

The role of CD23 on allergen-induced IgE levels, pulmonary eosinophilia and bronchial hyperresponsiveness in mice

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Summary

Background The role of Immunoglobulin (Ig)E in inflammation is the subject of considerable study and a number of studies have shown conflicting evidence for its role in eosinophil recruitment and bronchial hyperresponsiveness in a number of murine models. The low affinity IgE receptor, CD23, is known to act as a negative regulator of IgE production and we have used knockout mice deficient in CD23 to investigate the role of IgE in eosinophil recruitment and bronchial hyperresponsiveness in a murine model of airway inflammation. **Objective** To study the role of the low affinity FcεII receptor, CD23 in IgE production, lung inflammation and bronchial hyperresponsiveness.

Methods Wild-type and CD23 knockout C57Bl/6 mice (CD23^{-/-}) were immunized by intraperitoneal injection with ovalbumin on days 0 and 14 and challenged with aerosolized antigen on day 21 for a period of up to 1 week. Blood samples, bronchoalveolar lavage and lung tissue samples were obtained to determine serum IgE levels and inflammatory cell numbers, respectively. Furthermore, airway resistance was measured to increasing concentrations of aerosolized 5-hydroxytryptamine in order to evaluate the effect of CD23 deficiency on bronchial hyperresponsiveness to antigen challenge.

Results Sensitization of wild-type C57Bl/6 mice to ovalbumin resulted in elevated levels of total serum IgE and ovalbumin-specific IgE, which was significantly augmented in CD23 knockout C57Bl/6 mice (CD23^{-/-}). A significant increase in the percentage of eosinophils recovered in bronchoalveolar lavage fluid from wild-type and CD23^{-/-} mice was observed 24 h following 3 or 7 days aerosol exposure with ovalbumin (10 mg/mL). At 3 days, the increase in the percentage of eosinophils was significantly greater in CD23^{-/-} groups. Immunohistochemical analysis of lungs sections revealed the presence of CD3+, CD4+ and CD23+ cells in wild-type mice but a lack of immunofluorescence of CD23+ cells in CD23^{-/-} mice. In wild-type ovalbumin-immunized mice, bronchial hyperresponsiveness to aerosolized 5-hydroxytryptamine was observed following a 3-day antigen challenge, which was significantly greater in CD23^{-/-} ovalbumin-immunized mice.

Conclusion These studies demonstrate that CD23^{-/-} mice have increased capacity to produce IgE consistent with the view of a negative feedback role for membrane-bound CD23 and under such conditions, may account for the greater numbers of eosinophils recruited to the airways and bronchial hyperresponsiveness observed following acute but not chronic antigen challenge.

Keywords: asthma, bronchial hyperresponsiveness, CD23, eosinophils, IgE, knockout mice, murine

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Introduction

One of the hallmark features of atopic disease is the presence of Immunoglobulin (Ig) E, which has been suggested to play a pivotal role in the pathogenesis of the atopic state [1]. A number of studies have found a strong correlation between total serum IgE levels and bronchial hyper-responsiveness in asthma [2–5] and this trait appears to be specific for chromosome 5 [6] and chromosome 11q13 [7].

The role of IgE in allergic inflammation has been extensively studied in murine models of inflammation. Thus, antibodies directed against IgE can inhibit antigen-induced eosinophilia [8] and passive transfer of ovalbumin-specific IgE [9] or ovalbumin-specific B cells [10] to naive animals, resulted in increased airway smooth muscle sensitivity to electrical field stimulation and eosinophilia. In contrast, mice who are B cell deficient [11,12] or mice with a disrupted gene encoding the heavy chain of IgE [13] are still capable of developing bronchial hyperresponsiveness to muscarinic agonists and eosinophilia following antigen challenge of immunized animals. Similarly, mice lacking CD40 or IL-4 also retain the capacity to recruit eosinophils to the airways and develop bronchial hyperresponsiveness following antigen challenge [14]. Together these studies support the notion that IgE-dependent and -independent mechanisms are responsible for the recruitment of eosinophils and/or bronchial hyperresponsiveness in mice.

Recently, CD23 has received considerable attention with regard to its role in the regulation of IgE synthesis [1]. It has been demonstrated that mice deficient in CD23 produce higher levels of IgE than their wild-type counterparts [15] without any effect on B or T cell growth and differentiation [15–17]. Conversely, mice overexpressing membrane but not soluble CD23 exhibit a weaker IgE response [18]. In contrast, CD23 knockout mice were unable to demonstrate antigen-specific IgE-mediated antibody responses [16]; anti-CD23-inhibited IgE production in rats [19], although the effect on recruitment of eosinophils following antigen challenge in immunized mice is controversial [8,20]. We have investigated the role of CD23 on IgE production and eosinophilia following antigen challenge of immunized wild-type and CD23 knockout mice.

Materials and Methods

Mice

C57Bl/6 wild-type (local supplier) and CD23^{-/-} 6–8-week-old mice (breeding pairs obtained from Dr MC Lamers, Max-Planck-Institut für Immunbiologie, Freiburg, Germany) were immunized with ovalbumin (Sigma Chemical Co, Dorset, UK; 100 µg i.p.; type V) absorbed to a saturated solution of aluminium hydroxide [Al(OH)₃], 0.1 M on day 0

and 14. On day 21 mice were placed in a plexiglass container (12 L) and exposed to a nebulized solution of ovalbumin (10 mg/mL; De Vilbiss Ultraneb 90, De Vilbiss Healthcare Ltd, Heston, UK). Sham-immunized wild-type and CD23-deficient mice received Al(OH)₃ only (200 µL, 0.1 M) whereas in other studies, mice were injected on day 0 and 14 with sterile saline (0.9%). All mice were exposed three times daily for 20 min at hourly intervals during 3 consecutive days or every second day over a 7-day period and bronchoalveolar lavage performed 24 h after the last aerosol challenge with either saline (saline and sham-immunized mice) or ovalbumin (immunized mice).

Measurement of serum IgE

In some experiments, 100 µL of blood was obtained via the retro-orbital artery on day 0, 7, 14, 21 and 28 in sham and immunized wild-type and CD23 knockout mice. Each mouse provided only two blood samples and the procedure was approved by the Home Office (UK). Serum was collected and stored at –20 °C for later analysis of both total and specific IgE using an enzyme-linked immunosorbent assay (ELISA) protocol.

Total IgE Briefly, primary antibody LO-ME 3 (3 µg/mL; Serotec Inc, Oxford, UK) was diluted in 0.1 M carbonate/bicarbonate solution (pH 9.6) and placed overnight at 4 °C in 96-well plates (50 µL/well) then washed five times BPS-Tween. Samples and standards (purified mouse IgE kappa; Pharmingen, Oxford, UK) were diluted in ELISA buffer, incubated at room temperature for 2 h and then washed. Secondary antibody (OX-20 biotin; Serotec) was added to the wells and the plates incubated for a further 90 min. Extravidin alkaline phosphatase (Sigma Chemical Co, Dorset, UK) diluted in ELISA buffer (1.5 µg/mL) was added to the wells and incubated for 90 min before addition of p-nitrophenyl phosphate (Sigma Chemical Co., Dorset, UK) diluted in diethanolamine buffer (1 mg/mL) and absorbance was read at 405 nm.

Specific IgE Chicken ovalbumin (Grade V; Sigma Chemical Co, UK) was diluted in 0.1 M carbonate/bicarbonate solution (pH 9.6) and washed five times. Samples and standards (ovalbumin-specific IgG titre serum) were diluted in ELISA buffer and incubated at room temperature for 2 h and then washed. Secondary antibody antimouse IgE alkaline phosphatase (Sigma Chemical Co., Dorset, UK) diluted in ELISA buffer was added to the wells and the plates were incubated for a further 90 min. Extravidin alkaline phosphatase (Sigma Chemical Co., Dorset, UK) diluted in ELISA buffer (1.5 µg/mL) was added to the wells and incubated for 90 min before addition of p-nitrophenyl phosphate (Sigma Chemical Co, Dorset, UK), which was

diluted in diethanolamine buffer (1 mg/mL) and absorbance was read at 405 nm.

Bronchoalveolar lavage

Twenty-four hours after the last aerosolized challenge with either saline or ovalbumin, mice were anaesthetized with urethane (2 g/kg i.p.; Sigma Chemical Co, UK) and a cannula was inserted into the exposed trachea of anaesthetized mice where three 0.5 mL aliquots of warm sterile saline were injected into the lung and bronchoalveolar lavage fluid was collected. An aliquot (90 μ L) of haemolysis solution (0.1% methylene blue in 1% acetic acid) was added to 10 μ L of bronchoalveolar lavage fluid and the total number of cells enumerated with an improved Neubauer haemocytometer. For differential cell counts, cytopspin preparations were prepared from aliquots of BAL fluid (100 μ L) centrifuged at 300g for 1 min using a Shandon Cytospin 2 (Shandon Southern Instruments, Sewickley, PA, USA) at room temperature. Cells were then stained with a combination of haematoxylin and chromotrope 2R for 20 min. A total of 200 cells were counted to determine the proportion of neutrophils, eosinophils and monocytes using standard morphological criteria.

Tissue preparations

In other experiments, whole lungs from all groups were perfused with 10% formal saline (Sigma Chemical Co, UK) then embedded in paraffin and cut into 6 μ m sections, which were then stained with haematoxylin and eosin and specific eosinophil staining using histochemical methods (Lendrum). To determine eosinophil numbers, cells were counted around airways of approximately 1000 μ m in circumference and 50 μ m deep into the submucosa from the basal epithelium from two sections obtained from the same lung. This was performed with the aid of an eyepiece graticule at a magnification of $\times 400$.

In other cases, whole lung from all groups were inflated with 20% OCT-embedding medium (Lamb, London, UK) and snap-frozen in liquid nitrogen cooled isopentane then stored at -70°C for immunohistochemical studies. Cryostat sections (10 μ m) were cut from the frozen samples onto clean poly L-lysine-coated slides and air-dried in preparation for immunohistochemical staining. The method employed was an indirect method using diaminobenzidine (DAB) as a chromagen. Briefly, sections were fixed in ice-cold acetone for 10 min and allowed to air dry. Endogenous peroxidase activity was then quenched by immersion of slides for 1 h in 1% (v/v) water in PBS containing 0.02% (w/v) sodium azide. The slides were subsequently washed in PBS followed by incubation with 5% normal rabbit serum (Dako Ltd, Cambridgeshire, UK) for 20 min to prevent

nonspecific binding. Endogenous avidin and biotin were blocked by the application of a blocking kit (Vector, Burlingame, CA, USA). Slides were then washed in PBS and primary antibody was applied at a dilution of 1:25 for 1 h at room temperature; the antibodies used were rat antimouse CD4 (Caltag, Buckingham UK), and rat antimouse CD8 and CD23 (Serotec, UK). After washing the sections were incubated with biotinylated rabbit antirat IgG (1:200, Dako, UK) for 45 min then washed in PBS. Subsequently, slides were incubated with avidin horse radish peroxidase (1:500, Dako) for 45 min. The sections were again washed and the antigen visualized using DAB tablets (Sigma, UK) and the reaction terminated by washing with distilled water. The sections were counterstained in haematoxylin, dehydrated and mounted for microscopic examination. Immunopositive cells staining for CD4, CD8 and CD23 were enumerated as described earlier for eosinophils from two sections obtained from the same lung.

Preparation of mice for assessment of airways responsiveness

Sham- and ovalbumin-immunized mice (C57Bl/6 wild-type and CD23^{-/-}, 20–30 g) were anaesthetized with urethane (2 g/kg) 24 h following the last aerosol challenge on day 3 and day 7. Mice were laid supine and the tail vein was cannulated for the intravenous administration of drugs. A tracheostomy was performed and a cannula (white leuc) inserted into the trachea and attached to a Fleish pneumotachograph (size 00000). Flow was measured with a differential pressure transducer (± 2 cm H₂O, model MP 45–14–871, Validyne Engineering Corp, Northridge, CA, USA). Pleural pressure was estimated by placing a water-filled catheter in the lower third of the oesophagus. An index of transpulmonary pressure, the difference between mouth and pleural pressure, was recorded by a water/air differential pressure transducer (± 20 cm H₂O; Infodisp, Hants, UK) connected between the oesophageal cannula and the side of the pneumotachograph proximal to the animal. The position of the cannula was adjusted in order to maximize the recorded transpulmonary pressure, where it remained throughout the experiment. The flow was integrated to give a continuous recording of tidal volume. Breath by breath recording of total lung resistance (RL, cm H₂O/L/sec) was calculated by an online respiratory analyser and recorded on a personal computer (LFR version 3; Mumed Ltd, London, UK).

Mice instrumented in this fashion were neuromuscular-blocked with pancuronium bromide (0.2 mg/kg, Sigma UK) and mechanically ventilated at 150 breaths per minute with a tidal volume of 0.2 mL (SAR-830 A, Model 963217, CWE Inc, PA, USA).

Measurement of airways responsiveness to 5-hydroxytryptamine

Aerosols of saline or 5-hydroxytryptamine (5-HT, Sigma, UK) was generated from a DeVilbiss ultrasonic nebuliser (DeVilbiss Healthcare Ltd, Heston, UK). Aerosols were administered directly to the lungs via the endotracheal tube. The nebuliser was connected to a side-arm of the endotracheal tube which remained closed for measurements of changes in airways resistance following administration of aerosol. Aerosols of 5-HT were delivered via a second ventilator (tidal volume 1.2 mL, 15 s duration, 60 breaths per minute).

After measurement of baseline lung function, mice were exposed to an aerosol of sterile 0.9% physiological saline for 15 s, followed by a recording of respiratory parameters. Airways responsiveness was determined by the administration of increasing doubling concentrations of 5-HT (4, 8, & 16 mg/mL) for 15 s at each concentration with measurement of respiratory parameters made immediately after each concentration.

Statistical analysis

All results expressed as mean $\pm$ SEM. Analysis of variance (total cell counts) or Kruskal–Wallis test (% differentials) was used to determine differences between groups and analysis of covariance used to determine differences in concentration-effect curves to 5-HT. Comparisons between mean values were made using Student's nonpaired *t*-test with Bonferroni correction or Dunn's multiple comparison test (GraphPad Prism version 2.1 and Minitab version 12) and considered significant if $P < 0.05$.

Results

Serum IgE levels

CD23^{-/-} mice demonstrated significantly greater levels of serum total IgE (ng/mL) than their wild-type counterparts prior to immunization (CD23^{-/-}; 1247 ± 67 $n = 17$ vs wild-type; 5703 ± 1341 , $n = 21$, $P < 0.05$). Prior to immunization no ovalbumin-specific IgE was detected in the serum of CD23^{-/-} and wild-type mice (< 100 U/mL).

CD23^{-/-} mice demonstrated significantly raised total IgE (Fig. 1a) at all time points measured ($P < 0.05$, ANOVA) compared with immunized wild-type controls of one log order of magnitude difference. Similarly, CD23^{-/-} mice demonstrated significantly greater levels of ovalbumin-specific IgE than wild-type mice at day 21 (Fig. 1b, $P < 0.05$) of one log order of magnitude difference.

Bronchoalveolar lavage

Following 3 or 7 days challenge with ovalbumin there was a

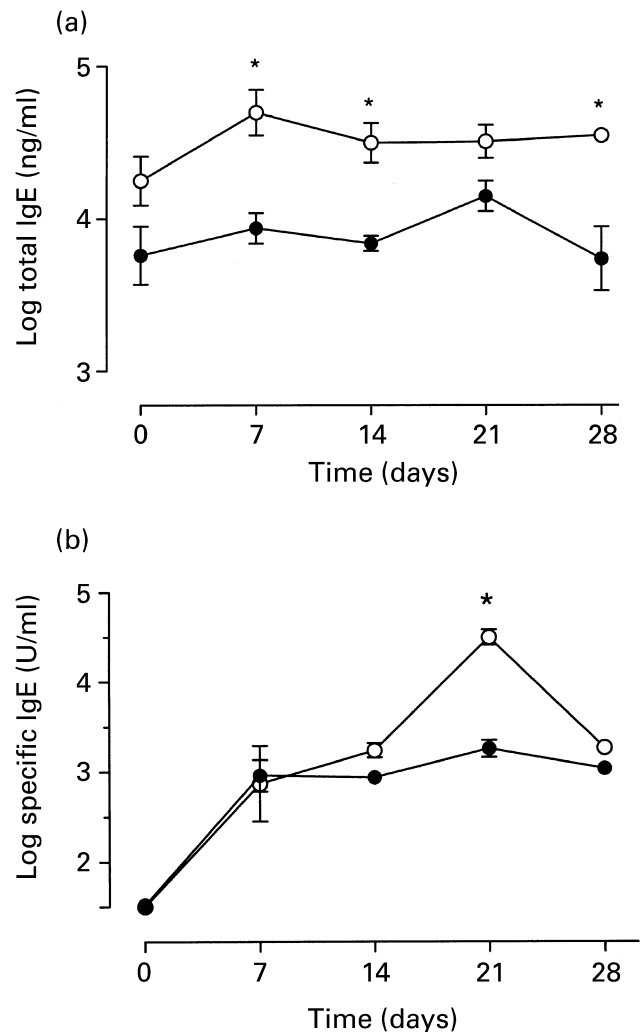


Fig. 1. Serum log total IgE (a) and specific IgE (b) in wild-type (●) and CD23^{-/-} mice (○). Each point represents the SEM of five mice.

significant increase in total cells recovered in lavage fluid in wild-type and CD23 gene disrupted mice compared with saline- or sham-immunized mice following saline aerosol challenge (Fig. 2). Differential cell counts revealed the presence of monocytes, lymphocytes and neutrophils in wild-type (Fig. 3) and CD23^{-/-} (Fig. 4) mice following 3 and 7 day ovalbumin challenge. There was a significant increase in the percentage of eosinophils recovered in bronchoalveolar lavage fluid following 3 and 7 day ovalbumin challenge (Fig. 5) compared with sham-immunized mice ($P < 0.05$). However, after 3 days challenge, ovalbumin-immunized CD23^{-/-} mice presented with a significant increase in the percentage of eosinophils recovered in BAL compared with wild-type ovalbumin-immunized mice ($P < 0.05$ Fig. 5). These differences were not observed after 7 days challenge, when the percentage of eosinophils was similar in both strains.

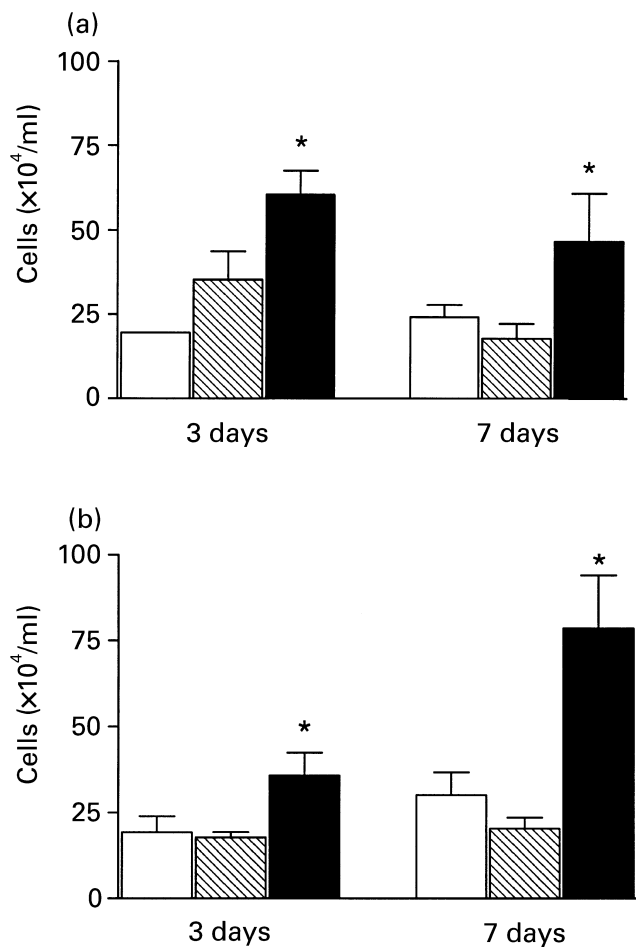


Fig. 2. Total cells ($\times 10\,000$ per ml) recovered in bronchoalveolar lavage in saline (open columns; $n = 5, 10$), sham-immunized (hatched columns; $n = 6, 9$) and ovalbumin-immunized (close columns; $n = 8, 22$) groups from (a) wild-type (b) $\text{CD23}^{-/-}$ mice following challenge (3 or 7 day, respectively). Vertical lines represent SEM (* $P < 0.05$ compared saline).

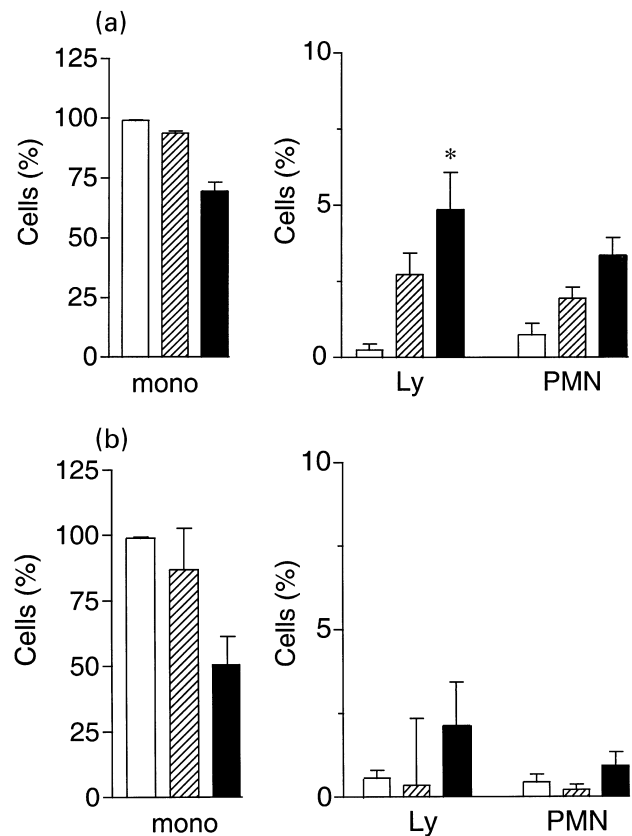


Fig. 3. Percentage of cells recovered in bronchoalveolar lavage fluid saline (open columns; $n = 5, 10$), sham (hatched columns; $n = 6, 20$) and ovalbumin-immunized (closed columns; $n = 8, 22$) groups from wild-type mice following (a) 3 day and (b) 7 day aerosol challenge. Monocytes, mono; lymphocytes, Ly; neutrophils, PMN; eosinophils, eos. Vertical lines represent SEM (* $P < 0.05$ compared with saline).

	Wild-type	$\text{CD23}^{-/-}$
3 day		
Saline	0 (3)	0.25 ± 0.25 (4)
Sham	0.57 ± 0.30 (7)	1.00 ± 0.52 (6)
Ovalbumin	$15.60 \pm 0.39^*$ (10)	$19.57 \pm 3.72^*$ (7)
7 day		
Saline	3.25 ± 0.84 (8)	1.00 ± 0.27 (16)
Sham	3.25 ± 0.56 (8)	1.00 ± 0.38 (15)
Ovalbumin	$31.75 \pm 3.70^*$ (8)	$29.15 \pm 4.80^*$ (13)

Results expressed as mean $\pm$ SEM. Values in parentheses indicate number of animals. Saline and sham groups were challenged with saline (0.9%) and ovalbumin group challenged with (10 mg/mL ovalbumin). Significant increase in eosinophil number compared with saline group and 3 day ovalbumin group (* $P < 0.05$).

Table 1. The number of eosinophils (cells/ 0.1 mm^2) enumerated in tissues sections from saline, sham- and ovalbumin-immunized mice following aerosol challenge

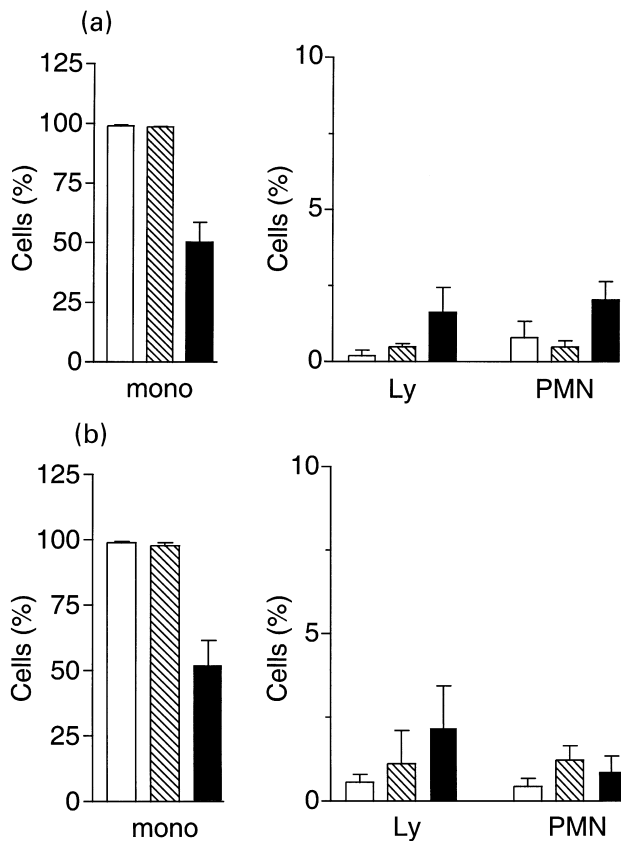


Fig. 4. Percentage of cells recovered in bronchoalveolar lavage fluid in saline (open columns, $n = 10, 12$), sham (hatched columns, $n = 9, 19$) and ovalbumin immunized (closed columns, $n = 11, 19$) groups from $CD23^{-/-}$ mice following (a) 3 day and (b) 7 day aerosol challenge. Monocytes, mono; lymphocytes, Ly; neutrophils, PMN; eosinophils, eos. Vertical lines represent SEM.

Tissue histology

Analysis of cell populations within the bronchial mucosa revealed significant increases in eosinophil (Table 1) and CD4, CD8 and CD23 positive cells (Table 2, 3) following 3 and 7 days ovalbumin challenge in ovalbumin-compared with sham-immunized wild-type and $CD23^{-/-}$ mice. However, while there was a trend to an increase in the number of eosinophils enumerated in bronchial mucosa between wild-type and $CD23^{-/-}$ mice following 3 day ovalbumin challenge, this did not achieve statistical significance. Furthermore, consistent with bronchoalveolar lavage data, there was a significant increase in the number of eosinophils enumerated in bronchial mucosa in wild-type mice (Table 1). There was a significant increase in the number of CD4 positive cells enumerated in bronchial mucosa between sham- and ovalbumin-immunized wild-type mice that was greater following 7 day challenge (Table 2, 3), a finding that was also evident in $CD23^{-/-}$ mice (Table 2, 3). The ratio of

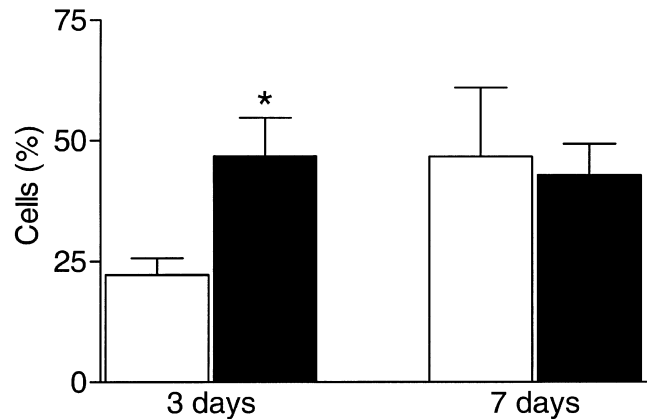


Fig. 5. Percentage of eosinophils recovered in bronchoalveolar lavage fluid following 3 or 7 day aerosol challenge in wild-type (open columns; $n = 9, 19$, respectively) and $CD23^{-/-}$ mice (closed columns; $n = 11, 19$, respectively). No eosinophils were enumerated in bronchoalveolar lavage fluid from sham-immunized mice. Vertical lines represent SEM.

CD4/CD8 positive staining cells was significantly greater in $CD23^{-/-}$ mice following 3 and 7 day antigen challenge ($P < 0.05$). There was an absence of CD23 positive staining in $CD23^{-/-}$ mice (Table 2, 3).

Bronchial hyperresponsiveness

Twenty-four hours after the last challenge (3 days), airway responsiveness to 5-HT was significantly increased in both wild-type and $CD23^{-/-}$ ovalbumin-immunized mice compared with sham controls ($P < 0.01$, ANCOVA, Fig. 6). Furthermore, airways hyperresponsiveness induced by antigen

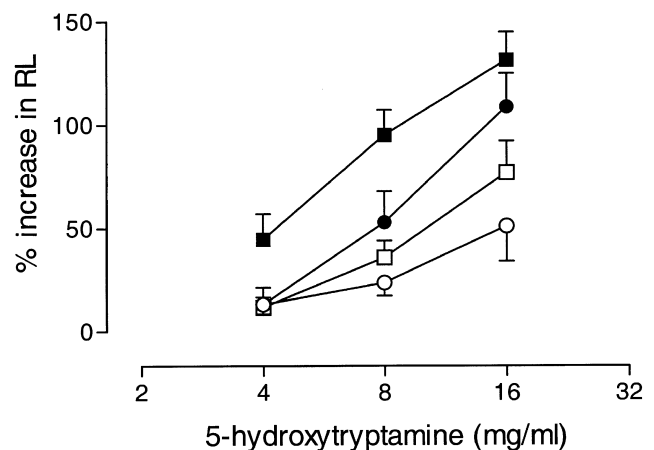


Fig. 6. Airways responsiveness to aerosols of 5-HT in wild-type (sham, $\circ$, $n = 11$; ovalbumin, $\bullet$, $n = 6$) and $CD23^{-/-}$ (sham, $\square$, $n = 11$; ovalbumin, $\blacksquare$, $n = 12$) immunized mice 24 h following 3 day challenge with saline (open symbols) and ovalbumin (10 mg/mL; closed symbols).

	CD4	CD8	CD23	CD4/CD8	n
Wild-type					
sham	18.33 ± 1.20	18.67 ± 3.38	0.67 ± 0.67	1.07 ± 0.18	3
ovalbumin	32.5 ± 2.62*	23.5 ± 1.98	7.00 ± 2.08*	1.42 ± 0.15	6
CD23 ^{-/-}					
sham	20.5 ± 0.7	12.75 ± 1.72	0	1.62 ± 0.19	8
ovalbumin	28.5 ± 4.75	11.0 ± 1.44	0	2.65 ± 0.35*	6

Results expressed as mean ± SEM. Significant increase in cell number compared with sham group (* $P < 0.05$).

	CD4	CD8	CD23	CD4/CD8	n
Wild-type					
sham	35.2 ± 4.24	24.0 ± 4.4	3.17 ± 0.7	1.58 ± 0.64	6
ovalbumin	79.0 ± 10.59*	37.2 ± 5.44	7.38 ± 1.89	2.16 ± 0.76*	8
CD23 ^{-/-}					
sham	21.0 ± 3.27	37.8 ± 6.36	0	0.64 ± 0.10	7
ovalbumin	62.6 ± 7.97*	52.6 ± 6.76*	0	1.26 ± 0.35*	11

Results expressed as mean ± SEM. Significant increase in cell number compared with sham group (* $P < 0.05$).

Table 2. The number of immunopositive cells (cells/0.1 mm²) enumerated in tissues sections from sham- and ovalbumin-immunized mice following 3 day aerosol challenge

Table 3. The number of immunopositive cells (cells/0.1 mm²) enumerated in tissues sections from sham- and ovalbumin-immunized mice following 7 day aerosol challenge

challenge was significantly greater in CD23^{-/-} compared with wild-type mice ($P < 0.01$, ANCOVA, Fig. 6). Baseline airways resistance (RL, cmH₂O/L/s) was significantly lower in CD23^{-/-} compared with wild-type mice (ANOVA, $P = 0.035$), however, there was no significant difference in baseline RL between sham- and ovalbumin-immunized wild-type mice (sham; 562 ± 23 , $n = 6$ vs ovalbumin-immunized; 623 ± 34 , $n = 11$, $P > 0.05$) and CD23^{-/-} mice (sham; 530 ± 37 , $n = 11$ vs ovalbumin-immunized; 494 ± 40 , $n = 12$, $P > 0.05$).

Discussion

We have demonstrated that both total and specific serum levels of IgE are significantly elevated in ovalbumin-immunized CD23^{-/-} mice compared with their wild-type counterparts. The percentage of eosinophils recovered in bronchoalveolar lavage fluid was significantly higher in CD23^{-/-} mice following 3 days but not after 7 days challenge, suggesting that CD23-dependent and -independent mechanisms are involved in eosinophil recruitment to the lung following allergen exposure. Furthermore, allergen-induced hyperresponsiveness was greater in CD23^{-/-} compared with wild-type mice.

CD23 is thought to mediate a number of effects including regulation of IgE synthesis, antigen presentation, proliferation

and differentiation of B cells and activation of monocytes, effects that can be ascribed to the membrane and soluble forms of CD23 [21]. Soluble CD23 (sCD23) augments spontaneous production of IgE [22] whereas antibodies directed against sCD23 has been shown to inhibit the synthesis of IgE by mononuclear cells from atopic subjects [23] and rats [19]. Similarly, monoclonal antibodies directed against CD23 produced a small decrease in IgE production in mice [24]. In contrast, CD23 efficiently focused protein antigens bound to IgE for antigen presentation to T cells and antigen-focusing to B cells in the mouse *in vitro* [25,26]. Studies in humans have shown that this process of antigen-focusing by CD23 [27,28] involves activation of CD21 on B cells [21]. In contrast, crosslinking of membrane-bound CD23 by antigen results in an inhibition of B-cell growth and differentiation in man [29–32]. Interestingly, monoclonal antibodies directed against CD23 inhibited IL2-R expression in CD8 but not CD4 positive cells and produced a modest inhibitory action on B-cell activation in mice [24].

We have shown that CD23-deficient mice produce higher levels of IgE than their wild-type counterparts, consistent with previous findings [15,20,24]. This appears to be unrelated to an alteration in T- or B-cell growth and differentiation [15–17]. Conversely, mice overexpressing membrane but not soluble CD23 exhibit a weaker IgE response [18]. In contrast, CD23 knockout mice do not present an

IgE-mediated enhancement of IgG1 production which suggests that CD23 can play a role in antigen-focusing [16]. Thus, it is clear that the overwhelming evidence supports the notion that the major action of CD23 *in vivo* is to impart a negative signal for IgE production in murine models of inflammation.

Our study has shown that repeated exposure of wild-type mice to ovalbumin induced a significant increase in total cells recovered from bronchoalveolar lavage fluid of a similar magnitude reported previously in C57Bl/6 mice [33–35]. The cell infiltrate was predominantly monocytic in sham-immunized mice. However, a significant increase in the percentage of eosinophils was observed in immunized mice following aerosol exposure to ovalbumin, consistent with previous studies in this strain of mice [33–35]. CD23^{-/-} mice like their wild-type counterparts also demonstrated a rich eosinophilic infiltrate following ovalbumin challenge in ovalbumin but not sham-immunized mice. However, the percentage of eosinophils recovered in bronchoalveolar lavage fluid was significantly greater following 3 but not 7 day ovalbumin challenge of CD23^{-/-} compared with wild-type mice, although this difference was not as apparent when eosinophils were enumerated from bronchial tissue. This may reflect differences in eosinophil traffic through different airway compartments as has been previously reported [14]. In our model the timing of the aerosol challenge plays an important role in influencing eosinophil recruitment in the CD23^{-/-} compared with wild-type mice and suggests that both IgE-dependent and -independent mechanisms can operate to recruit eosinophils to the lung during airway inflammation. A number of studies have shown that IgE is implicated in eosinophil recruitment to the airways. Thus, antigen challenge of mice passively sensitized with IgE was associated with the recruitment of eosinophils to the lung [9]; conversely, treatment of mice with anti-IgE attenuated eosinophil recruitment to the airways [8]. However, it is also clear that IgE-independent mechanisms play a role in the recruitment of eosinophils to the lung. Indeed, mast-cell-deficient mice [8,36,37], CD40-deficient mice [14], IgE-deficient mice [13] and B-cell-deficient mice [11,12] still retain the capacity to recruit eosinophils following antigen challenge. Moreover, a study utilizing a relatively short period of exposure to antigen [38] compared with other studies [8,36,37], demonstrated a significant impairment in the recruitment of eosinophils to the lung following antigen challenge in mast-cell-deficient mice. While IL-4 has been shown to be important for Th2 cell commitment and isotype switching of B cells to the IgE phenotype [39], the role of IL-4 in allergen-induced recruitment of eosinophils is controversial. Thus, eosinophil recruitment is significantly attenuated in IL-4 gene-disrupted mice [34,40]; in mice treated with monoclonal antibodies to IL-4 during antigen immunization [40] or prior to antigen challenge [41]; and in

mice treated with IL-4 receptor antibodies prior to antigen challenge [42]. In contrast, other studies have shown that treatment with antibodies directed against IL-4 prior to antigen sensitization [43] or prior to antigen challenge [40,43] had no effect on eosinophil recruitment to the airways. Interestingly, a recent study has shown that eosinophil recruitment to the lungs still occurs in IL-4-deficient mice [14]. It would therefore appear that both IL-4-dependent and -independent mechanisms can be expressed in different murine models of inflammation. The reason for this is unclear, but cannot be explained entirely by the use of different strains of mice since a number of the conflicting data has arisen using mice of a similar genetic background [14,34,36].

We also showed that following the 7 day ovalbumin challenge the number of CD4 + T-lymphocytes was increased in wild-type and CD23^{-/-} mice. The presence of CD4 + T lymphocytes in airway tissue is consistent with the view that recruitment of eosinophils during chronic inflammation is dependent upon CD4 + T lymphocytes [44,45]. Interestingly, while there was a small increase in the number of CD4+ cells following 3 day aerosol challenge, this did not achieve statistical significance and may indicate that IgE-dependent mechanisms play a role at this time. CD23^{-/-} positive staining cells were only found in wild-type mice, confirming the effectiveness of this gene disruption in CD23^{-/-} mice.

We have also demonstrated airways hyperresponsiveness to aerosolized 5-HT in ovalbumin-immunized wild-type and CD23^{-/-} mice following 3 day challenge with ovalbumin. The increased airways responsiveness to 5-HT following ovalbumin challenge was significantly greater in CD23^{-/-} compared with wild-type mice. We also attempted to investigate airways hyperresponsiveness following 7 day exposure to ovalbumin. However, most of the ovalbumin-immunized mice did not survive exposure to 5-HT. The mechanism by which a lack of expression of CD23 may predispose immunized mice to heightened airways responsiveness remains to be established. Since, ovalbumin-immunized CD23^{-/-} mice have a higher titre of IgE and higher percentage of eosinophils in the bronchoalveolar lavage, these factors may be responsible for the increased airways hyperresponsiveness in CD23^{-/-} mice. There is considerable evidence supporting the view that eosinophil recruitment to the airways is essential for the expression of bronchial hyperresponsiveness and tissue damage. Monoclonal antibodies directed against IL-5 attenuate eosinophil recruitment in allergic mice and bronchial hyperresponsiveness [33,44,46,47]. Conversely, eosinophil recruitment is compromised in IL-5 gene-disrupted mice and bronchial hyperresponsiveness induced by antigen is inhibited [33] and if IL-5 is overexpressed in the lung, then bronchial hyperresponsiveness is observed in nonsensitized mice [48].

However, other studies have suggested that the link between eosinophils and hyperresponsiveness is not so clear. It is of interest that eosinophils recruited to the airways following antigen challenge in IL-5 transgenic mice do not exhibit bronchial hyperresponsiveness [49]. In contrast, monoclonal antibodies directed against IL-5-inhibited antigen-induced eosinophilia but not hyperresponsiveness [50]. It remains to be established whether differences in the activation state of the eosinophil is an important determinant of the conflicting results reported in these studies. Interestingly, eosinophils recruited to the lung do not appear to demonstrate evidence of deposition of extracellular granule products in mice despite the presence of bronchial hyperresponsiveness [51,52]. Moreover, although a recent study has suggest a possible interaction between IgE and eosinophils in the development of bronchial hyperreactivity in mice [53], it is of further interest that murine eosinophils appear to lack both high and low affinity IgE receptors, but do express IgG receptors [54].

Thus, we have demonstrated that CD23 plays a major role in the negative feedback control of IgE production in C57Bl/6 mice, and this gene disruption does appear to modulate eosinophil recruitment to the airways following acute but not chronic exposure to antigen and is associated with a greater degree of bronchial hyperresponsiveness.

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