

V β 8⁺ T lymphocytes are essential in the regulation of airway hyperresponsiveness and bronchoalveolar eosinophilia but not in allergen-specific IgE in a murine model of allergic asthma

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Summary

Background There is increasing evidence that in allergic asthma the inflammatory process is regulated by T lymphocytes. In BALB/c mice the majority of ovalbumin responsive T lymphocytes express the V β 8.1⁺ and V β 8.2⁺ T-cell receptor.

Objective We analysed the contribution of V β 8⁺ T lymphocytes during the sensitization and challenge phase in the regulation of antigen-specific IgE, airway hyperresponsiveness and cellular infiltration in the airways in a murine model of allergic asthma.

Methods Mice strains genetically lacking (SJL/J and SJA/9) and expressing (BALB/c) the V β 8⁺ T cell receptor were used. In addition, prior to the sensitization and prior to the challenge BALB/c mice were treated with antibodies to V β 8. Mice were sensitized with ovalbumin, followed by repeated challenge with ovalbumin or saline aerosols.

Results In ovalbumin challenged BALB/c mice treated with control antibody a significant increase in eosinophils in the bronchoalveolar lavage, airway hyperresponsiveness and increased serum levels of ovalbumin-specific IgE were observed compared to control mice. Treatment of BALB/c mice with antibodies to V β 8 prior to the sensitization or prior to the challenge period completely inhibited the ovalbumin induced infiltration of eosinophils and airway hyperresponsiveness, while ovalbumin-specific IgE was slightly decreased. In SJA/9 and SJL/J mice ovalbumin challenge did not induce eosinophilic infiltration and airway hyperresponsiveness. In SJL/J mice ovalbumin challenge induced an upregulation of ovalbumin-specific IgE, however, in SJA/9 mice no upregulation was observed.

Conclusion It is demonstrated that V β 8⁺ T lymphocytes are essential for infiltration of eosinophils in the airways and development of airway hyperresponsiveness in a murine model of allergic asthma. In contrast, although V β 8⁺ T lymphocytes seem to be important for the extent of IgE levels, no essential role for V β 8⁺ T lymphocytes in the induction of antigen-specific IgE was observed.

Keywords: mouse, T lymphocytes, eosinophils, IgE, airway hyperresponsiveness

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Introduction

Chronic airway inflammation is currently considered to play a crucial role in the pathogenesis of allergic asthma [1].

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Allergic asthma is associated with allergen-specific IgE, increased airway responsiveness to bronchoconstrictive stimuli and infiltration of T lymphocytes and eosinophils in the airway tissue. There is increasing evidence for an important regulatory role for T helper 2 lymphocytes (TH₂ lymphocytes) [2] in the inflammatory process of allergic asthma. *In vitro* studies show that TH₂ lymphocytes produce

cytokines such as IL-4 and IL-5 whereas Th1 lymphocytes produce IL-2 and IFN γ [3]. It is known that IL-4 released from activated T cells stimulates B cells to proliferate and to undergo isotype switching to produce IgE antibodies [4]. IL-5 enhances migration, differentiation and effector function of eosinophils [5]. However, the precise role of TH₂ lymphocytes in allergic asthma remains difficult to establish. Murine models reminiscent of the immunological and pathophysiological features of human allergic asthma may be helpful to unravel the regulatory role of T lymphocytes.

Recently, we have developed a murine model of allergic asthma in which BALB/c mice are sensitized with ovalbumin without using an adjuvant and later on repeatedly exposed to ovalbumin aerosols [6]. In this model symptoms can be observed that resemble those found in patients with allergic asthma, such as presence of antigen-specific IgE in serum [7], an increase in *in vivo* airway responsiveness to methacholine and serotonin [8], and presence of peribronchial inflammatory infiltrates, which is reflected by an increased number of eosinophils in bronchoalveolar lavage fluid.

Evidence suggests that in mice the immune response to ovalbumin is mediated via T cells which utilize a limited set of T-cell receptors [9,10]. In addition, ovalbumin reactive T cell hybridomas isolated from ovalbumin-sensitized BALB/c mice expressed predominantly V β 8 and less frequently V β 2 and V β 14 T-cell receptors [11,12]. It has been shown that *in vitro* IgE production by B lymphocytes was stimulated only in the presence of V β 8⁺ T cells whereas V β 2⁺ T-cells lack the capacity to stimulate IgE production [10]. Furthermore, SJL/J mice which do not express the V β 8⁺ T-cell receptor were not able to produce IgE [10,11]. In addition, passive transfer of activated V β 8⁺ T cells to naive recipient mice induced antigen-specific IgE production. These data suggest an important role for V β 8⁺ T cells in IgE synthesis. However, the precise role of V β 8⁺ T cells in the sensitization and effector phase of allergic airway inflammation and development of *in vivo* airway hyper-responsiveness to bronchoconstrictive mediators remains unclear.

In the present study we wanted to investigate the role of V β 8⁺ T lymphocytes in a murine model of allergic asthma by examining *in vivo* airway responsiveness to methacholine, presence of inflammatory cells in bronchoalveolar lavage fluid and serum levels of ovalbumin-specific IgE. BALB/c mice were *in vivo* depleted from V β 8⁺ T cells by a monoclonal antibody directed to the V β 8⁺ T-cell receptor prior to the sensitization and prior to the challenge period [13]. In addition SJL/J and SJA/9 mice, which have a genetic deletion involving the T cell receptor V β 8 gene segment, were used.

Materials and methods

Animals

Specific pathogen free male BALB/c mice (6–8 weeks) were obtained from the breeding colony of the Central Animal Laboratory, Utrecht, the Netherlands, specific pathogen free male SJL/J mice (6–8 weeks) were obtained from the breeding colony of the Erasmus University, Rotterdam, the Netherlands and IFFA Credo, L'Arbresle, France, and specific pathogen free male SJA/9 mice (6–8 weeks) were obtained from the breeding colony of Erasmus University, Rotterdam, the Netherlands. The mice were housed in macrolon cages and provided with food and water *ad libitum*.

Immunization protocol

Active sensitization was performed [7] by giving seven intraperitoneal injections of 10 μ g ovalbumin (grade V) in 0.5 mL pyrogen free saline on alternate days (1 injection per day). This sensitization procedure has been shown to induce high levels of total IgE antibodies in the serum of BALB/c mice, of which 80% is ovalbumin-specific IgE [7]. Three weeks after the last injection, the mice were exposed to eight ovalbumin (2 mg/mL) or eight saline aerosols for 5 min on consecutive days (one aerosol per day). The aerosol was generated with an ultrasonic nebulizer (Devilbiss, Ultra neb 2000, particle size 3–5 μ m) connected to a plexiglas exposure chamber (5 L). In an additional experiment mice were treated with control or anti-V β 8 antibodies before the sensitization period, the aerosol was generated with a jet nebulizer (pari IS-2 Jet nebulizer, PARI Respiratory Equipment, Richmond, VA, USA, particle size 2–3 μ m) driven by compressed air at a flow rate of 6 L/min. In pilot experiments it was found that all parameters were similar, only the eosinophilic infiltration is more extensive using the latter nebulizers. Aerosol was given in groups of maximal six animals.

From each mouse *in vivo* airway responsiveness, V β 8⁺ T cells present in spleen, ovalbumin-specific IgE in serum and cellular infiltration in the bronchoalveolar lavage were determined 24 h after the last challenge. All experimental groups consisted of at least six mice.

Treatment with anti-V β 8⁺ T-cell monoclonal antibodies

Two different treatment protocols were used. One day before the sensitization procedure started, naive BALB/c mice were injected intraperitoneally with 0.4 mg (total injection volume 0.4 mL) anti-V β 8⁺ (clone F23.1 rat anti-mouse V β 8.1⁺, V β 8.2⁺ T-cell receptor IgG2a, kindly donated by M. Sitkovsky, NIAID, NIH, Bethesda, USA)

or control antibody (rat IgG, Sigma, St. Louis, MO, USA). This was followed by the sensitization and challenge period as described above.

In a different set of experiments, mice were sensitized with ovalbumin and one day before the first ovalbumin or saline inhalation, they were intraperitoneally injected with 0.3 mg (total injection volume 0.3 mL) anti-V β 8⁺ or control antibody (rat IgG, Sigma, St. Louis, MO, USA). The dose given is known to cause depletion of V β 8⁺ T cells for 4–8 weeks [14]. In addition, the specificity of the monoclonal antibody is restricted to the V β 8.1⁺ and V β 8.2⁺ T cell receptors [13,14].

Measurement of V β 8⁺ T cells in spleen

At the end of the experiment spleens were removed and put in cold PBS after which a single cell suspension was prepared with a 70 μ m cell strainer (Falcon, Lelystad, The Netherlands). Erythrocytes were lysed by 1 min treatment of the pellet fraction with ice-cold isotonic ammonium chloride solution (155 mm NH₄Cl, 10 mm KHCO₃, 0.1 mm EDTA, pH 7.4). The cells (5×10^5 in 25 μ L) were preincubated with PBS containing 5% mouse serum (4 °C, 15 min). Thereafter cells were incubated with anti-V β 8⁺-PE (clone MR5–2, PharMingen, San Diego, CA, USA) in PBS containing 5% mouse serum on ice for 30 min. After washing, the cells were analysed by flow cytometry under identical setting for all samples (FACScan, Becton-Dickinson). At least 10 000 cells were measured.

Murine ovalbumin-specific IgE ELISA

After measuring the airway responsiveness blood samples were obtained from the mice via a cardiac puncture and rotated for 10 min at 350 g. Serum was collected and samples were kept at –20 °C till measured. Serum levels of ovalbumin-specific IgE were determined as described previously [8,15]. In short, microplates were coated with rHuFc ϵ RI-IgG (2 μ g/mL, kindly donated by P.M. Jardieu, Genentech, South San Francisco, CA, USA) at 4 °C for 24 h. The ELISA was performed at room temperature. The plates were blocked with ELISA buffer (containing 50 mm Tris, 2 mm EDTA, 136.9 mmol/L NaCl, 0.05% Tween 20 and 0.5% BSA, pH 7.2) and left to incubate for 1 h. After removal of the ELISA buffer, plates were incubated for 2 h with appropriately prediluted serum samples and serial twofold dilutions (starting 1:10) of an ovalbumin-specific IgE reference standard in duplicate. The standard was obtained by intraperitoneal immunization of mice with ovalbumin according to previously published methods [16] and arbitrarily assigned a value of 1000 experimental units/mL (EU/mL) ovalbumin-specific IgE. Followed by 1 h incubation with 10 μ g/mL ovalbumin in ELISA buffer.

After incubation, goat antiovalbumin horseradish peroxidase conjugated (1: 5000, Jackson, West Grove, PA, USA) was added for 1 h followed by application of the substrate 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) (0.5 mg/mL) to develop colouring. Ten minutes after addition of the substrate the optical density was measured at 405 nm using a Titertek Multiskan (Flow Labs, Irvine, UK).

Antibody titres of samples were calculated by comparison with the ovalbumin-reference standard and values were expressed in EU/mL.

Airway responsiveness *in vivo*

Airway responsiveness was measured *in vivo* 24 h after the last aerosol exposure using an air-overflow pressure method [6,8,17]. With this method the bronchial resistance to inflation is measured. Mice were anaesthetized by intraperitoneal injection of urethane (2 g/kg), and placed on a heated blanket (30 °C). The trachea was cannulated and a small polyethylene catheter was placed in the jugular vein for intravenous administrations. The spontaneous breathing was suppressed by intravenous injection of tubocurarine chloride (3.3 mg/kg). When it stopped, the tracheal cannula was attached to a respiration pump (Sanders Brinie, Enschede, The Netherlands). The inflation volume of the pump was 0.8 mL per beat with 190 beats per min of which the mice inhale $\approx$ 0.1 mL. PO₂ and pCO₂ levels of animals ventilated in this way stay in the physiological range. A pressure transducer (Validyne, Northridge, CA, USA) was located between the trachea and the respiration pump in order to measure changes in the bronchial resistance to inflation. Pressure signal was recorded breath-by-breath on a Gould Brush 2400 recorder (Godart, Utrecht, The Netherlands). At time intervals of 4 min and after the response had returned to baseline level, doubling doses of methacholine ranging from 20 μ g/kg to 640 μ g/kg were administered. Concentrations of methacholine were prepared in saline and kept on ice for the duration of the experiment. At the end of the dose–response curve, the maximal response was determined by clamping the tracheal cannula. The increase in air-overflow pressure was measured at its peak and expressed as percentage increase of the maximal response.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed in the same animals that were used for airway responsiveness measurements. In pilot experiments it was found that combining the two techniques had no effect on total number of cells derived from lavage nor on the appearance of different cell types. Directly after the dose–response curve with

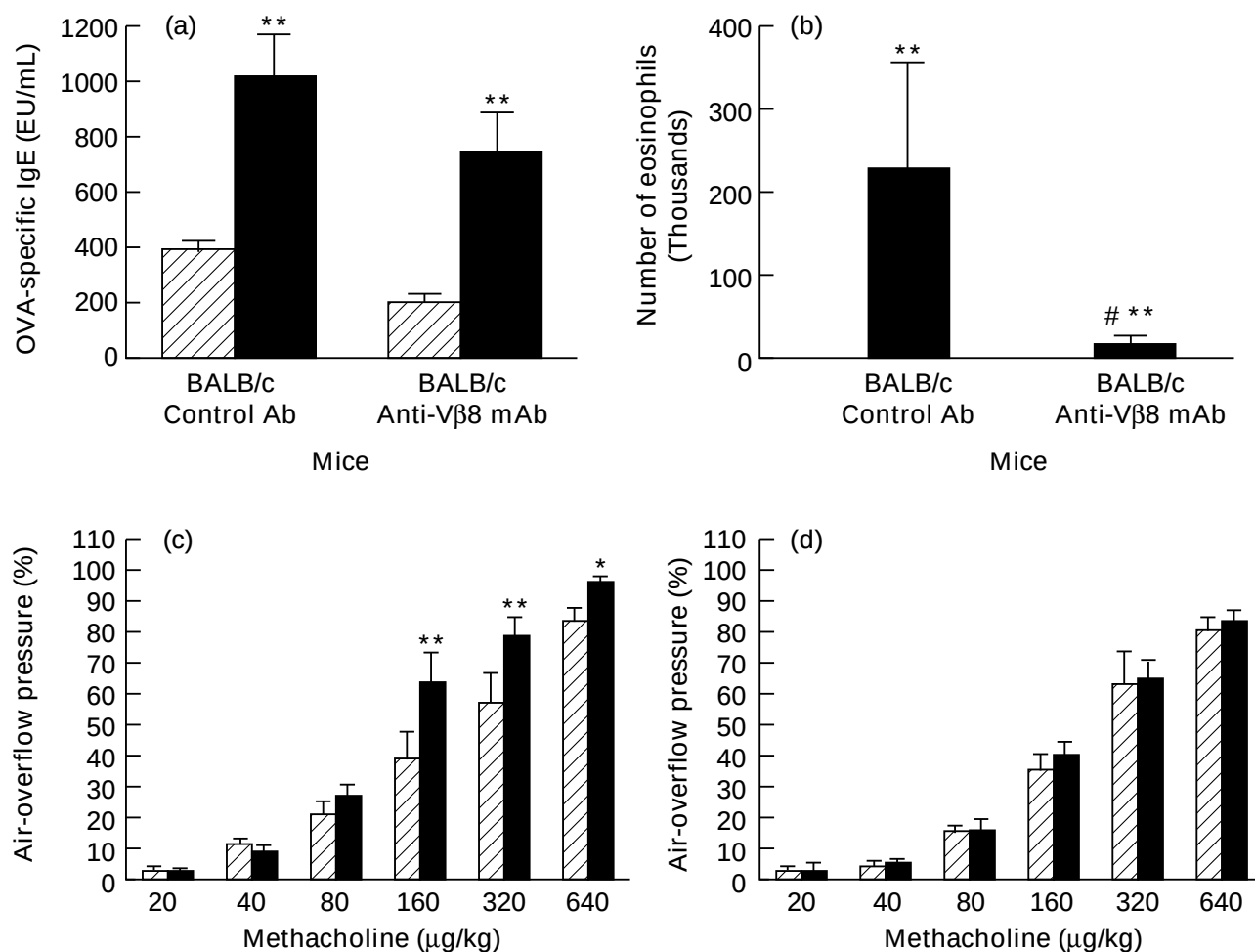


Fig. 1. *In vivo* parameters of saline (hatched bars) and ovalbumin (black bars) challenged BALB/c mice treated prior to the ovalbumin sensitization with control Ab or anti-Vβ8 mAb. (A) Ovalbumin-specific IgE serum levels, (B) numbers of eosinophils recovered in the bronchoalveolar lavage and (C and D) airway hyperresponsiveness *in vivo* to methacholine (μg/kg) in (C) control Ab and (D) anti-Vβ8 MoAb treated mice. Values are expressed as mean ± SEM (six mice per group). * $P < 0.05$; ** $P < 0.01$ as compared with saline challenged mice of the same treatment. # $P < 0.05$ as compared to control Ab treated mice with the same challenge.

methacholine was finished the animals were lavaged five times through the tracheal cannula with 1 mL aliquots of pyrogen free saline warmed to 37°C. The BAL cells were washed with cold PBS (400 g, 4°C, 5 min) and the pellet was resuspended in 150 μL cold PBS. Using a Bürker–Türk chamber total numbers of BAL cells were counted. For differential BAL cell counts cytospin preparations were made and stained with Diff-Quick (Merz & Dade AG, Düringen, Switzerland). After coding, all cytospin preparations were evaluated by one observer using oil immersion microscopy. Cells were identified and differentiated into mononuclear cells, neutrophils and eosinophils by standard morphology. Per cytospin preparation at least 200 cells were counted and the absolute number of each cell type was calculated. To evaluate differences between ovalbumin-challenged mice and saline-challenged controls for the

different treatments, total BAL cell number and the numbers of the various BAL cell types were tested with an analysis of variance. For cell types with a very low number in control animals (i.e. neutrophils and eosinophils) a Poisson distribution was assumed.

Histology

In a separate experiment mice were sensitized with ovalbumin and treated with control Ab or anti-Vβ8 MoAb prior to the challenge period as described above. Lungs were lavaged and slowly inflated with 4% paraformaldehyde/PBS solution. The lungs were removed from the body and kept in paraformaldehyde till embedding. Embedding was performed as described previously [18]. The sections were counter stained with haematoxylin and eosin.

Chemicals

Ovalbumin (chicken egg albumin crude grade V), 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid), tubocurarine chloride, cell cultured tested BSA (fraction V) were purchased from Sigma Chemical Company (St. Louis, MO, USA), PBS from Gibco Life Technologies (Merelbeke, Belgium), mouse serum from Cedar Lane (Ontario, Canada), saline from B. Braun Medical B.V. (Oss, The Netherlands) and urethane and methacholine (acetyl- β -methylcholine) from Janssen Chimica (Beerse, Belgium).

Data analysis

Unless stated otherwise data are expressed as mean $\pm$ SEM and evaluated using an analysis of variance followed by a *post hoc* comparison between groups. A probability value of 0.05 was considered statistically significant. Statistical analyses were carried out using Systat, version 5.03 or GLIM, version 4.0 (NaG Inc., Oxford, UK).

Results

$V\beta 8^+$ T cells present in spleen

To compare the levels of $V\beta 8^+$ T cells in different mouse strains flow cytometric analysis was performed on the spleen. The spleens of control Ab treated BALB/c mice contained $5.5 \pm 0.5\%$ $V\beta 8^+$ T cells which is $21.8 \pm 1.8\%$ of the total T-cell population. $V\beta 8^+$ T cells were removed from BALB/c mice by treatment *in vivo* with the anti- $V\beta 8$ MoAb F23.1 (anti- $V\beta 8^+$ treatment), one day before the sensitization or challenge period. At time of section, respectively, $77.2 \pm 1.9\%$ and $95.8 \pm 0.9\%$ of the $V\beta 8^+$ T cells were depleted.

In SJL/J mice no $V\beta 8^+$ T cells were found in the spleen. While both SJA/9 and SJL/J mice genetically lack $V\beta 8^+$ T cells, only $V\beta 8^+$ T cells presence in spleen of SJL/J mice was examined. The numbers of $V\beta 8^+$ T cells in SJL/J, control and anti- $V\beta 8^+$ treated BALB/c mice were not different between ovalbumin and saline challenged mice (data not shown).

Levels of ovalbumin-specific IgE

At the end of the experiment serum was obtained in order to measure levels of ovalbumin-specific IgE. In control Ab treated BALB/c mice the level of ovalbumin-specific IgE was significantly ($P < 0.05$) potentiated after ovalbumin challenge compared with saline challenge (Figs 1a and 2).

Treatment with anti- $V\beta 8^+$ MoAb prior to the sensitization or prior to the challenge period in ovalbumin challenged BALB/c mice showed a tendency (respectively

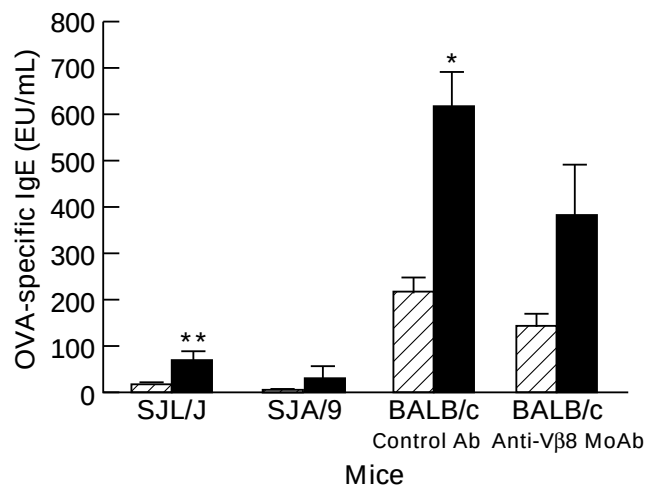


Fig. 2. Ovalbumin-specific Immunoglobulin E (IgE) levels in serum of SJL/J, SJA/9 and BALB/c mice treated with control Ab or anti- $V\beta 8$ MoAb prior to the challenge period. Serum was taken from ovalbumin sensitized mice after repeated ovalbumin (black bars) with saline (hatched bars) challenge. Values are expressed as mean $\pm$ SEM (at least six mice per group). * $P < 0.05$; ** $P < 0.01$ as compared with saline challenged mice of the same strain or treatment.

$P = 0.07$ and $P = 0.12$) to decrease the levels of ovalbumin-specific IgE in serum compared to control Ab treated ovalbumin challenged BALB/c mice. In mice treated with antibodies to $V\beta 8^+$ prior to the sensitization period (Fig. 1a), ovalbumin challenge significantly increased ($P < 0.01$) the levels of ovalbumin-specific IgE in serum, but treatment with antibodies to $V\beta 8^+$ prior to the challenge period did not show an increase ($P < 0.06$) of ovalbumin-specific IgE levels upon ovalbumin challenge compared with saline challenged mice (Fig. 2). The levels of ovalbumin-specific IgE in anti- $V\beta 8^+$ saline challenged BALB/c mice were not significantly different from control Ab treated saline challenged BALB/c mice.

In SJL/J mice repeated ovalbumin challenge resulted in a significant increase ($P < 0.05$) of ovalbumin-specific IgE (63 ± 14 EU/mL) compared with the saline challenged (8 ± 4 EU/mL) mice (Fig. 2).

In SJA/9 mice low levels of ovalbumin-specific IgE were present after saline challenge (3 ± 2 EU/mL). Unlike BALB/c and SJL/J mice, ovalbumin challenge in SJA/9 mice caused no significant increase of ovalbumin-specific IgE (28 ± 24 EU/mL) compared with saline challenged control mice (Fig. 1b).

Airway responsiveness *in vivo*

Airway responsiveness *in vivo* to methacholine was measured 24 h after the last aerosol challenge. For all mice a

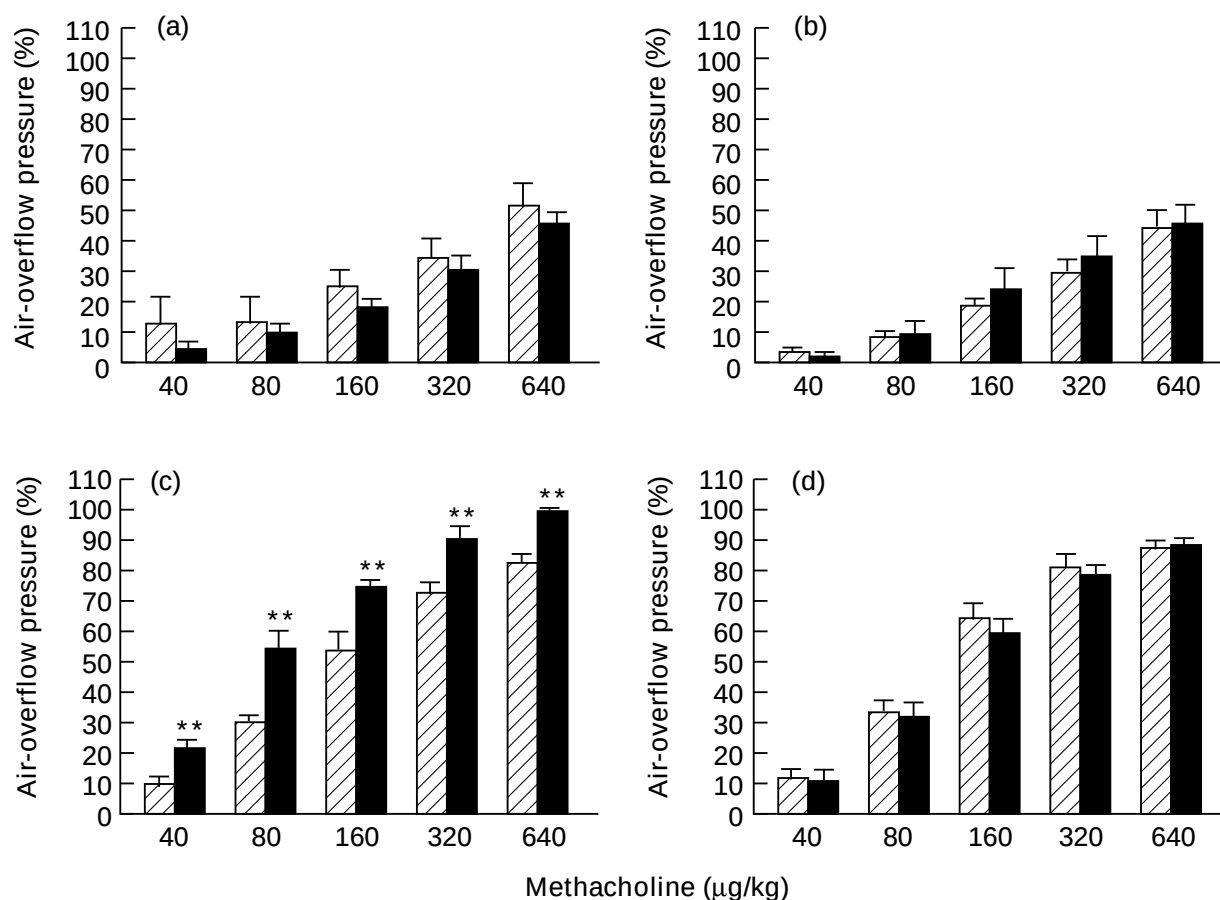


Fig. 3. Airway responsiveness *in vivo* to methacholine ($\mu\text{g/kg}$) in (A) SJA/9, (B) SJL/J and BALB/c mice treated with (C) control Ab or (D) anti-V $\beta 8$ MoAb prior to the challenge period. Expressed is the percentage air-overflow pressure of ovalbumin (black bars) or saline (hatched bars) challenged mice as measured 24 h after the last challenge. Values are expressed as mean $\pm$ SEM (at least six mice per group). * $P < 0.05$; ** $P < 0.01$ as compared to the saline challenged mice of the same treatment group.

complete dose–response curve of methacholine (ranging from 40 $\mu\text{g/kg}$ to 640 $\mu\text{g/kg}$) was made. In ovalbumin challenged control Ab treated BALB/c mice a significant increase in airway responsiveness ($P < 0.05$) to methacholine was observed at doses ranging from 160 $\mu\text{g/kg}$ to 640 $\mu\text{g/kg}$ (Fig. 1C) or 40 $\mu\text{g/kg}$ to 640 $\mu\text{g/kg}$ (Fig. 3C) compared with their saline challenged controls. No difference was observed between saline and ovalbumin-challenged BALB/c mice treated with anti-V $\beta 8^+$ prior to the sensitization or prior to the challenge period in the airway responsiveness at all doses of methacholine used (Figs 1D and 3D). Treatments with anti-V $\beta 8^+$ resulted in completely abolishment of the airway hyperresponsiveness to methacholine in ovalbumin challenged BALB/c mice (Figs 1D and 3D). In addition, no significant differences were observed in the methacholine dose–response curves between the different groups of saline challenged BALB/c mice.

No difference in airway responsiveness *in vivo* at all doses of methacholine (40 $\mu\text{g/kg}$ to 640 $\mu\text{g/kg}$) was observed between ovalbumin and saline challenged SJL/J or SJA/9 mice (Fig. 3A,B).

Bronchoalveolar lavage

Bronchoalveolar lavage was performed 24 h after the last challenge. In control Ab and anti-V $\beta 8^+$ treated BALB/c, SJL/J and SJA/9 mice, no significant differences were found between ovalbumin and saline challenged mice, neither in the total numbers of cells recovered nor in the numbers of mononuclear cells and neutrophils (Table 1).

No eosinophils were observed in the bronchoalveolar lavage of all groups of saline challenged mice. In control Ab treated BALB/c mice ovalbumin challenge caused a significant increase ($P < 0.01$) in the number of infiltrating eosinophils in the bronchoalveolar lavage compared with

Table 1. Number of mononuclear cells (MNC) and neutrophils and total number of cells recovered in the bronchoalveolar lavage (BAL) of different mouse strains (SJA/9, SJL/J and BALB/c mice treated with control Ab or anti-V β 8 prior to the challenge period) sensitized with ovalbumin and repeatedly challenged with ovalbumin (OVA) or saline (SAL).

Mouse strain	Challenge	MNC ($\times 10^5$)	Neutrophils ($\times 10^4$)	Total BAL cells ($\times 10^5$)
SJL/J	SAL	3.44 $\pm$ 0.70	1.21 $\pm$ 0.47	3.56 $\pm$ 0.74
	OVA	5.00 $\pm$ 0.89	1.55 $\pm$ 0.71	5.16 $\pm$ 0.95
SJA/9	SAL	1.78 $\pm$ 0.21	0.12 $\pm$ 0.07	1.80 $\pm$ 0.21
	OVA	1.89 $\pm$ 0.31	0.11 $\pm$ 0.07	1.91 $\pm$ 0.32
BALB/c control				
Ab	SAL	2.69 $\pm$ 0.41	0.05 $\pm$ 0.03	2.70 $\pm$ 0.41
	OVA	3.14 $\pm$ 0.38	0.04 $\pm$ 0.04	3.40 $\pm$ 0.35
BALB/c anti-V β 8 ⁺				
V β 8 ⁺	SAL	3.03 $\pm$ 0.36	0.18 $\pm$ 0.09	3.05 $\pm$ 0.36
	OVA	2.95 $\pm$ 0.51	0.07 $\pm$ 0.04	3.02 $\pm$ 0.53

their saline challenged mice (Figs 1B and 4). This eosinophilic infiltration was significantly decreased (both $P < 0.05$) by anti-V β 8⁺ treatment both prior to sensitization (Fig. 1B) and prior to the challenge period (Fig. 4). In ovalbumin challenged SJL/J mice a low number of infiltrated eosinophils in the bronchoalveolar lavage were observed, due to a few eosinophils in two out of nine mice. In both saline and ovalbumin challenged SJA/9 mice no eosinophilic infiltration in the bronchoalveolar lavage was observed.

Airway histology

The histology of the lungs was analysed to determine inflammatory cell infiltration. In all saline challenged mice no cellular infiltration was observed (Fig. 5A,C). In the control treated ovalbumin challenged mice a considerable number of inflammatory cells was observed in lung tissue (Fig. 5B). The cellular inflammation was mainly patchy and concentrated around the bronchioli. In mice treatment with anti-V β 8⁺ MoAb prior to the challenge period inflammatory cell infiltrate was almost completely absent in the lung tissue (Fig. 5D). So, in addition to decreased eosinophilic infiltration in the bronchoalveolar lavage, treatment with anti-V β 8⁺ MoAb also inhibited cellular inflammation in lung tissue of ovalbumin challenged mice.

Conclusions and discussion

In the present study it is demonstrated that V β 8⁺ T cells are essential for the infiltration of eosinophils in the respiratory

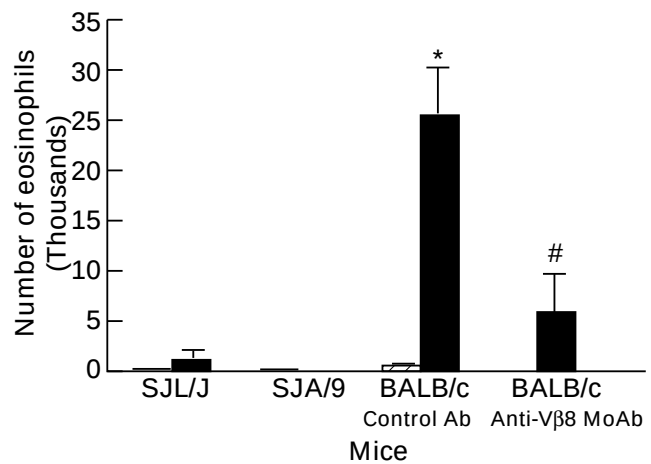


Fig. 4. Number of eosinophils recovered in the bronchoalveolar lavage of SJL/J, SJA/9 and BALB/c mice treated with control Ab or anti-V β 8 MoAb after ovalbumin (black bars) or saline (hatched bars) challenge. Values are expressed as mean $\pm$ SEM (at least six mice per group). * $P < 0.05$ as compared with saline challenged BALB/c mice. # $P < 0.05$ as compared with control Ab treated ovalbumin challenged BALB/c mice.

airways and the development of airway hyperresponsiveness in a murine model of allergic asthma. In contrast, although V β 8⁺ T cells seem to be important for the extent of IgE levels, no essential role for V β 8⁺ T cells in the induction of antigen-specific IgE was observed.

It has been shown in BALB/c mice that the peptide OVA323–339 represents a main epitope of ovalbumin for B and T lymphocytes [9]. OVA323–339 is efficiently recognized by cells expressing the V β 8⁺ T-cell receptor [19], and therefore the *in vivo* response to ovalbumin is thought to be predominantly mediated by V β 8⁺ T cells. Consequently, in this study we used BALB/c mice which express the V β 8⁺ T-cell receptor and SJL/J and SJA/9 mice which genetically lack the V β 8⁺ T cell receptor [20]. In addition, we depleted *in vivo* V β 8⁺ T cells from BALB/c mice with antibodies to V β 8⁺ prior to the sensitization period and prior to repeated exposure to ovalbumin or saline aerosols.

We observed increased serum levels of ovalbumin-specific IgE in ovalbumin challenged mice compared to saline challenged mice. These data show that repeated ovalbumin inhalations induce an IgE memory response. Memory B cells with the help of memory T cells play a role in the secondary IgE response [21] which is important for a more rapid and stronger antigen-specific response than upon the first antigen exposure. In SJA/9 mice no significant ovalbumin-specific IgE in serum was detected, which is in line with studies describing that SJA/9 mice are unable to produce detectable levels of IgE [22,23]. Despite the lack of

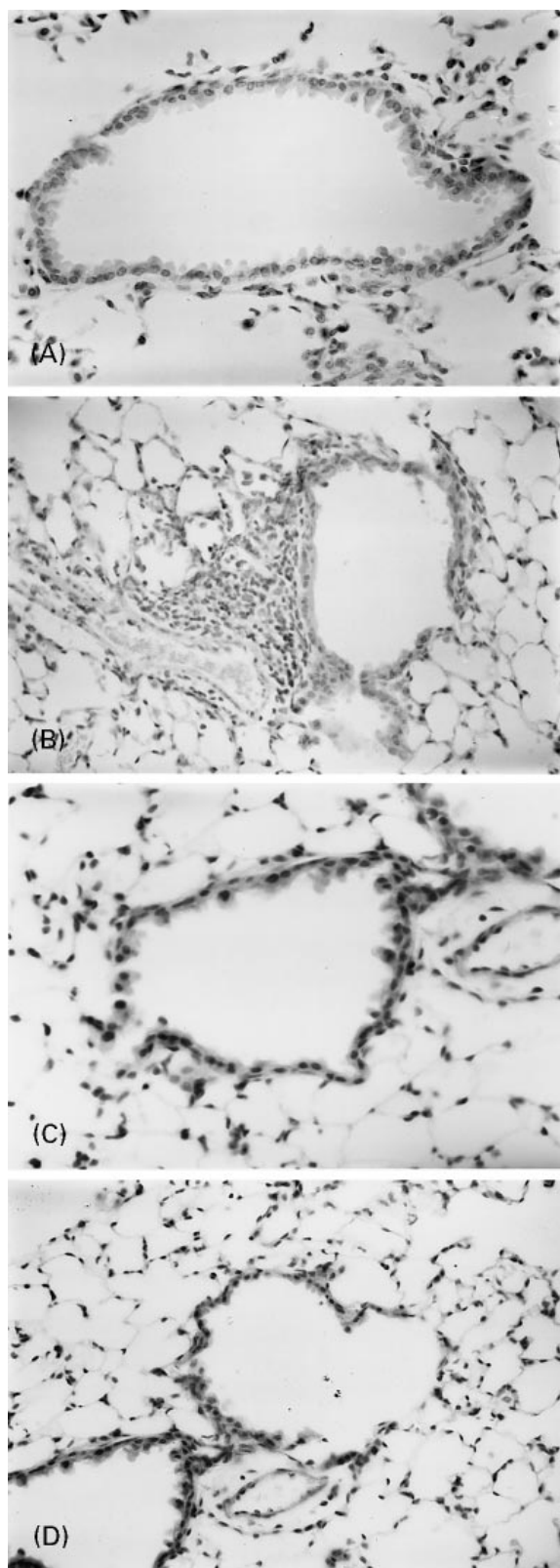


Fig. 5. Lung sections of (A) saline and (B) ovalbumin challenged mice treated with control Ab, and of (C) saline and (D) ovalbumin challenged mice treated with anti-V β 8 MoAb prior to the challenge period.

V β 8⁺ T cells in SJL/J mice repeated ovalbumin aerosols increased the level of antigen-specific IgE in serum as compared with saline challenged mice, suggesting that V β 8⁺ T cells are not totally essential for the induction of ovalbumin-specific IgE. In addition, in BALB/c mice treated with anti-V β 8 MoAb prior to the sensitization, no altered serum levels of ovalbumin-specific IgE were observed in ovalbumin challenged mice compared with control mice. These data suggest a minor and not essential role for V β 8⁺ T cells in IgE production, and that additional mechanisms are involved in the IgE synthesis. In contrast, a role for V β 8⁺ T cells in IgE synthesis has been described by Renz *et al.* [11], who showed that transfer of activated V β 8⁺ T cells induced antigen-specific IgE production in naive recipients. In the present study, in sera of ovalbumin challenged SJL/J mice a lower level of ovalbumin-specific IgE was observed compared with ovalbumin challenged BALB/c mice. This could be explained by a deficient IL-4 production in SJL/J mice. It is known that IL-4 is obligatory for B cells to proliferate and to undergo isotype switching to produce IgE [4,24]. SJL/J mice exhibit a relatively low capacity to produce IL-4 *in vitro* compared with BALB/c mice [25]. In addition, *in vitro* IgE production in SJL/J mice can be restored by administration of exogenous IL-4 [26]. Therefore, besides a role for V β 8⁺ T cells, deficient IL-4 synthesis may explain the low levels of ovalbumin-specific IgE in SJL/J mice compared with BALB/c mice. Low IL-4 and IgE responses of SJL/J mice are thought to be associated with the absence of the CD4⁺ NK1.1⁺ T cell population [26]. CD4⁺ NK1.1⁺ T cells promptly produce IL-4 *in vivo* in response to different stimuli [27,28], however, these cells are not essential for IL-4 mediated IgE production [29,30]. In addition, CD4⁺ NK1.1⁺ T cells bear predominantly the V β 8⁺ T cell receptor [31] and V β 8⁺ T cells from sensitized mice have been shown *in vitro* to be able to produce IL-4 [9]. Therefore, it can be speculated that the absence of V β 8⁺ T cells in SJL/J mice is responsible for the low IL-4 synthesis and IgE production.

In the present study anti-V β 8⁺ treatment after sensitization of BALB/c mice attenuated the increase of ovalbumin-specific IgE in serum after ovalbumin challenge compared to control Ab treated mice. Thus, the role of V β 8⁺ T cells in the memory IgE response is also not prominent. Consequently, it can be suggested that besides V β 8⁺ T cells other memory T cells or mechanisms are involved in the upregulation of the IgE response. A possible explanation is that the secondary IgE response is not mainly regulated by memory T cells [32]. Taken together, it seems that V β 8⁺ T lymphocytes are able to stimulate B lymphocytes to produce antigen-specific IgE, however, other cells and/or mechanisms also appear to be involved.

In the present study *in vivo* airway hyperresponsiveness to methacholine, infiltration of eosinophils in the

bronchoalveolar lavage and cellular infiltration in airway tissue were observed in ovalbumin challenged BALB/c mice. Anti-V β 8⁺ treatment of BALB/c mice prior to the sensitization or challenge period resulted in absence of airway hyperresponsiveness and almost completely decreased eosinophilic infiltration in the bronchoalveolar lavage after ovalbumin challenge. In addition, in SJA/9 and SJL/J mice neither airway hyperresponsiveness nor eosinophilic infiltration in the bronchoalveolar lavage were observed. To our knowledge, we are the first to show that *in vivo* airway hyperresponsiveness and eosinophilic infiltration are dependent on the presence of V β 8⁺ T cells. Our data on *in vivo* airway hyperresponsiveness to exogenous methacholine are in agreement with results obtained by Renz *et al.* [11], who demonstrated that passive transfer of activated V β 8⁺ T cells from sensitized mice to naive recipients increased tracheal responsiveness as measured *in vitro* by electric field stimulation. However, cellular infiltration in the airways was not investigated in this study. The precise mechanism by which V β 8⁺ T cells are involved in eosinophilic infiltration and airway hyperresponsiveness is at present unclear. It is known that IL-5 promotes eosinophilopoiesis *in vivo*, prolongs eosinophil survival, modulates eosinophil function *in vitro* and induces eosinophil chemotaxis *in vitro* and *in vivo* [5]. In previous studies we have demonstrated that *in vivo* treatment with antibodies to IL-5 completely abolished the eosinophilic infiltration in the bronchoalveolar lavage [6]. Based on the observations in the present study it can be suggested that V β 8⁺ T cells are an important source of IL-5. The role of IL-5 in airway hyperresponsiveness remains elusive. Data as presented by us and others [6,33,34] using antibodies to IL-5, suggest that IL-5 does not play an important role for the onset of airway hyperresponsiveness in BALB/c mice. However, in other studies using IL-5 knock out mice neither eosinophilia nor airway hyperresponsiveness were observed [35]. Recently, we have demonstrated that *in vivo* treatment with antibodies to IFN γ completely abolished the airway hyperresponsiveness [6]. Since V β 8⁺ T cells appear to be involved in both eosinophilic infiltration in the lungs and airway hyperresponsiveness it can be speculated that V β 8⁺ T cells are an important source of IL-5 and IFN γ in our animal model. Recently, Garlisi *et al.* observed that T lymphocytes are responsible for most of the IFN γ and IL-5 mRNA expression in both bronchoalveolar lavage and lung cells [36].

The correlation between serum levels of antigen-specific IgE, airway hyperresponsiveness and eosinophilic infiltration in the airways remains elusive. In the present study serum levels of antigen-specific IgE are not directly correlated with airway hyperresponsiveness or eosinophilic infiltration in the lavage. This is in agreement with results obtained by Eum *et al.* who observed in BALB/c mice high

IgE levels in serum with eosinophilic infiltration in the bronchoalveolar lavage but without airway hyperresponsiveness [37]. In the present study low levels of antigen-specific IgE in serum without eosinophilic infiltration in the bronchoalveolar lavage were observed in SJL/J mice, whereas in BALB/c mice eosinophils in the bronchoalveolar lavage were accompanied with high levels of antigen-specific IgE in serum. Therefore, it can be suggested that high IgE levels are required for eosinophilic infiltration in the airways. In agreement herewith, Coyle *et al.* observed that treatment with antibodies to IgE prevented the recruitment of eosinophils into the lungs [38]. However, in IgE knock out mice eosinophilic infiltration in the lavage was observed [39]. Therefore, the precise mechanism of action of IgE in the development of airway hyperresponsiveness and eosinophilic inflammation remains to be elucidated.

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