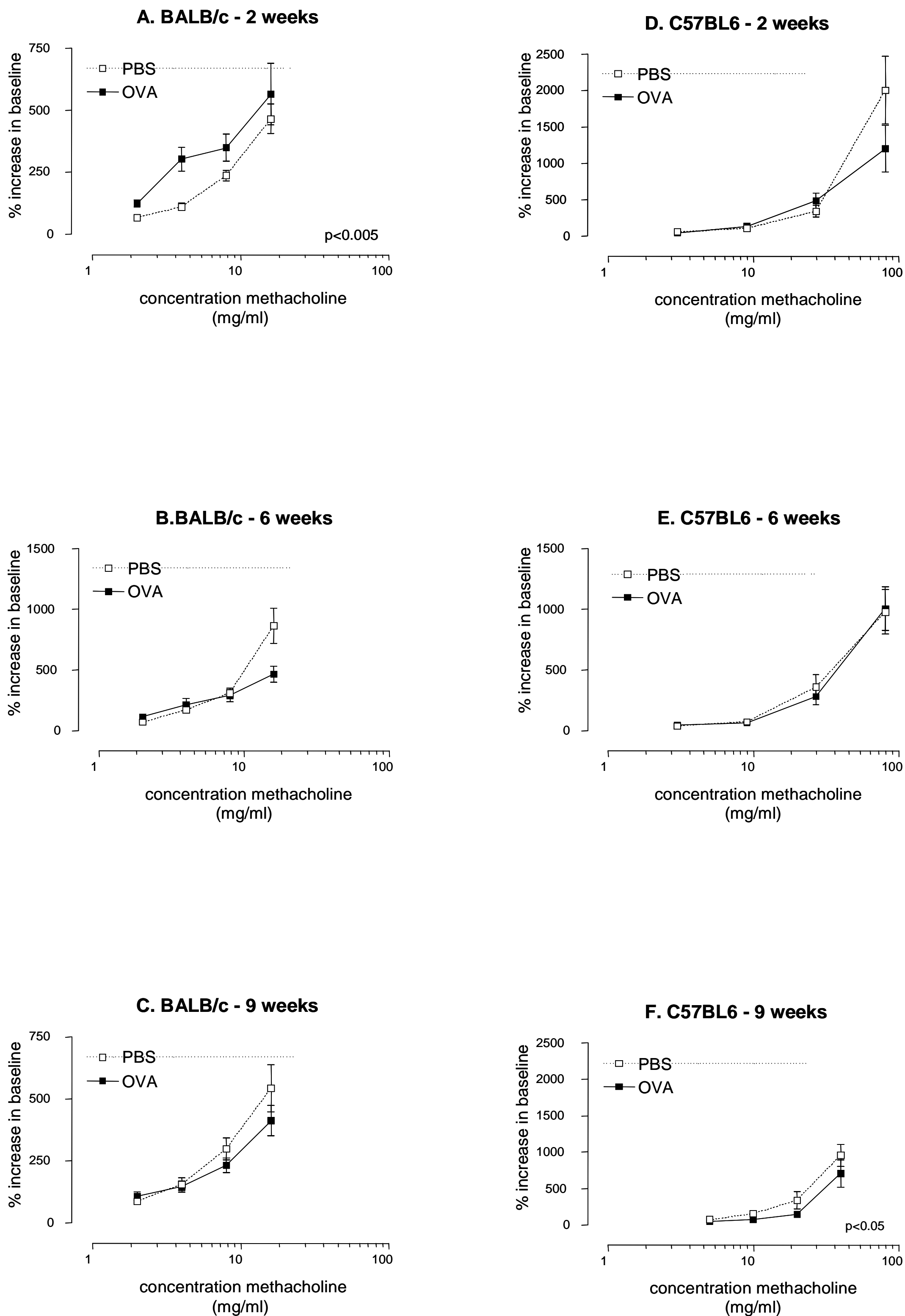


Figure 7: AHR to nebulized methacholine in sensitized BALB/c (A, B and C) and C57BL/6 mice (D, E and F) exposed to OVA (closed squares) during 2 weeks (A and D), 6 weeks (B and E) and 9 weeks (C and F). ANOVA OVA vs PBS at 2 weeks: $p<0.005$.

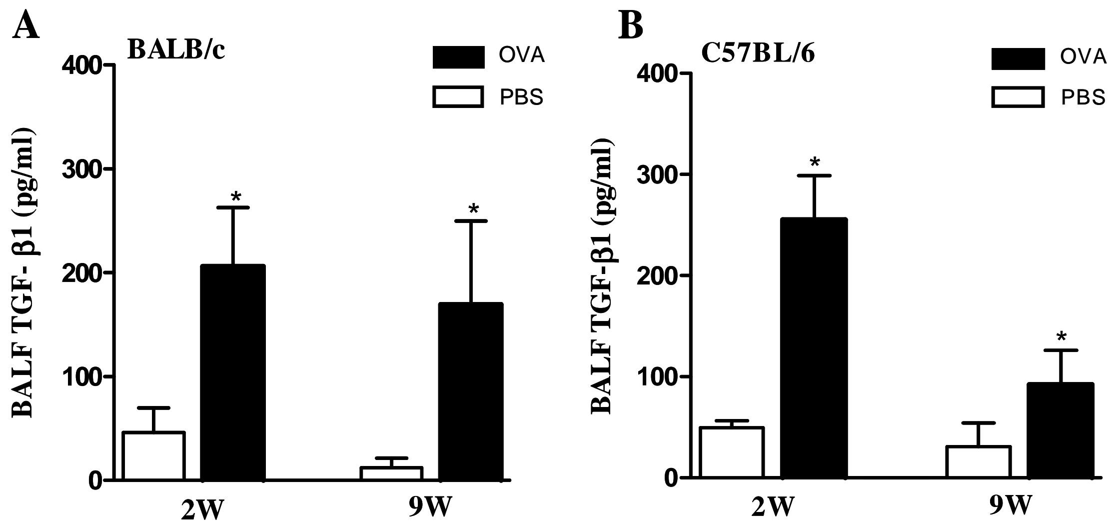


Figure 8: Levels of TGF-β_1 in the BALF of C57BL/6 (B) and BALB/c (A) mice in sensitized to OVA and exposed to OVA or PBS aerosols for 2 weeks and 9 weeks. *p<0.05 OVA vs PBS

DISCUSSION

We here document that in the two most commonly used mouse strains to model asthma, C57BL/6 mice and BALB/c mice, genetic background and the duration of allergen exposure have a profound impact on different aspects of the resulting pathological phenotype. Although the acute response in both strains was predominated by eosinophilic airway inflammation, only the BALB/c strain developed a significant airway smooth muscle hyperplasia and AHR. In an attempt to mimic more closely the features of chronic asthma, the response to longer periods of allergen exposure was predominated by airway remodelling while inflammation and AHR decreased in both strains. Although other groups have earlier described strain differences in response to various durations of OVA aerosol exposure [9], the current study gives a more detailed insight into the relationship of inflammation, remodelling and responsiveness in the two most commonly used mouse strains.

Inflammation, AHR and airway remodelling are thought to be interdependent processes. A large variety of mouse models focus on a limited number of characteristics of asthma to study the underlying pathogenesis [14;15]. To study inflammation, these models mainly rely on (hyper-) acute, short-term exposure protocols. The reason for this is that the use of short-term exposure protocols is practical but also avoids the establishment of respiratory tolerance that can develop after prolonged allergen exposure [11-14]. However, long-term exposure protocols are usually applied to study the main features of airway remodelling [4;5;14;16]. In these models, inflammation and lung function tests are often either absent or partially described [4;5;20]. We chose to provide here a comprehensive analysis encompassing not only the assessment of inflammation and responsiveness, but also a thorough characterisation of remodelling characteristics.

Here, we found that inflammation and remodelling behave differently in both strains, especially in the acute models, based on short-term allergen exposure (*Protocol A*). Both strains developed eosinophilic inflammation, as evident from BALF analysis and quantification of peribronchial eosinophilia. However, airway eosinophilia was more pronounced in the C57BL/6 mice in the acute model. This is in line with one earlier study, that also found that C57BL/6 mice are more susceptible to the development of airway eosinophilia [21]. In contrast, Shinagawa *et al.* found a more pronounced eosinophilia in BALB/c mice after repeated (chronic) intranasal OVA instillation [9]. We presume the

method of antigen exposure is critical in this respect, as we administered OVA 1% by aerosol whereas Shinagawa *et al.* administered OVA 0.1% intranasally. Serum OVA-IgE levels were more elevated in the BALB/c mice than in C57BL/6 after prolonged OVA challenge (*Protocols B* and *C*) confirming earlier findings that showed that BALB/c mice are IgE – prone [22]. This is also underscored by the fact that BALB/c mice, but not C57BL/6, have measurable IgE titres already after sensitization only.

In the BALB/c mice we observed a significant increase in airway smooth muscle mass (ASM) already after 2 weeks of OVA challenge ('acute' - *Protocol A*), in contrast to the C57BL/6 strain, where longer durations of allergen exposure were required to obtain such an ASM increase. In addition, AHR was present in BALB/c mice at that time point. This histological observation now adds up to the earlier descriptions that inbred mouse strains exhibit significant genetic variability in the airway calibre and development of AHR after antigen sensitization and exposure [9;23]. Although the contraction of ASM is the ultimate translation of the asthma pathology into physiologic disturbance in patients, it remains an unresolved issue if a larger smooth muscle mass is really the cause of the AHR [24;25]. Some studies indicate a clear link between ASM and AHR in both animal models and humans, since both AHR and increased ASM can persist after resolution of the airway inflammation [26-28], and mathematical models predict that airways with increased ASM narrow to a much greater extent than airways with less ASM volume [29]. One could speculate that the lack of AHR after 2 weeks of OVA exposure in C57BL/6 mice could be related to the absence of ASM hyperplasia. However, other factors could be involved. Firstly, the C57BL/6 strain developed a certain degree of interstitial inflammation, which was earlier also reported by another group [30]. Secondly, the airway anatomy of both strains is different [31]. These factors could both be of potential relevance to explain why AHR was observed only in the BALB/c strain.

Prolonged allergen challenge (*Protocols B* and *C*) results in increased fibronectin and collagen deposition in the airway wall in both strains which is concordant with findings described by other authors [20;26;32-35]. We here document that this structural remodelling occurs in parallel with a decrease of the inflammatory cells in the BALF compartment to baseline in both strains and loss of AHR in BALB/c mice. We and others previously described that prolonged OVA challenge in these mice results in the establishment of respiratory tolerance, as they are unresponsive to new sensitizations [12]. To date, the precise mechanisms of the development of respiratory tolerance in mice repeatedly challenged with

OVA aerosols have not been elucidated, and both regulatory T cell-dependent [36] and regulatory T cell-independent [37] mechanisms have been proposed. One factor that may be important in the down-regulation of allergic airway inflammation and AHR over time could ageing of the mice, because some studies found that the capability of T cells to differentiate into Th-2 cells was reduced in aging mice [38;39]. In contrast, one recent study found that older mice developed increased allergic inflammation, but less pronounced AHR [40]. Moreover, we earlier documented that mice aged over 20 weeks can easily develop Th-2 mediated airway inflammation [12]. Thus, the precise effect of ageing on allergic airway disease remains a matter of debate [39-41].

The exact role of the eosinophils in remodelling, inflammation and AHR is speculative. It is possible that the eosinophils could contribute to the initiation of collagen synthesis by fibroblasts during the acute inflammatory phase [42]. In our opinion, the few remaining eosinophils in the bronchial wall upon prolonged allergen exposure represent a small but measurable fraction deemed to vanish shortly thereafter. In the BALB/c mice, the decrease of eosinophilic inflammation and increase of fibrosis in the airway wall occurs in parallel with the loss of AHR. It is thus tempting to speculate that the remodelling process could occur in an attempt to protect against allergen-induced AHR and inflammation. Collagen and fibronectin deposition could indeed increase airway wall stiffness and oppose against extended narrowing of the airway wall. Moreover, the sub-epithelial fibrosis could decrease the amount of allergen exposure by shielding off the immune system from the allergens (‘wash-away’ effect).

TGF-β_1 is a key molecule involved in the regulation of both inflammation and remodelling in both humans and mouse models [43-46], and therefore represents a potential therapeutic target of interest. Increases in TGF-β_1 are a constant finding for both mouse strains used here, in the acute and in the chronic exposure models. It could therefore be speculated TGF-β_1 is activated quite early as an endogenous “anti-asthma” molecule to control inflammation [45;46], to promote remodelling [43;44;47] and thus to inhibit AHR [48]. Previous studies using neutralising antibodies for TGF-β_1 gave however conflicting results on its role. One study found that anti-TGF-β_1 antibody treatment could antagonize matrix deposition, smooth muscle cell proliferation and mucus production without affecting the airway inflammation [47]. In contrast, in a recent report – based on an intranasal OVA administration- it was

shown that anti-TGF-β_1 treatment did not inhibit remodelling, but rather induced an exacerbation of the allergen induced airway changes suggesting that this mediator is not *per se* responsible of airway remodelling [49]. These authors even warned against the use of therapeutic strategies aimed at interfering with TGF-β_1.

The current study has some limitations. Firstly, for measuring airway responsiveness, we used the whole body plethysmograph, a method that has been criticised. There is evidence that Penh and airway resistance do not correlate under certain conditions, although plethysmography remains a valuable tool for explorative analysis [19;50]. When Penh can be used as a surrogate for invasive measurements, it can decrease cost, time and number of animals required for experiments. Secondly, in this study we could not provide in-depth explorations into the precise mechanisms that regulate the relationship between airway inflammation, remodelling and responsiveness. Although the limitations of this study should be taken into account, the current data provide researchers with clues to choose an appropriate asthma model for the particular aspects of the disease they want to investigate.

In conclusion, genetic background has an impact on the different aspects of the acute allergic phenotype. These differences are far less pronounced when studying chronic allergen exposure, albeit the latter models are characterized mainly by remodelling but not inflammation or AHR.

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