CD23 Deficient Mice Develop Allergic Airway Hyperresponsiveness Following Sensitization with Ovalbumin

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The low affinity receptor for IgE (CD23) is reported to regulate immune and inflammatory events and as a result, it may have a role in the development of allergic airway inflammation and hyperresponsiveness (AHR). To test this hypothesis CD23-deficient mice were studied following different modes of allergic sensitization. Mice were actively sensitized either intraperitoneally with ovalbumin (OA)/alum or via the airways (10 days exposure to OA aerosol with no adjuvant). Passive sensitization was performed by intravenous injections of OA-specific IgE. Airway responsiveness, serum IgE and IgG levels were assessed together with airway inflammation. Passive sensitization followed by airway challenges resulted in increased OA-specific IgG and IgE in the serum of wild-type mice only, while both the CD23+/+ and CD23−/− groups developed tracheal smooth muscle hyperresponsiveness to electrical field stimulation, indicating that IgE/CD23-mediated immune functions may not be necessary for the development of allergic changes. Active sensitization of both CD23+/+ and CD23−/− mice resulted in increased serum levels of OA-specific IgE and IgG, airway eosinophilia and significant AHR when compared with nonsensitized mice. The genetic deficiency of CD23−/− mice not only failed to prevent but was associated with a significant increase of these responses. These results indicate that CD23 may not be essential for the development of allergen-induced AHR and further, that its presence may have some inhibitory effects on the allergic response.


In allergic asthma, a close relationship has been demonstrated between IgE levels and the degree of AHR (1). Expression of IgE is thought to be controlled by both heavy-chain switching and the selection of IgE-committed cells. Some of the regulatory circuits for this process are mediated through the low affinity IgE receptor (CD23) either by stimulating B cells to produce more IgE or, when bound to IgE, by blocking its production (2). CD23 (FceRII) is a type II membrane glycoprotein expressed on various cell types, including mature B cells, follicular dendritic cells, γδ T cells, subsets of CD4+ and CD8+ T-cells after activation and eosinophils (3). In addition to regulating IL-4-induced IgE production, CD23 has been implicated in cellular adhesion (4), antigen presentation (5–6), growth and differentiation of B and T cells (7–8), and rescue from apoptosis (9). The expression of CD23 is strikingly increased in allergic disorders (3, 10). Although it is possible that CD23 has an important role in IgE-mediated priming and activation of cells expressing this receptor, the relationship of CD23 expression to disease and the specific functions of CD23-positive cells is still unclear. Overproduction of IgE in response to common environmental antigens may be responsible for amplifying immune reactions which in turn lead to sustained inflammation and airway hyperresponsiveness (AHR). However, the in vivo relevance of many of these observations has recently been questioned by a series of studies on gene-targeted mice lacking expression of CD23, since these mice showed no other phenotypic difference, they had normal T and B cell development and demonstrated an increased production of IgE upon parasite infection (11).

While IgE appears to be involved in a network of immune and inflammatory events relating to immediate hypersensitivity reaction, the role of IgE in allergic inflammation is not clear. Using different approaches to sensitization and challenge, which show differences in IgE dependency for development of AHR, we investigated whether CD23 is functionally important to the inflammatory changes which develop following allergic sensitization leading to AHR. In studying IgE dependency for the development of AHR, we demonstrated that 10-day exposure to ovalbumin (OA) exclusively via the airways triggers an
IgE-dependent development of tracheal smooth muscle hyperresponsiveness to electrical field stimulation (12, 13). We also utilized passive sensitization where the inflammatory changes induced following limited allergen challenges are mediated exclusively by O A-specific IgE (14). In contrast to 10-day OA exposure or passive sensitization, after systemic sensitization and repeated airway challenge the development of A H R has been shown to be IgE-independent (15), although these mice produce high levels of IgE. These alternative approaches to sensitization and challenge indicated that CD 23 may not be essential for the development of allergen-induced A H R.

**METHODS**

**Mice**

CD 23 deficient (CD 23−/−) and wild type (CD 23+/+) mice were generated as previously described (5). The gene-targeted 129/Ola strain was crossed with C 57 B l/6, then the CD 23 deficient F 1 heterozygous mice were backcrossed to C 57 B l/6 mice twice. Those heterozygous mice were then intercrossed to produce homozygous wild-type and CD 23 deficient mice. Confirmation for the functional disruption of the CD 23 gene was performed by Southern Blot and Northern Blot analysis and has been published in detail in an earlier report (10). Briefly, genomic DNA was prepared from ES cells and adult tail tips. To ascertain targeting of the CD 23 allele, 15 μg of DNA was digested with Sph 1 or A a l I and probed with a 0.4-kb B st E I /XI b a I or a 0.6-kb B am H I/X b a I genomic DNA fragment. Incorporation of the single neomycin-resistance gene was further confirmed using a neo probe. For analysis of CD 23 mR N A, total RNA was prepared from spleen B cells stimulated with L P S (10 μg/ml) in the presence of L -4 (60 μg/ml, G enzyme) for 36 h, electrophoresed, transferred to H y b o n-D (A mersham) and hybridized with a CD 23 cDNA fragment or a β-actin probe. For analysis of CD 23 expression we have performed F A C Scan analysis. The antibody used for identifying CD 23 was a rat monoclonal antibody (B 3 B 4 ) kindly provided by Dr. D. H. C onrad (V irginia C ommonwealth U niversity, R ichmond, V A ) (16). A detailed F A C Scan analysis of splenocytes and thymocytes as well as immunohistochemical analysis of germinal centers of spleen sections from homozygous CD 23 deficient and wild-type mice were published by F ujiwara and coworkers (5). Mice were housed in pathogen-free conditions and were maintained on an ovalbumin (O A)-free diet. Experiments were performed on age- and sex-matched groups between the age of 8-12 wk.

A ll experimental animals used in this study were under a protocol approved by the I nstitutional A nim al Care and U se Committee of the National J ewish M edical and R esearch Center.

**Sensitization and Airway Challenge**

Mice were exposed to OA following three different protocols: (1) Mice were exposed for 10 consecutive days to an aerosol containing 1% OA using a D eV ibiss A erosonic 5000 nebulizer (DeV ibiss H ealth C are I nc., S omerset, P A ) as previously described (12-13); (2) Mice were actively immunized by intraperitoneal injection of 20 μg of O A (G rad e V ; S igma C hemica l Co., S t. L ouis, M O ) together with 20 mg al um (I nject A l um; Pierce, R ockford, I L ) in 100 μl PBS (phosphate-buffered saline), or with PBS alone on Day 1 and Day 14. On Days 24, 25 and 26 mice received an aerosol challenge for 20 min with a 1% O A/PBS solution; (3) Mice were passively sensitized with O A-specific IgE as previously described (14). Briefly, mice were injected intravenously with 100 μl of hybridoma supernatant containing 200 μg of O A-specific IgE antibody on three consecutive days (Days 1, 2, 3) prior to four aerosol challenges with 1% O A on Days 5, 6, 7 and the day of challenge, A ss a control, mice received T N P-specific IgE (A T C C, R ockville, M D ). A ll mice were killed 48 h after their last OA exposure.

**Electrical Field Stimulation of Trachea In Vitro**

A irway responsiveness to electric field stimulation was determined 48 h after the last aerosol challenge of mice as described previously (12). Briefly, tracheae were removed and 0.5 cm long preparations were placed in K rebs-H enseil solution suspended by triangular supports transducing the force of contractions. Electrical field stimulation with an increasing frequency from 0.5–40 Hz was applied and the contractions measured. Frequencies resulting in 50% of the maximal contractions (E S 0 5 ) were calculated from linear plots for each individual animal and were compared between the different groups.

**In Vivo Measurement of Bronchial Responsiveness to Methacholine (MCh)**

Bronchial responsiveness was assessed as a change in airway function after challenge with aerosolized M Ch via the airways using a modification of methods previously described in rats (17-18) and in mice (12, 19). Mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (70 to 90 mg/kg). A stainless steel 18 G tube was inserted as a tracheostomy cannula and was passed through a hole in the Plexiglas chamber containing the mouse. A four-way connector was attached to the tracheostomy tube, with two ports connected to the inspiratory and expiratory sides of a ventilator (Model 683; Harvard A pparatus, S outh N ativck, M A ). V entilation was achieved at 160 breaths per minute and a tidal volume of 0.15 ml with a positive end-expiratory pressure of 2-4 cm H 2 O . The Plexiglas chamber was continuous with a 1.0-liter glass bottle filled with copper gauze to stabilize the volume signal for thermal drift.

Transrespiratory pressure was detected by a pressure transducer. Changes in lung volume were measured by detecting pressure changes in the plethysmographic chamber. Flow was measured by digital differentiation of the volume signal. Lung resistance (R L ) and dynamic compliance C d y n were continuously computed by a M acintosh computer software (L abview; N ational Instruments, A us tin, T X ) by fitting flow, volume, and pressure to an equation of motion.

The aerosolized bronchoconstrictor agents were administered through a bypass tubing via an ultrasonic nebulizer placed between the expiratory port of the ventilator and the four-way connector. A aerosolized agents were administered for 10 s with a tidal volume of 0.5 ml. After a dose of inhaled PBS was given, the subsequent values of R L were used as baseline. Starting 3 min after saline exposure, increasing concentrations of M Ch were given by inhalation (10 breaths), with the initial concentration set at 0.4 mg/ml. Increasing concentrations were given at 5-7 min intervals. H yerinifications of twice the tidal volume were applied between each M Ch concentration and performed by manually blocking the outflow of the ventilator in order to reverse any residual atelectasis and ensure a constant volume history prior to challenge. From twenty seconds up to three minutes after each aerosol challenge, the data of R L and C d y n were continuously collected and maximum values of R L and minimum values of C d y n were taken to express changes in murine airway function.

**Serum Collection**

V enous blood was collected from the tail vein before and at different time points during the sensitization period into serum separator tubes (M icrotainer; B ecton-D ickinson, F ranklin L akes, N J ). Serum samples were stored at −20° C pending analysis.

**ELISA for Immunoglobulins**

Serum antibody levels were determined as previously described (12). Briefly, ELISA plates (D y natech, C hallt i vy, V A ) were coated with OA (20 μg/ml N aH C O 3 buffer, p H 9.6) or with polyclonal goat anti-mouse IgE 3 μg/ml (T he B inding Site L td., S an D iego, C A ) and incubated overnight at 4° C. Plates were blocked with 0.2% gelatin buffer (p H 8.2) for 2 h at 37° C. Serum was diluted 1:10. Standards containing O A-specific IgE and IgG were generated as described (20). For total immunoglobulin, commercial standards were used (P harmingen, S an D iego, C A ). E LISA data were analyzed with the M icropol iate M anager software program for the M acintosh (B io-R ad L ab s, R ichmond, V A ).

**Mononuclear Cell Culture for Proliferation Assay**

Spleens were removed and placed in sterile PBS. Single-cell suspensions were prepared and mononuclear cells were purified by density gradient centrifugation (Lymphocyte Separation M edium; O rganon T eknika, D urham, N C ). Cells were washed, counted and resuspended in culture medium (R PM I 1640; G IB C O B RL, G aithersburg, M D ), containing heat-inactivated fetal calf serum (F CS 10%; H yclone, L o- gan, U T ), L-glutamine (2 mM), 2-mercaptoethanol (5 mM), H EP E S
buffer (15 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml), all from GIBCO BRL. Cells were plated at 2 × 10³/ml in 96-well round bottom tissue culture plates in triplicate and incubated with medium alone, OA (100 ng/ml) or the combination of phorbol 12,13-dibutyrate (10 nM) and ionomycin (0.5 µM) for 48 h in a humidified atmosphere of 5% CO₂ at 37°C. Cell supernatants were harvested and stored at −20°C pending cytokine ELISA assays. Cell proliferation was assessed by uptake of [³H]-thymidine which was added to cell culture wells for the last 16 h of the incubation period. A tetra incubation, cells were harvested onto a glass-fiber filter paper using a cell harvester apparatus and the incorporated radiolabel was counted using a β-spectrometer. Results were expressed as mean counts of triplicate cultures.

**BAL and Lung Digest Differential Cell Count**

A tetra measurement of lung function parameters, lungs were lavaged with 1 ml aliquots of 0.9% wt/vol. of sterile NaCl (room temperature) through a polyethylene syringe attached to the tracheal cannula. Lavage fluid was centrifuged (500 × g for 10 min at 4°C), and the cell pellet was resuspended in 0.5 ml of RPMI tissue culture medium.

Lung digestion was performed after exsanguination and perfusion of the lungs following the protocol previously described from our laboratory (14). Cells from BAL or lung digests were resuspended in RPMI and counted with a hemocytometer. Differential cell counts were made from cytospin preparations as described (14, 18). Cells were identified as macrophages, eosinophils, neutrophils and lymphocytes by standard morphology and at least 500 cells counted under 400 magnification. The percentage and absolute numbers of each cell type were calculated.

**Immunolabeling of Eosinophils**

Immunocytochemistry was performed as described previously (20). Briefly, lung tissue was removed and fixed in 10% formalin solution. Four µm thick sections were cut, deparaffinized and treated with porcine trypsin for 30 min, 37°C. After washing 3 times, 10% goat serum solution on Days 25, 26, and 27. Mice were bleed on Days 0, 7, 14, 21, and 28 and assessed for total IgG (A) and total IgE levels (B). Data represent the mean ± SEM from three experiments with n = 3, 4 mice in each experiment.

**RESULTS**

**Kinetics of Immunoglobulin Production in Wild-Type and CD23-Deficient Mice**

In mice sensitized (i.p.) and challenged with OA, serum samples were obtained on Days 0, 7, 14, 21, and 28. Immunoglobulin values were presented on a log scale throughout. Total IgG levels remained relatively constant (approximately 100 ng/ml) during this time period (Figure 1A). Total IgE levels plateaued at ~50 ng/ml by Day 14 and continued through Day 28 (Figure 2B). In the CD23−/− mice, OA-specific IgG present in the samples before sensitization. After the first injection it rapidly increased with an additional increase after the booster on Day 14, and plateaued on Day 21. CD23+/+ and CD23−/− mice showed identical changes. OA-specific IgE concentrations in the wild type mice reached a plateau by Day 14 and declined through Day 28. In the CD23−/− mice, OA-specific IgE reached significantly higher levels (p < 0.05, n = 12) on Days 14 and 21 when compared with CD23+/+.
mice (Figure 2B). Without sensitization, aerosol exposure to OA for 3 consecutive days did not affect serum IgE levels in either group (data not shown).

Ten days OA-aerosol exposure also resulted in significant increases in OA-specific IgE levels which were comparable in the two groups of mice (Figure 2B). Mice that received 10 day-PBS exposure had no OA-specific immunoglobulins (data not shown).

Effects of Passive Sensitization with OVA-Specific and TNP-Specific IgE on Immunoglobulin Levels Following Airway Exposure to OA

We determined whether passive sensitization with OA-specific IgE would potentiate antibody responses following OA exposures of the airways. Mice received 100 μl of hybridoma supernatant intravenously, containing either OA-specific or TNP-specific IgE on three consecutive days prior to aerosol

Figure 2. Ovalbumin-specific immunoglobulin levels during the course of intraperitoneal sensitization and 10 day ovalbumin exposure. Mice were sensitized, challenged and bled as described in Figure 1 for OA-specific IgG (A) and IgE (B) production (lineplots). In a second protocol mice were exposed to ovalbumin aerosol for 10 consecutive days and assayed on Day 12 (bargraph). Ovalbumin specific immunoglobulin production is expressed in ELISA U/ml. *p < 0.05: comparisons were made between CD23+/− and CD23+/+ mice (n = 8–15).

Figure 3. OA specific IgG (A) and IgE (B) levels in passively sensitized mice. Mice received intravenous injections of hybridoma supernatants containing OA-specific (solid bars) and TNP-specific (hatched bars) IgE prior to four aerosol challenges (neb) with 1% OA. Control groups received OA-specific IgE alone (grey bars) or 4 d nebulization alone (open bars). Serum was assayed on Day 10 (n = 8–16). *p < 0.05, **p < 0.01 CD23+/− versus CD23+/+, ##p < 0.05 OA-IgE alone versus OA-IgE + 4 days OA nebulization.
challenge with 1% OA on four consecutive days. Serum samples were collected for immunoglobulin assays 48 h after the last challenge. Figure 3 illustrates the levels of O-A-specific IgG or IgE in the serum of either CD23\(^{+/+}\) and CD23\(^{-/-}\) mice on a log scale. Four airway challenges with OA alone, failed to induce O-A-specific IgG or IgE production. In passively sensitized mice, O-A-specific IgG was found only in the CD23\(^{+/+}\) mice, while the CD23\(^{-/-}\) mice had no detectable O-A-specific IgG in their serum (Figure 3A). Mice passively sensitized with IgE without OA-airway challenge failed to develop IgG or IgE antibodies. There were significant differences in the IgG and IgE levels between mice receiving OA-IgE alone and mice receiving OA-IgE as well as four airway challenges in the CD23\(^{+/+}\) group but not in the CD23\(^{-/-}\) animals. O-A-specific IgE was found in the serum of both CD23\(^{+/+}\) and CD23\(^{-/-}\) groups. Following O-A-IgE injection and four days O-A nebulization, the O-A-specific IgE levels in CD23\(^{-/-}\) mice were 25 ± 3 EILISA U/ml as opposed to 365 ± 24 EILISA U/ml in the CD23\(^{+/+}\) mice. This difference is statistically significant (p < 0.01). Values in the control groups were less than 10 EILISA U/ml (Figure 3B).

**Proliferative Response of Mononuclear Cells from Sensitized CD23\(^{-/-}\) and CD23\(^{+/+}\) Mice**

We compared the capability of lymphocytes from CD23\(^{-/-}\) and CD23\(^{+/+}\) mice to proliferate in response to antigen. Mononuclear cell preparations from the spleens of both groups responded with dose-dependent proliferative responses following intraperitoneal sensitization. A indicated in Table 1, there were no significant differences between the groups. Mononuclear cells from nonsensitized mice did not show any antigen-induced proliferation (data not shown).

**BAL and Lung Digest Cellular Content in Wild Type and CD23-Deficient Mice**

Animals exposed to OA over 10 d demonstrated significant increases in eosinophil numbers in the lung digest. Although there was a trend for higher eosinophil counts in CD23\(^{-/-}\) mice it did not reach statistical significance (p = 0.065) (Figure 4). Following this protocol in this mouse strain we were not able to find significant inflammatory changes in the BAL samples when compared with control mice receiving 10 d PBS. The BAL cellular content in these mice was virtually identical to the control values in both of the CD23\(^{+/+}\) and CD23\(^{-/-}\) groups.

The numbers of total leukocytes and macrophages recovered from BAL of intraperitoneally sensitized mice were significantly higher than in nonsensitized animals, in both the CD23-deficient and wild-type animals (Figure 5). While there were no eosinophils and neutrophils in nonsensitized mice, the sensitized and challenged animals from both the CD23\(^{+/+}\) and CD23\(^{-/-}\) groups demonstrated a marked increase in inflammatory cells, particularly eosinophils. The proportion of

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<th>OA (µg/ml)</th>
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<tr>
<td>CD23(^{+/+})</td>
<td>0.96 ± 0.05</td>
<td>1.18 ± 0.05</td>
<td>1.25 ± 0.1</td>
<td>5.36 ± 0.7</td>
<td>18.4 ± 2.2</td>
<td>30.3 ± 9.9</td>
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<tr>
<td>CD23(^{-/-})</td>
<td>0.79 ± 0.04</td>
<td>1.12 ± 0.2</td>
<td>1.21 ± 0.2</td>
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<td>20.3 ± 4.7</td>
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TABLE 1
TRITIATED THYMIDINE UPTAKE OF SPLENIC MONONUCLEAR CELLS FROM CD23\(^{-/-}\) AND CD23\(^{+/+}\) MICE

[\(^{3}H\)TdR Uptake (cpm × 10\(^{-3}\))]

Mononuclear cell preparations from spleens of sensitized and challenged CD23\(^{+/+}\) and CD23\(^{-/-}\) mice were purified and cultured for 96 hours in the presence of medium alone, ovalbumin or PI in triplicates. For statistical analysis the geometrical mean of triplicate culture results were used. Data here represent the mean ± SEM from four independent experiments.

PI = phorbol 12,13-dibutyrate and ionomycin.

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Figure 4. Lung digest cellular composition of mice receiving 10 days OA exposure. Mice received OA aerosol exposure for 20 min on 10 consecutive days. Lung digestion was performed on Day 12 as described in Figure 5. **p < 0.01 OA-challenged mice versus PBS-challenged mice. Crossed bars = CD23\(^{+/+}\) OA challenged (n = 8); open bars = CD23\(^{+/+}\) PBS challenged (n = 5); solid bars = CD23\(^{-/-}\) OA challenged (n = 8); hatched bars = CD23\(^{-/-}\) PBS challenged (n = 6).
eosinophils reached approximately 63% in the wild-type and 72% in the CD23−/− mice.

The number of eosinophils in the lung digests obtained both from sensitized and challenged CD23+/+ and CD23−/− mice were significantly elevated when compared with controls. Furthermore, CD23−/− mice had significantly higher eosinophil levels than the wild-type mice (Figure 6).

**Tissue Eosinophil Influx Following Sensitization and Challenge of CD23+/+ and CD23−/− Mice**

In order to directly examine the level of eosinophilia in the peribronchial tissue we performed immunolabeling of eosinophil major basic protein (MBP) on formalin fixed, paraffin embedded tissue sections. The tissue samples were taken 48 h after the last OA aerosol challenge. In both CD23+/+ and in CD23−/− mice we failed to detect any positively stained cells following three OA nebulizations alone (Figure 7A). However, sensitized mice which received aerosol challenges developed a marked eosinophilia in both groups. The eosinophils (MBP+ cells) were accumulated in the peribronchial and perivascular submucosal tissue, while the lung parenchyma remained relatively eosinophil free. A few cells could also be seen penetrating the airway epithelium, traveling through to the lumen (Figure 7B). We observed the following morphological changes...
after sensitization and challenge of mice: (1) thickening of the submucosal tissue probably due to edematous changes and a large number of inflammatory cells; (2) the inflammatory cell infiltrate consisted mainly of eosinophils; (3) lymphoid tissue proliferation at certain sites. No basement membrane thickening, epithelium disruption or airway hyperplasia was observed probably due to the acute nature of our model (the mice were sacrificed 48 h after the last OA challenge). In all of these parameters, we were unable to identify any differences between CD23+/− and CD23−/− mice.

**Tracheal Smooth Muscle Reactivity in CD23+/− and CD23−/− Mice**

To monitor airway responsiveness in the different groups following all three modes of sensitization/challenge (10 d OA exposure, i.p. OA/alum injections and OA challenges, passive sensitization with IgE and OA challenges), we used electric field stimulation of tracheal preparations. ES50 values from individual dose-response curves were calculated and the ratio (%) relative to naive controls are depicted in Figure 8. A decrease in ES50 represents an increase in responsiveness (13).

As shown in Figure 8A, passively sensitized mice which received OA-specific IgE prior to four OA-aerosol challenges demonstrated significant decreases in ES50 when compared with mice which were injected with TNF-specific IgE or which received nebulized OA alone. Further, there were no differences between the CD23+/− and CD23−/− groups.

A active sensitization of CD23+/− and CD23−/− mice following 10 days OA exposure as well as sensitization with OA/alum and OA aerosol challenges resulted in significant and comparable decreases in ES50 as illustrated in Figure 8B. Mice receiving 3 d OA exposure alone, demonstrated no difference from responsiveness of naive controls. Mice which were i.p. sensitized but challenged with PBS instead of OA showed no significant differences from the control animals (data not shown).

**Airway Responsiveness to Methacholine in Wild Type and CD23-Deficient Mice**

Figure 9 presents the dose-response curves of pulmonary resistance (Rl) plotted against concentrations of inhaled MCh in mice sensitized with OA/alum (or injected with PBS) followed by airway challenges. Nonsensitized but OA-challenged animals from the CD23−/− and CD23+/− groups were not significantly different and demonstrated changes in Rl in response to increasing concentrations of MCh, which were similar to normal C57/B6 mice (data not shown). Sensitization and challenge with OA resulted in significant increases in airway responsiveness. CD23−/− mice were more responsive than the CD23+/− mice as demonstrated by a two-way ANOVA test (p < 0.01). In addition, there was a significant difference between these two groups at 50 mg/ml MCh (p < 0.01) (Figure 9A).

PC100 and 200 (provocative concentrations of MCh which cause 100 and 200% increases in lung resistance above baseline) were calculated by log-linear transformation of the dose-response curves. Each of these PC values was significantly lower in CD23−/− mice than in their wild-type littermates (Figure 9B).

We have additionally compared the changes of dynamic compliance (Cdyn) in these mice. This parameter has several determinants including airway resistance to flow, tissue resistance to deformation and elastic recoil of the lung tissue; thus, it is useful in detecting changes in the small airways and peripheral tissue. While sensitization and challenge resulted in significant decreases in the Cdyn values (ANOVA p < 0.01) in both the CD23+/− and CD23−/− mice (Figure 9C), there were no significant differences detectable by Cdyn between CD23+/− and CD23−/− mice.

**DISCUSSION**

CD23 has been widely implicated in the synthesis of IgE as well as in IgE-mediated immune and inflammatory functions (2–4). AAs a result, CD23 may have a pivotal role in the development and maintenance of allergic inflammation. We tested this hypothesis in a mouse model of allergic AHR and found that following sensitization and challenge with OA, neither production of IgE nor the development of airway inflammation and AHR were inhibited in CD23−/− mice.

We compared the allergic responses in CD23+/− and CD23−/− mice using three different sensitization protocols. First, mice received nebulization with OA for 10 consecutive...
Figure 8. Passive and active sensitization to ovalbumin causes airway hyperresponsiveness measured by electrical field stimulation. Airway responsiveness was studied by electrical field stimulation of tracheal smooth muscle preparations. Results are expressed as the percent ES50 values of naive control mice used in the same experiments. Mean ES50 values of control naive mice were 3.58 ± 0.26 Hz in CD23−/− mice (n = 10) and 3.75 ± 0.27 Hz in CD23+/+ mice (n = 10). (A) Groups of mice were passively sensitized with OA-specific IgE (solid bars, n = 16) or TNP-specific IgE (crossed bars, n = 6) prior to four exposures to OA. Control mice required four OA nebulizations alone (open bars, n = 6). (B) Mice were actively sensitized by intraperitoneal injection followed by aerosol challenges as described in Figure 1 (solid bars, n = 14), or received 10 days of OA exposure (hatched bars, n = 14). Control mice received three OA nebulizations alone (open bars, n = 9). Comparisons were made between sensitized and nonsensitized mice. *p < 0.05.

Figure 9. Airway responsiveness to MCh in CD23+/+ and CD23−/− mice. Groups of mice were sensitized (i.p.) as described in Figure 1. Control mice received three aerosol challenge to OA alone. Increasing concentrations of nebulized methacholine (MCh) were administered through the tracheal cannula. (A) Data points represent the mean increase in Rl relative to the baseline resistance, measured in response to PBS in individual mice in each group. *p < 0.05, **p < 0.01 sensitized versus nonsensitized mice, *p < 0.05 CD23−/− versus CD23+/+ mice. Solid circles = CD23−/− sensitized and challenged (n = 12); open circles = CD23−/− challenged alone (n = 8); solid squares = CD23+/+ sensitized and challenged (n = 12); open squares = CD23+/+ challenged alone (n = 8). (B) PC100 and 200 (provocative concentrations of MCh which cause 100 and 200% increases, respectively, in lung resistance above baseline). Crossed bars = CD23+/+ sensitized and challenged (n = 12); solid bars = CD23−/− sensitized and challenged (n = 12); *p < 0.05, **p < 0.01 CD23+/+ versus CD23−/− mice. (C) Changes in dynamic compliance (Cdyn) following nebulized MCh. Data points represent the percentage decrease relative to baseline values which were measured following PBS nebulization. *p < 0.05, **p < 0.01 sensitized versus nonsensitized mice.
days. This approach does not involve adjuvant treatment and antigen exposure is solely via the airways (12), mimicking natural conditions more closely. Further, the development of tracheal smooth muscle hyperresponsiveness to electrical field stimulation in this model appears dependent on IgE production and eosinophilic infiltration of the airways (12-14, 20). Mice in this study produced moderately elevated O A-specific IgE levels, some eosinophilic inflammation in the peribronchial regions and tracheal smooth muscle hyperresponsiveness to electrical field stimulation, in both the CD 23−/− and CD 23+/− groups. These data suggest that CD 23 has no profound effect on the allergic changes in the 10 d O A-exposure model.

In a second protocol, we further amplified the allergic responses in order to be able to study and compare the kinetics of immunoglobulin production in CD 23 deficient and wild-type mice, as well as to examine changes in airway inflammation and airways responsiveness to nebulized M Ch in vivo. It has previously been shown that high levels of IgE and eosinophilic airway inflammation can be achieved by airway challenges of systemically (i.p.) sensitized animals (17–18, 21). Following this approach, the mice produced marked increases in O A-specific IgE and IgG, a significant airway inflammatory cell infiltration with predominance of eosinophils, tracheal hyperreactivity to electrical field stimulation and increased responsiveness to M Ch in vivo, again, in both groups of mice. However, the changes were significantly higher in the CD 23-deficient mice suggesting an inhibitory effect of CD 23 when high levels of IgE are involved in the allergic response.

Finally, in order to study the importance of CD 23/IgE-mediated events on the development of allergic response independently, we used a third protocol in which CD 23−/− and CD 23+/− mice were passively sensitized with O A-specific IgE prior to O A challenges. A similar method has previously been described in our laboratory and was shown to induce tracheal smooth muscle hyperresponsiveness to electrical field stimulation (14). The role of CD 23 in enhancing antigen presentation by binding antigen-IgE complexes seems to be the most consistent feature among human and murine immune responses. The absence of upregulation of O A-specific IgG1 and IgE production in CD 23−/− mice following administration of anti-O A IgE supports these observations. However, this mechanism does not seem to play a role in the development of tracheal smooth muscle hyperresponsiveness.

There are a number of studies suggesting that CD 23 is involved in increased IgE production presumably by interacting with its ligand, CD 21 on the B cell surface (21). Further, in vivo anti-CD 23 antibody treatment of rats was shown to inhibit specific IgE immune responses (22) supporting a role for CD 23 as an enhancer of IgE production. However, the function of CD 23 may be more complex as shown by seemingly controversial data. A transgenic CD 23+/− mice in our studies produced significantly higher amounts of O A-specific IgE while their O A-specific IgG levels remained the same when compared with their CD 23+/− littermates. These data suggest that presence of CD 23 in this mouse model may exert a specific inhibitory effect to a certain extent on IgE production, following systemic (i.p.) sensitization. It has indeed been demonstrated that while a soluble form of CD 23 increases the spontaneous as well as IL-4-induced IgE synthesis by B cells (23), ligation of the membrane form of CD 23 inhibited IgE production (24). In addition, in humans, most of the regulatory function of soluble CD 23 is ascribed to its IgE binding capacity but in mice there is no evidence to support that soluble CD 23 retains IgE binding (25). Other laboratories using CD 23-deficient mice have demonstrated that these animals have normal T and B cell development and function with normal or enhanced IgE production following sensitization to antigen (5) or parasite infection (26, 27). Further, using heterozygous (CD 23−/+ ) populations of gene-targeted mice, an inverse relationship was revealed between the expression of CD 23 and levels of IgE produced after parasite infections (26). Taken together, these data suggest that in murine systems, the inhibitory, membrane bound form of CD 23 may be the dominating IgE-regulatory factor.

A nitogen-IgE complexes bound to CD 23 are described to facilitate antigen presentation to specific T cells, thereby amplifying T cell responses in vitro (5, 6). We have previously shown that such in vitro upregulation of T cell function following exogenous IgE treatment could be abolished after addition of anti-CD 23 antibody, and was absent in cells from CD 23 deficient mice (28). In vivo studies have also demonstrated an impaired capability of CD 23−/− mice to utilize the enhancing effects of antigen-specific IgE treatment in immunoglobulin production (5). We found that passive sensitization with O A-specific IgE (but not anti-TNP IgE) antibody prior to repeated inhalational exposures to O A was associated with significantly enhanced immune responses including O A-specific immunoglobulin (IgG and IgE) production in CD 23−/− but not in CD 23+/− mice. These data confirmed that in the absence of CD 23, the enhancing effects of IgE on certain immune functions are indeed impaired. Interestingly, following this sensitization protocol, CD 23−/− mice were still perfectly capable of developing tracheal smooth muscle hyperresponsiveness to electrical field stimulation, indicating that facilitated antigen presentation through IgE-CD 23, may be of little importance in this murine model.

Sensitized and challenged mice developed a marked increase in the number of eosinophils both in the B A L fluid and in the airway submucosal tissue. In addition, quantitative evaluation of whole lung digests revealed a significant increase in the eosinophilia of CD 23−/− mice when compared to wild-type littermates. The higher number of eosinophils and the fact that CD 23−/− mice were able to produce significantly larger amounts of O A-specific IgE may account for their increased R 1 response to M Ch. Immunocytochemistry of the lung tissue showed that eosinophils were accumulated in the peribronchial-perivascular area in sensitized and challenged mice while the parenchyma was relatively eosinophil-free. While the precise mechanism whereby eosinophils affect function is not known, it is suggested that eosinophil granule products increase airway responsiveness to nonspecific stimuli in vivo. CD 23 may play a role in IgE-mediated eosinophil degranulation (29). Our experiments however, did not support this hypothesis since the absence of CD 23 did not inhibit M Ch responsiveness in the CD 23−/− mice. Recently, two different m R N A species were described in humans, CD 23a and CD 23b (30). Both forms have been identified on B cells, but only CD 23b was found on other cell types such as T cells, dendritic cells, platelets and eosinophils (3, 11). The presence of form b has been confirmed in the mouse, and it is not known whether eosinophils in this species express CD 23.

Although availability of immunologically relevant transgenic or ‘knockout’ mice have proven important for studying models of allergic sensitization (31), site-specific alterations of the mouse genome can often result in surprising phenotypes (32). While constructing the mutations carrying loss-of-function or nullalleles of the gene of interest is technically the simplest, there are a number of limitations to this approach. These include a redundancy in the genome that allows multiple genes to perform overlapping functions, as well as the ability of the genes to compensate for the absent function of the targeted gene. Since surface expression of CD 23 was com-
pletely absent in the homozygous gene-deficient mice in the present study, the findings of increased functional consequences (such as heightened R_1) may be explained by compensatory mechanisms of other membrane structures that are able to bind and mediate functions of IgE in a similar fashion to that of CD23. Several different classes of such IgE-binding molecules have been found (the best known are FcεRI and Mac-2). These molecules are structurally unrelated, they are encoded by different genes and may be detected on the cell surface, in the cytoplasm and the nucleus of various cell types, particularly after activation (33). Further studies are needed to clarify the involvement of these IgE-binding molecules in IgE-mediated functions and the possible regulatory relationships between them.

In the absence of functionally active CD23 in the CD23−/− mice we observed enhanced allergic responsiveness following i.p. sensitization and repeated airway challenge. There are at least two possible explanations: (1) The absence of CD23 may activate another, functionally similar gene which compensates or overcompensates for the loss of CD23. Evidence for this is still missing, although such compensation has been described following targeting of other genes (32). (2) If ligation of CD23 plays an inhibitory role on IgE production, its absence would lead to enhanced responses. Our findings on the effects of anti-CD23 antibody (B3B4) which suppresses IgE production and inhibits the development of airway inflammation and hyperresponsiveness suggest that binding of CD23 with this antibody results in negative regulatory signals (manuscript in preparation). This is supported by studies on CD23 transgenic mice which demonstrate a severely impaired capability to produce IgE (34).

In conclusion, the present experiments demonstrate that CD23 deficient mice are not impaired in their ability to produce IgE or to develop allergic airway inflammation and AHR following active allergic sensitization and challenge. The allergen-induced responses are in fact enhanced in these mice, implying that a negative regulatory effect of CD23 on allergic inflammation may be deficient in the CD23−/− animals.

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References
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