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Impact of boosting for the strength of asthma parameters and dendritic cell numbers in a C57BL/6 model of allergic airway inflammation

Christina Hartwig^{a,*}, Hanne Constabel^b, Detlef Neumann^c, Heinz Gerd Hoymann^d, Thomas Tschernig^a, Georg M.N. Behrens^b

^aFunctional and Applied Anatomy, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

^bClinical Immunology and Rheumatology, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

^cInstitute for Pharmacology, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

^dFraunhofer Institute of Toxicology and Experimental Medicine, Department of Immunology, Allergology and Immunotoxicology, Nikolai-Fuchs-Strasse 1, 30625 Hannover, Germany

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Abstract

Background: Murine models assist in elucidating the pathogenesis of allergic asthma and evaluation of new therapeutic strategies. We aimed to assess the requirement of boosting needed in the BL/6 murine asthma model and its influence on DC populations in lungs and bronchial lymph nodes.

Methods and results: Two injections of OVA + alum – one sensitization and one booster – followed by two aerosol challenges were sufficient to induce a distinct asthma-like inflammation in BL/6 mice, including significant increased immunoglobulin (IgE) level, influx of eosinophils in the airway lumen, and evident histopathology. Using this protocol, CD11c^{high}MHC-II⁺ DC counts in lungs and lymph nodes doubled with no changes of CD8⁺ DC in the lungs but increase in lung-draining lymph nodes.

Conclusions: Given the site-specific changes of dendritic cell (DC) subpopulations during allergic asthma we propose a distinct regulation of antigen transport and antigen presentation in the murine asthma model.

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Keywords: Asthma; Ovalbumin; Sensitization; Dendritic cells

Introduction

Allergic diseases of the airway such as asthma or allergic rhinitis are on the rise throughout the world, especially in western countries (Beasley et al., 2000). They are classified as atopic diseases, defined by an increased allergen-specific immunoglobulin-E (IgE) level

in blood serum. One of the most important diseases is bronchial asthma, that is defined as a chronic inflammation of the bronchial airways and characterized by reversible airway obstruction, increased mucus production and infiltration of the airway with eosinophils, neutrophils, mast cells and T-lymphocytes (Busse and Lemanske, 2001). Less well studied are ocular allergies, nevertheless they contribute an essential element of allergic diseases (Groneberg et al., 2003).

OVA-based murine asthma models assessing airway eosinophilia, bronchial inflammation and airway

*Corresponding author. Tel.: +49 511 532 6740;

fax: +49 511 532 2948.

E-mail address: hartwig.christina@mh-hannover.de (C. Hartwig).

hyper-responsiveness (AHR) have been developed on a BALB/c background to study both asthma pathogenesis as well as therapeutic intervention. Far less publications describe OVA-based models in C57BL/6 (BL/6) mice (Moisan et al., 2005; Medoff et al., 2006; Vermaelen and Pauwels, 2003; Szema et al., 2006; Wu et al., 2001; Kodama et al., 2000). Nearly all published protocols differ in dose, frequency, route or number of OVA applications. Zhang et al. (1997) tested different asthma protocols on BALB/c and BL/6 mice, using an intra-peritoneal (i.p.) and intra-nasal sensitizations in combination with several intra-nasal challenges. They found that sensitized BALB/c mice that received only two intra-nasal challenges of OVA had no significant increase in airway responsiveness. Increasing the number to four or six challenges resulted in significant elevated airway reactivity, characterized by lower doses of methacholine (MCh) required to reduce C_{dyn} (dynamic compliance) and GL (lung conductance) by 50% from basal values. OVA-treated BALB/c mice always had a significant greater number of inflammatory cells in their bronchoalveolar lavage (BAL)-fluid compared with control mice independent of the number of challenges. Herz et al. (1998) explained these findings with different immunological phenotypes in BALB/c and BL/6 mice. They postulate that two pathways can regulate the induction of increased airway responsiveness. The first depends on the presence of allergen-specific IgE/IgG1 and allergen, the second is mediated by allergen-independent inflammation. After OVA-sensitization, C57BL/6 mice prefer a humoral immune response with small increases in IgE/IgG1 production but strong IgG2a production. The IgG2a expression is stimulated by IFN γ production, which is enhanced in the BAL-fluid of the C57BL/6 mice. In contrast, in the BALB/c strain the IL-4 production is significantly increased which positively regulates the IgE and IgG1 expression.

Given that allergen-specific CD4⁺ T lymphocytes play a pivotal role in the initiation of asthma, priming of CD4⁺ T cells by lung dendritic cells (DC) has been demonstrated in murine asthma models as essential prerequisite. In addition, individual DC subsets including plasmacytoid DC might have specific functions in determining tolerance or allergic immune responses in the lungs (De Heer et al., 2004; Lambrecht et al., 2000; Vermaelen et al., 2001). In this study, we demonstrate the effects of several OVA-injections on asthma parameters in an acute C57BL/6 asthma model. We systematically evaluated the strength of common markers such as eosinophilia, IgE titre, AHR and cytokine level. Finally, we determined the influence of asthma development on the numbers and composition of DC subsets in lungs and lung-draining lymph nodes.

Material and methods

Animals

Six to eight-week-old female C57BL/6 mice (Charles River, Sulzfeld, Germany) were used throughout the study. Mice were fed with ovalbumin-free laboratory food and tap water *ad libitum* and kept in a regular 12 h dark/light cycle at a temperature of 21 ± 2 °C and 50% humidity under specified pathogen free conditions. All experimental procedures were performed according to a protocol approved by the appropriate governmental authority and animal committee.

Sensitization and allergen challenge of mice

Eight animals per group were sensitized to OVA Grade VI and challenged with OVA Grade V (Sigma, Deisenhofen, Germany). The study groups are described in Fig. 1. The first OVA-injection was the initial sensitization and the second or third OVA-injection was the first or second booster. The first asthma group was sensitized with 10 μ g OVA absorbed to 1.5 mg aluminium hydroxide (alum, Pierce Biotechnology, Rockford, USA) i.p. on day 1 and with 1.5 mg alum, solved in phosphate buffered sodium chloride (PBS) on days 14 and 21. The second asthma group was treated with 1.5 mg alum on day 1 and with 10 μ g OVA on days 14 and 21. The third asthma group was treated with OVA on days 1, 14 and 21. The control group was sham sensitized with 1.5 mg alum on days 1, 14 and 21. On days 28 and 29, all mice were challenged with 1% OVA/PBS aerosol for 20 min. Allergen exposition was performed by dispersing of the relevant agent in a plexiglass box, in which mice can move freely. No sickness or restrictions were noticed during the experiments.

Measurement of lung function

Twenty-four hours after the last allergen challenge, AHR to inhaled MCh was assessed in all mice. Pulmonary function was measured non-invasively with

Groups	Sensitization day 1	1st booster day 14	2nd booster day 21	Challenge day 28 and 29
Control (n = 8)	Alum	Alum	Alum	1% OVA 20 min
S1 (n = 8)	Alum	Alum	OVA + Alum	1% OVA 20 min
S2 (n = 8)	Alum	OVA + Alum	OVA + Alum	1% OVA 20 min
S3 (n = 8)	OVA + Alum	OVA + Alum	OVA + Alum	1% OVA 20 min

Fig. 1. Schematic presentation of the study design. Mice were sensitized and boosted by intra-peritoneal injection of OVA or PBS absorbed to the adjuvans alum [Al(OH)₃]. All groups were challenged with OVA.

a glass-made head-out body plethysmograph system for four mice as previously described (Glaab et al., 2005; Hoymann, 2006). Briefly, the conscious, spontaneously breathing mice were placed in the body plethysmograph while the head of each animal protruded through a neck collar into a ventilated head exposure chamber. Tidal midexpiratory flow (EF_{50}) was calculated from the pneumotachograph flow signal for each breath (HEM 3.5, Notocord). EF_{50} (ml/s) is defined as the tidal flow at the midpoint (50%) of expiratory tidal volume and has been established as a measure of bronchoconstriction (Glaab et al., 2002; Vijayaraghavan et al., 1994).

Animal dissection

After testing the lung function, the mice were killed with an i.p. overdose of a mixture of 2% rompun (Bayer Vital GmbH, Leverkusen, Germany) and ketamin (A. Albrecht GmbH, Aulendorf, Germany). Mice were bled by heart puncture and serum was prepared and frozen at -80°C . The lungs of each animal were lavaged in situ, first with a 1 ml aliquot of sterile PBS and secondly with 0.8 ml PBS. Some lungs were fixed in 4% formalin via the trachea using 15 cm water columns and embedded in paraffin. Non-fixed lungs of four mice and lung-draining lymph nodes of all mice were weighed and stored in cold medium.

Differential cell count

Total cell counts in BAL-fluid were scored using a Zeiss (Telaval 31) microscope and viability was determined by trypan blue (Sigma, Steinheim, Germany) dye exclusion using a Neubauer chamber. Leucocyte subsets (eosinophils, neutrophils, macrophages or lymphocytes) were counted in BAL-fluid using cytopsins (centrifuged preparations) stained with Diff-Quik (Medion Diagnostics GmbH, Dünningen, Germany). A total of 400 cells in each sample were counted.

DC isolation, flow cytometric analysis and sorting

DCs were isolated from mouse lungs as described elsewhere (Henri et al., 2001; Vremec et al., 2000; Wilson et al., 2003). In brief, the lungs were cut into small fragments, digested with collagenase and DNase I (Sigma, Deisenhofen, Germany) and enriched by gradient centrifugation using Nycodenz reagents (Axis-Shield, PoC, Oslo, Norway). DCs were then enriched by negative depletion with an antibody cocktail containing anti-Gr1, anti-B220, anti-erythrocytes, anti-CD19 and anti-CD3, using magnetic separation. To prevent DC maturation during the isolation protocol, the procedure was carried out on ice, except for an initial 20 min digestion with collagenase/DNase, which was performed

at room temperature. This protocol excluded B220⁺ “plasmacytoid DC” from the DC preparation (O’Keeffe et al., 2002). DCs were labelled with CD11c-FITC and MHCII-PE (used as markers for DCs, both BD Biosciences, Pharmingen, Germany) and CD8-APC monoclonal antibodies (BD Biosciences, Pharmingen, Germany). Analytical and preparative fluorescent-activated cell sorting was carried out employing a FACSaria (Beckton Dickinson, San Jose, CA), or a Mo-Flu (Cytomation, Fort Collins, CO) instrument. Culture of DC in vitro was carried out at a cell density of 2.5×10^4 cells/ml in RPMI 1640 supplemented with 10% FCS and 25 mM HEPES.

T cell stimulation

For OT-II (OVA-specific CD4⁺ T cells) stimulation, single cell suspensions from the lymph nodes were treated with mAbs against M1/70, F4/80, Ter 119, RB6, M5/114, and 53–6.72 (anti-CD8). The antibody-coated cells were incubated with anti-rat IgG-coupled magnetic beads (Biomag[®] Quiagen, Germany) following the manufacturer’s protocols. For the experiments using soluble OVA, DC were plated in U-bottom 96-well plates at 1×10^4 cells/well with the indicated concentrations of OVA (Sigma, St. Louis, MO) for 45 min at 37°C in complete medium. The DCs were washed three times and resuspended in 200 μl of complete medium containing 5×10^4 carboxyfluorescein diacetate succinimidyl ester (CFSE)-labelled OT-II cells. For proliferation analysis after 60 h of culture, OT-II cells were stained with CD4-APC (BD Biosciences, Pharmingen, Germany), and samples were analysed by flow cytometry. The total number of dividing ($\text{CD4} + \text{PI}^{\text{neg}}\text{CFSE}^{\text{low}}$) cells was determined in duplicate.

Histology

The paraffin blocks were cut into 4 μm slices and stained with hematoxylin/eosin (Merck, Darmstadt, Germany). From each mouse lung, six sections (containing hilar structures and periphery) of the right and left lung were evaluated. Microphotographs were performed using a Nikon Eclipse 50i microscope with a Nikon Digital Sight DS-U1 Camera.

Cytometric bead array (CBA)

The cytokine levels of IL-13, IL-4, INF- γ and IL-12p70 in sera were measured, using a CBA system (BD Biosciences, San Diego, USA). The assay was performed according to the manufacturer’s instructions. Before measurement standard curves had been established.

Mouse anti-OVA IgE ELISA

To define serum levels of OVA-specific IgE, an ELISA-analysis was carried out using a mouse-specific anti-IgE-antibody. Briefly, OVA-coated microtiter plates (Nunc, Roskilde, Denmark) were incubated with test sera, diluted 1:200 in PBS containing 5% BSA, and serial dilutions of a standard serum obtained from a hyper-immunized mouse. Mouse IgE was detected by subsequent incubation with a biotinylated rat anti-mouse IgE antibody (BD Biosciences, Pharmingen, Germany) and peroxidase-coupled streptavidine (Biosource International, Camarillo, USA). Peroxidase-dependent conversion of TMB was quantified by reading at 405 nm. Titres were calculated according to the standard serum, which was set at 1E6 U/ml.

Statistical analysis

All values are expressed as mean $\pm$ SEM. Statistical analysis was performed using the *U*-test (Mann–Whitney). A *p*-value <0.05 (*) and <0.01 (**) was considered as statistically significant.

Results

Antigen-induced inflammation and eosinophilia in BAL-fluid

First, we wished to determine the impact of the frequency of sensitization on the overall cell number and composition in the BAL, especially the development of eosinophilia. Single or double boosting in BL/6 mice lead to an approximately 3- or 6-fold increase of inflammatory cells, respectively. Mice boosted once had a mean of $0.83 \pm 0.64 \times 10^6$ cells ($p = 0.026$ versus control), mice boosted twice a mean of $1.19 \pm 0.54 \times 10^6$ cells ($p = 0.0003$ versus control), and alum-only-treated mice $0.21 \pm 0.05 \times 10^6$ cells (control). In all mice that were sensitized to OVA analysis of the BAL-fluids revealed a strong eosinophilia as compared with the control animals (Fig. 2). The influx was strongest in mice which were boosted twice and no eosinophils were detected in the control. Macrophages were non-significantly increased in mice boosted once but almost tripled in mice that received a second booster. A significant increase in lymphocytes was detected in mice which were sensitized and boosted once or twice. Finally, mice that were sensitized only but not boosted developed only minimal increase in eosinophils and lymphocytes, and no changes in number of macrophages. These animals were not included in the following analyses.

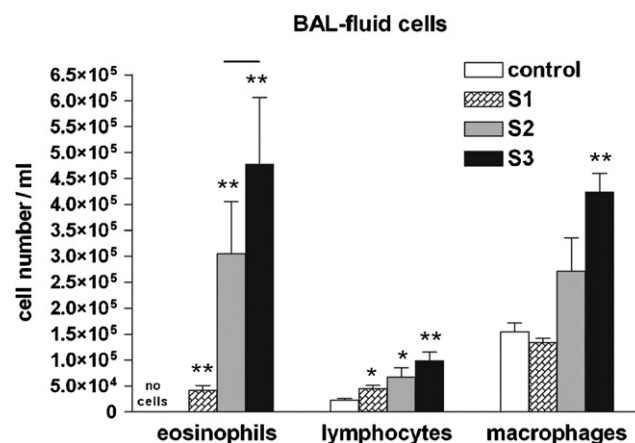


Fig. 2. Cell numbers in BAL-fluid. Lungs were lavaged in situ with PBS 24 h after the last challenge. Total cell counts were scored using light microscopy. Eosinophils, macrophages and lymphocytes were determined in BAL-fluid. Mice were divided into control (sham sensitized with PBS but challenged with OVA), S1 (one OVA-injection), S2 (two OVA-injections) and S3 (three OVA-injections). The bars represent the mean value $\pm$ SEM of eight mice. Significant differences between control and mice sensitized to OVA are shown by one ($p < 0.05$) or two ($p < 0.01$) asterisks.

Histology

Next we studied the tissue inflammation in the lungs. Macroscopic measurement revealed that the lungs of control mice had a mean weight of 288 mg, while those mice boosted once or twice had a mean weight of 318 mg. In the sham-sensitized mice microscopic lung morphology appeared unaffected (Fig. 3A). The mice boosted once showed a peribronchial infiltration only with no cellular infiltrate around the alveola (Fig. 3B). The lungs of the mice boosted twice, however, revealed alveolitis and a massive peribronchial and perivascular inflammation, among others with eosinophils (Fig. 3C). Thus, together with the results of the BAL these data confirm initial inflammatory cell infiltration in the lungs of BL/6 mice after the first booster which is efficiently further enhanced by the second booster.

Antigen-induced IgE in serum

An important feature of allergic asthma includes the production of allergen-specific IgE, which has also been demonstrated in mice. Sensitization to OVA followed by aerosol challenge of the mice-induced production of allergen-specific serum IgE in single ($2.63 \pm 2.43 \times 10^4$ IU/ml; $p = 0.001$) and double boosted mice ($5.47 \pm 4.92 \times 10^4$ IU/ml, $p = 0.0002$). No OVA-specific IgE antibodies could be detected in sham-sensitized control mice.

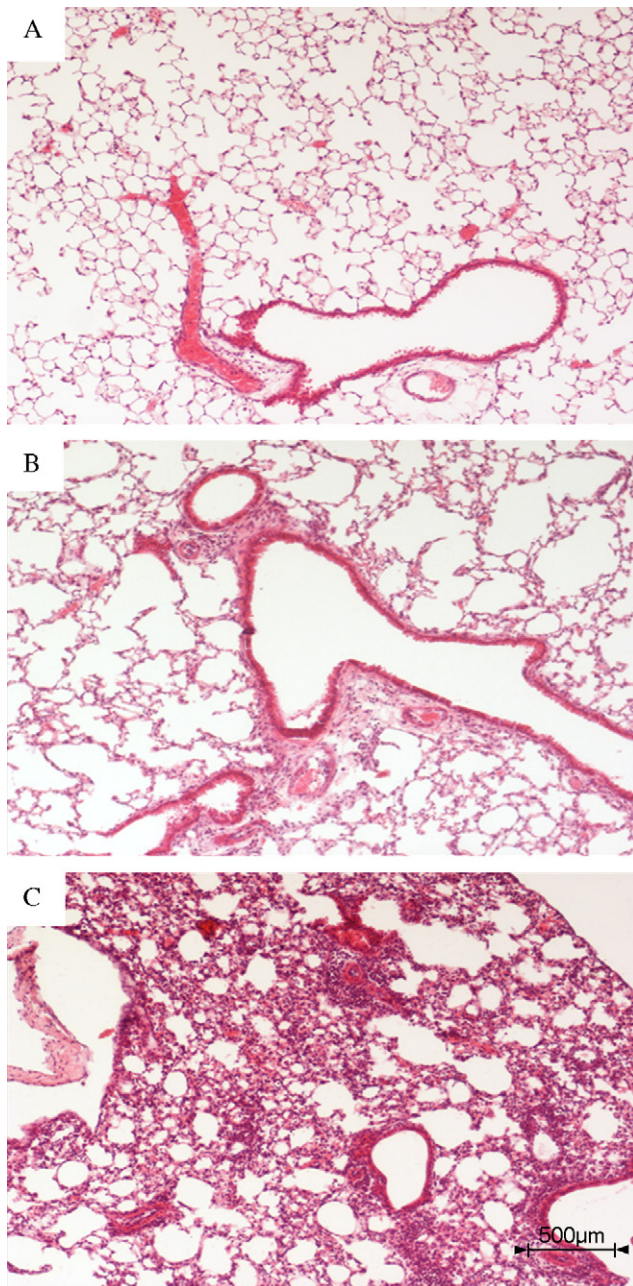


Fig. 3. (A–C) Histology of the lungs. Lung histology represents the actual status 24 h after the last challenge. A control lung of a mouse sensitized only with PBS and adjuvans (A), a mouse boosted once (B) and a mouse boosted twice with OVA (C) are shown. No pathohistological signs of inflammation could be detected in A, whereas in B a slight influx of inflammatory cells is detectable, in C a clear and massive cell influx is seen. Bar = 500 μ m.

Antigen-induced airway hyper-responsiveness

Aberrant lung function is the main clinical symptom of an allergic asthma-like response. Existing methods for measuring respiratory function in mice include invasive

and non-invasive approaches. The invasive recordings of pulmonary resistance, conductance and dynamic compliance are well established and show only small variations within the measurement. The non-invasive method is subjected to a greater variability but includes other advantages like the lacking of artefacts (generated by the anaesthesia and surgical trachostomy) or the possibility of using large numbers of animals within the experiment. It is shown that with both methods, allergic responses may be evidenced (14). To treat the mice with care, we decided to use the non-invasive head-out body plethysmograph system.

In order to analyse the influence of several OVA-injections during the sensitization phase on lung physiology airway responsiveness to MCh was determined. Surprisingly, no distinct AHR could be found in all experimental groups independently of the number of OVA-injections. If at all, only small and non-significant differences between mice sensitized sham or with OVA were detected (control = ED₅₀ 16.96 $\pm$ 7.22/boosted once = EF₅₀ 14.15 $\pm$ 4.87/boosted twice = EF₅₀ 17.15 $\pm$ 4.87). However, it is known that AHR is difficult to induce and to measure in C57Bl/6 mice (10).

Serum cytokines

The serum levels of IL-12p70 were about six-fold increased in the mice boosted once but decreased to about threefold the levels of sham-sensitized animals in mice boosted twice. In all groups only low IL-13 and IFN- γ were measured and no differences between control and boosted mice could be detected. IL-4 was not detected in either of the groups analysed (Fig. 4).

DCs isolation and phenotype-analyses of lung tissue and draining lymph nodes

A number of studies have focus on the role of DC in the development of allergic airway inflammation (for review see Lambrecht and Hammad, 2003; De Heer et al., 2005). The analysis of DC distribution in lung parenchyma and airways or lung-draining lymph nodes is hampered by the large fraction of autofluorescent macrophages and overlapping surface expression of lineage defining molecules (e.g. CD11c). Thus, we first confirmed the phenotypic and functional characterization of DCs enriched from the lungs and lung-draining lymph nodes. Enrichment of lung-derived cells by density centrifugation and magnetic bead depletion as described lead to a prevailing CD11c⁺ cell population consisting of cells with high and low MHC II expression. After sorting into CD11c^{high}MHC-II^{low} and CD11c^{high}MHC-II^{high} cells were short-term cultured for up to 24 h and microscopically analysed (Fig. 5A) or used for T cell stimulation (Fig. 5B). As depicted in Fig. 6,

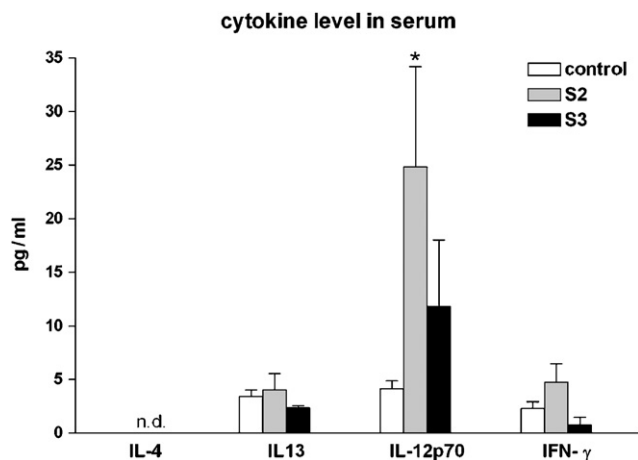


Fig. 4. Cytokine status in the serum. IL-4, IL-13, IL-12p70 and IFN- γ were measured using mice serum, 24 h after the last challenge. Groups are sham-sensitized mice (control), mice with two OVA-injections (S2) and mice with three OVA-injections (S3). The bars represent the mean value $\pm$ SD of eight mice.

CD11c^{high}MHC-II⁺ cells from the lungs presented with features typical for DCs including a variable cell size, the development of dendrites which expressed MHC-II molecules, and cluster formation at 5 h (data not shown). In contrast, CD11c^{high}MHC-II^{low} cells were monomorphic, did not have dendrites and were adherent to cell culture dishes. Based on this, lung CD11c^{high}MHC-II⁺ cells were classified as DC and CD11c^{high}MHC-II^{low} cells as macrophages.

Next we used these experimental settings to study the influence of sensitizing on DC frequency in lungs and lung-draining-lymph nodes. Lung CD11c^{high}MHC-II⁺ DCs were about three-fold higher as compared with the control mice, irrespective whether the mice were boosted once or twice. Interestingly, these changes were the result of increased numbers of CD11c^{high}MHC-II⁺CD8⁻ DC as CD8⁺ DC in the lungs remained constant (Fig. 7). In lung-draining lymph nodes, we observed a similar although less distinct (two-fold) increase of DCs. This increase, however,

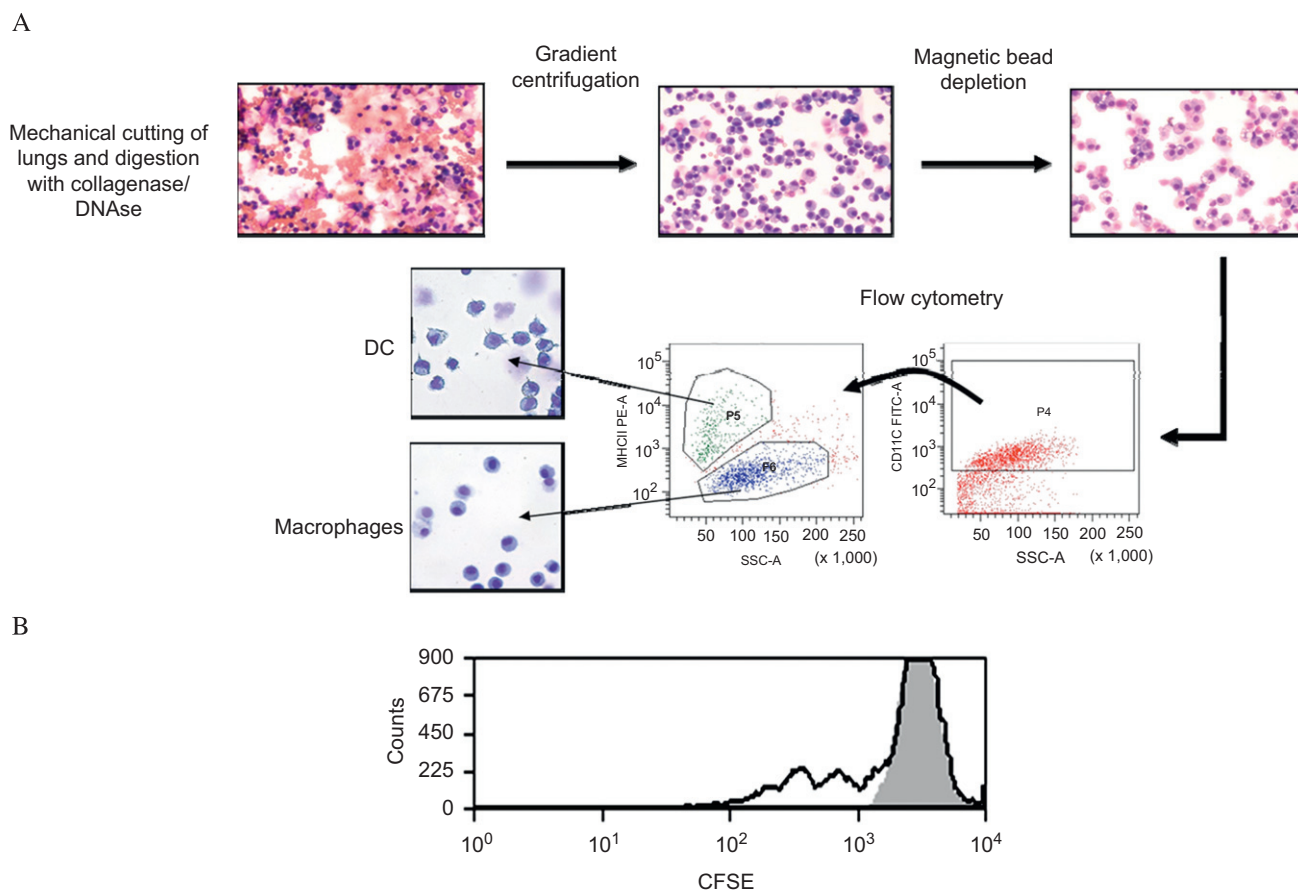


Fig. 5. (A, B) Scheme of DC isolation (A) and a representative example of T cell stimulation in flow cytometry analysis (B). A single, high CFSE fluorescence (B) was monitored if DCs and T cell were co-cultured without antigen (grey area). In contrast different generations of T cell with a decreasing rate of CFSE were detectable in co-culture of T cells and antigen pulsed DCs (black line).

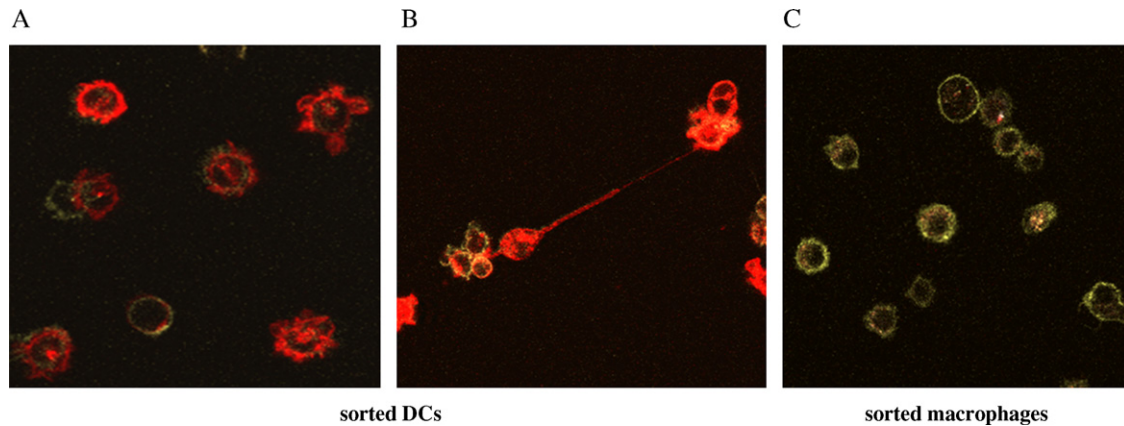


Fig. 6. (A–C) Cell analysis by confocal fluorescence microscopy. Isolated DCs and macrophages were short term cultured and stained with CD11c (FITC, green) and MHC-II (PE, red). After 1 h, cell populations were analysed via confocal fluorescence microscopy using a $40\times$ magnification. A distinct membrane-associated fluorescence for CD11c could be monitored in the DC and in the macrophages culture (A–C). Only a limited MHC-II expression could be recognized on macrophages (C), whereas DCs show a massive expression membrane-associated (A) and on dendrites (B).

comprised both $CD8^{+}$ and $CD8^{-}$ DC to a similar degree and was different to the changes in the lungs. Taken together we observed a divergent increase of DC subpopulations in the lungs and lung-draining lymph nodes in our allergic asthma model, which was not affected by the number of boosters during sensitization.

Discussion

We aimed to study the influence of the frequency of boosters during sensitization in a murine BL/6 asthma model on airway inflammation and composition of DC populations. The results of the BAL-fluid cell counts demonstrate that already a single OVA-injection of BL/6 mice leads to a significant influx of cells, specifically eosinophils in the lung which is further enhanced by repetitive OVA-injections as described for BALB/c mice (Rückert et al., 2005).

Lung inflammation needs booster in the sensitization phase

As has been shown recently, differences in effector cell influx in histopathology of murine asthma is even more strain dependant, with BALB/c mice having more infiltrating cells than BL/6 mice in allergic inflammation (Singh et al., 2005). In our study, clear histological evidence of allergic inflammation in BL/6 mice such as peribronchial and perivascular infiltrates could be detected after two OVA-injections. Additional boosting led to a pronounced alveolitis and further inflammation. It seems to be important to consider these differences of sensitization procedures on the effector

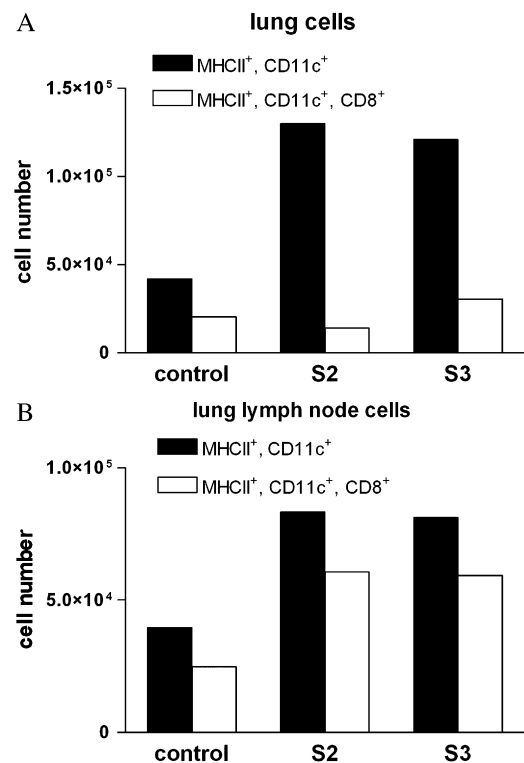


Fig. 7. (A, B) Flow cytometric analysis of lung and lung-draining lymph node DCs. DCs from lung and draining lymph nodes were isolated 24 h after the last challenge. Cells were labelled with CD11c, MHC II and CD8 and analysed by flow cytometry. Mice groups are sham-sensitized mice (control), mice with two OVA-injections (S2) and mice with three OVA-injections (S3). Absolute cell counts of DCs per mouse, in lung tissue (A) and lung-draining lymph nodes (B) are shown (pooled data, $n = 4$ mice, therefore no SD given). Black bars represent the double positive cells for CD11c and MHC II and white bars show the CD11c, MHC II and CD8 positive cells.

phase when using BL/6 mice for interventional studies to prevent asthma.

Repeated booster influence IgE response

Induction of an antigen-specific IgE response is a condition precedent the development of allergic reactions, although it is not a prerequisite for AHR or cellular infiltration (Mehlhöf et al., 1997). Using IgE level as readout, the protocol with one booster is sufficient to prove a significant increase in BL/6 mice. Nelde et al. (2001) showed that both OVA application via the airways and epicutaneous or intra-peritoneal sensitization leads to allergic IgE response. In addition, few exposures with high-dose antigen seem to be less efficient than multiple exposures with low doses in BALB/c mice. Basically, the IgE synthesis is strongly influenced by the route and the frequency of antigen application. These observations are in agreement with our findings that mice, which were sensitized three times showed a more than two fold higher serum IgE titres than mice which were boosted once. Again, the genetic background of the mice plays a major role for detection of antigen-specific IgE: BL/6 mice show an approximately 40% decreased titre of OVA-specific circulating IgE compared with BALB/c mice (Zhang et al., 1997).

The animal model influences the cytokine profile most

Airway inflammation strongly correlates with a dysregulated production of cytokines secreted by allergen-specific Th2 T-helper cells. Our data showed unaffected low serum level of IL-13 and IFN- γ in all groups, whereas the IL-12p70 level was higher in mice sensitized to OVA than in sham-sensitized mice. The increased IL-12p70 level confirms the finding that BL/6 mice typically show a Th1 immune response and but could also indicate a supportive effect of IL-12 in asthma development. In a murine model it was recently demonstrated that although IL-12 counteracts Th2 sensitization, it contributes to full-blown allergic airway disease upon airway allergen exposure in the post-sensitization phase, with enhanced recruitment of CD4⁺ T cells and eosinophils (Meyts et al., 2006). Interestingly, IL-12 levels obtained from BAL-fluid of BL/6 mice were found unaffected in a model similar to ours (Koya et al., 2006).

Lung function is a difficult parameter in BL/6 mice

We were unable to show any significant differences in AHR to MCh between sham- and OVA-treated mice. Similarly, some authors (Medoff et al., 2006; Shinagawa and Kojima, 2003) did not find AHR in BL/6 mice. In

contrast, Szema et al. (2006) measured airway resistance and dynamic lung compliance in BL/6 mice using a whole-body plethysmograph. Comparing OVA treated with control mice, OVA-treated mice show a significant elevated resistance and also a significant decreased compliance. Zosky et al. (2004) compared asthma protocols (BALB/c) with one, three or six challenges in relation to the MCh responsiveness 24 and 48 h after last challenge, using a low-frequency forced-oscillation technique. Briefly, six challenges increased the responsiveness 24 h (as well as 48 h) after the last challenge in all measured parameters.

Compartment-specific rise in lymph node CD8⁺ DC

DCs are a critical cell population contributing to the balance of mucosal inflammation and tolerance (Maldonado-Lopez et al., 1999). The role of DC during the sensitization and effector phase in mice as a relevant factor for the progress of the allergic airway inflammation has been demonstrated recently (De Heer et al., 2004). Among the different DC subsets that have been characterized so far, CD8⁺ DC and plasmacytoid DC have been proposed as to be important for the development of mucosal tolerance (Lambrecht et al., 2000; Hammad et al., 2004; Stock et al., 2004; Van Rijt et al., 2005). Our data support the finding of only low CD8⁺ DC numbers in the lung (for reference see discussion by Hammad) and that non-CD8⁺ DC contribute to the DC increase during allergic asthma, most probably through influx of new cells. CD8⁺ DC have been proposed as a blood-derived lymph node-resident DC, which contribute to cytotoxic T-cell responses via cross-presentation of antigens carried into the lymph node by other DC subpopulations (Villadangos and Heath, 2005). Whether this concept holds true for MHC-class II-mediated antigen presentation has not been evaluated yet. In contrast to the lungs, CD8⁺ DC in the lymph node increased during the allergic inflammation and represented a significant population of all DC. This rise in lymph node CD8⁺ DC might be a result of increased influx, survival, reduced egress or a combination of all. In any case, the divergent changes of CD8⁺ DC between the lungs and lymph nodes during the pulmonary inflammation would argue for a distinct role of this DC subset in murine asthma and the concept of CD8⁺ DC being a lymph-node resident DC population.

In conclusion, two OVA-injections – one sensitization and one booster – followed by two aerosol challenges are sufficient to induce a distinct asthma-like inflammation in BL/6 mice, including significant increased IgE level, influx of eosinophils in the airway lumen, and evident histopathology. Site-specific changes of DC subpopulations during allergic asthma indicate a distinct

regulation of antigen transport and antigen presentation which has important implications for interventional strategies aimed to treat asthma. We think that BL/6 mice serve as a useful model to further explore the role antigen presentation on classical asthma parameters.

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