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Allergen-Induced Airway Hyperreactivity Is Diminished in CD81-Deficient Mice

Jun Deng,* V. Pete Yeung,† Daphne Tsitoura,† Rosemarie H. DeKruyff,† Dale T. Umetsu,† and Shoshana Levy2*

We demonstrated previously that CD81−/− mice have an impaired Th2 response. To determine whether this impairment affected allergen-induced airway hyperreactivity (AHR), CD81−/− BALB/c mice and CD81+/+ littermates were sensitized i.p. and challenged intranasally with OVA. Although wild type developed severe AHR, CD81−/− mice showed normal airway reactivity and reduced airway inflammation. Nevertheless, OVA-specific T cell proliferation was similar in both groups of mice. Analysis of cytokines secreted by the responding CD81−/− T cells, particularly those derived from peribronchial draining lymph nodes, revealed a dramatic reduction in IL-4, IL-5, and IL-13 synthesis. The decrease in cytokine production was not due to an intrinsic T cell deficiency because naive CD81−/− T cells responded to polyclonal Th1 and Th2 stimulation with normal proliferation and cytokine production. Moreover, there was an increase in T cells and a decrease in B cells in peribronchial lymph nodes and in spleens of immunized CD81−/− mice compared with wild-type animals. Interestingly, OVA-specific Ig levels, including IgE, were similar in CD81−/− and CD81+/+ mice. Thus, CD81 plays a role in the development of AHR not by influencing Ag-specific IgE production but by regulating local cytokine production. The Journal of Immunology, 2000, 165: 5054–5061.

Asthma is a chronic airway disease characterized by inflammation, airway hyperreactivity (AHR), and reversible airway obstruction (1). The lung inflammatory responses in asthma are tightly associated with airway hyperresponsiveness, and Th2 cells play a critical role in initiating and sustaining asthmatic lung inflammation. In asthma patients, activated CD4+ T cells with predominant Th2 type cytokine (IL-4 and IL-5) expression are present in bronchoalveolar lavage (BAL) fluid and in lung biopsies (2–5). In murine models, the importance of CD4+ Th2 cells in the development of pulmonary allergic inflammation has also been demonstrated. Adoptive transfer of Ag-specific Th2 cells into naive mice induced AHR and lung inflammation upon Ag exposure (6–8). Absent or reduced levels of IL-4 in IL-4−/− mice or in mice treated with anti-IL-4 Ab inhibited both airway eosinophilia and AHR (9, 10). Mice lacking the STAT6 transcriptional factor, important in mediating IL-4-downstream events (11, 12), are protected from allergen-induced bronchial eosinophilia, inflammation, and AHR (13, 14). In addition, neutralization of IL-13, another Th2 cytokine, with the soluble IL-13 receptor α2, significantly attenuated the asthma phenotype in OVA-sensitized and -challenged BALB/c mice (15, 16). We reasoned that because Th2 cells are necessary and sufficient for the induction of AHR, factors affecting Th2 immune responses might influence the development of asthma. One such factor is the CD81 tetraspanin protein.

CD81 (TAPA-1, the target of an anti-proliferative Ab) is a widely expressed cell surface protein involved in a variety of biological responses that has been studied mostly in the context of the immune system (17). On T cells, it associates with CD4 and CD8 and was shown to be involved in T cell differentiation (18–20). On B cells, it associates in a B cell-specific complex with CD19, CD21, and the IFN-inducible Ag Leu13 (21–23), with integrins, and with other tetraspanins (26, 27). Functional studies suggest that CD81 is involved in cell motility, adhesion, proliferation, and differentiation (17). CD81-deficient (CD81−/−) mice have an impaired humoral immune responses to protein Ags (28–30). Chimeric mice, in which only the B cells lacked CD81, were also deficient in Th2 responses as evidenced by their reduced production of Ag-specific IgG1 and IL-4 (31).

The goal of this project was to study the role of CD81 in the context of a physiological Th2-dependent response. For this purpose, we compared the effect of allergen exposure on the development of AHR, airway eosinophil inflammation, and cellular and humoral immune responses in CD81−/− mice and their wild-type littermates. Our results indicate that expression of CD81 is essential for the development of AHR and for local cytokine production although it has no effects on T cell proliferation or on specific IgE response to the allergen.

Materials and Methods

Animals

CD81−/− mice were generated as described (28) and backcrossed six times to BALB/c mice, obtained from Stanford Medical Center Division of Laboratory Animal Medicine (Stanford, CA). After the fourth backcross, CD81−/− mice could no longer reproduce and had to be maintained as heterozygous animals. Heterozygous mice from the sixth backcross were crossed once more to BALB/cByJ mice obtained from The Jackson Laboratory (Bar Harbor, ME). Heterozygous mice from the seventh backcross were bred to produce CD81+/+ and CD81−/− littermates.

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Immunization protocols

Mice were immunized i.p. with 100 μg OVA complexed with aluminum potassium sulfate on days 0 and 14 and challenged intranasally with OVA (50 μg in PBS) on days 14, 25, 26, and 27 (OVA/OVA). Control mice received the same initial immunization but were challenged intranasally with PBS (OVA/PBS). AHR to methacholine was measured on day 28. On the day of AHR measurement, left lungs were removed and bronchoalveolar lavage fluid was collected.

Measurement of OVA-specific Igs

Blood was collected from the tail vein in serum separator tubes (Becton Dickinson, San Jose, CA), and serum was obtained by centrifugation at 1500 × g for 5 min. Serum Ab levels were measured by ELISA. OVA-specific IgG1 was determined by coating plates with 5 μg/ml of OVA overnight and blocking 1 h with 5% milk in PBS. The OVA-specific Ab levels were determined using the following Abs and reagents: mouse anti-OVA IgG1 mAb from Sigma (St. Louis, MO), and goat anti-mouse IgG1-peroxidase. The detection range was 100 – 0.195 ng/ml for IFN-γ, 5000 – 7.5 pg/ml for IL-4, and 5000 – 40 pg/ml for IL-5 and IL-13.

Ag-specific proliferation assay

Splenocytes and peribronchial LN lymphocytes were cultured in 96-well plates (2.5 × 10^5 cells/well) with or without OVA (100 μg/ml) for 4 days. For the last 20 h of culture, cells were pulsed with [3H]thymidine (Amersham, Arlington Heights, IL) in 50 μl of medium. The cells were harvested onto filters using a 96-well harvester (Wallac, Turku, Finland) and read on a 96-well format scintillation counter (Wallac).

Flow cytometry

Mouse splenocytes and peribronchial LN cells were stained with PE-conjugated anti-CD3, biotin-conjugated goat anti-mouse IgG1, and mouse IgE, respectively. The stained cells were analyzed by flow cytometry (Becton Dickinson Immuno cytometry Systems, San Jose, CA).

T cell purification

Single cell suspension from peripheral LN of naive CD81^-/- and CD81^+/- mice was incubated with anti-mouse Thy1.2 Ab-conjugated magnetic beads at 6^-12°C for 15 min. The cells were passed through a MACS-MS^+ separation column and bound cells were collected as the T cell fraction. T cell purity, analyzed by FACScan using FITC-conjugated anti-CD3 Ab, was >95%. All Ab-conjugated beads, columns, and magnetic separators were purchased from Miltenyi Biotec (Auburn, CA).

In vitro T cell differentiation assays

Purified T cells (1 × 10^6) were stimulated on plates coated with anti-CD3 (2 μg/ml) and anti-CD28 (5 μg/ml) in the presence of mouse IL-2 (10 ng/ml). Mouse IL-12 (10 ng/ml) and anti-IL-4 mAb (11B11, 10 μg/ml) were added for Th1 differentiation, and mouse IL-4 (1000 U/ml) and anti-IL-12 mAb (C17.8, 10 μg/ml) were added for Th2 differentiation. Cells were stimulated for 7 days, then washed extensively with PBS/2% FBS and restimulated at 10^5 cells/ml on anti-CD3 and anti-CD28 coated plates in the absence of exogenous cytokine. Supernatants were harvested 24 h later and tested for cytokine levels. For cell proliferation, cells were plated on anti-CD3 and anti-CD28 coated 96-well plates (1 × 10^3 to 2.5 × 10^5/well), and [3H]thymidine incorporation during a 24-h restimulation period was determined.

Statistic analysis

The difference of AHR among groups was analyzed with ANOVA using Prism (Intuitive Software for Science, San Diego, CA) and significant ANOVA values between groups were checked by Bonferroni’s multiple comparison tests. Values of p < 0.05 were considered significant.

Results

Reduction of allergen-induced AHR in CD81^-/- mice

To address whether CD81 plays a role in the development of allergen-induced AHR, mice were immunized i.p. and challenged intranasally with OVA. In wild-type mice, OVA immunization and challenges (OVA/OVA) significantly increased AHR. This effect does not bind mouse IgG1 to capture total IgG2a or IgE in the serum sample. OVA-specific IgG2a or IgE were detected by binding to OVA-biotin. Total IgE was measured by using R35-72 as a capture and R35-92 biotin as a detecting Ab. The standard curves were generated using a 2-fold dilution over 15 wells of purified 200 ng/ml anti-OVA IgG2a, 100 ng/ml anti-OVA IgE, and 500 ng/ml total IgE. The sensitivity of these assays was 6.25 ng/ml for OVA IgG2a and OVA IgE, and 15 ng/ml for total IgE.
was dependent upon the intranasal challenge because mice primed with OVA and challenged with PBS (OVA/PBS) showed very little AHR (Fig. 1). In marked contrast, OVA-sensitized and -challenged (OVA/OVA) CD81−/− mice did not develop a significant increase in AHR and were comparable to their PBS-challenged controls (OVA/PBS). These results demonstrate that CD81 is important in the development of allergen-induced AHR (Fig. 1).

Reduction of allergen-induced lung inflammation, eosinophilia, and mucin production in CD81−/− mice

Examination of lung tissue of wild-type mice stained with hematoxylin and eosin revealed widespread patchy inflammatory infiltrates in the lung after sensitization and intranasal challenge (OVA/OVA) (Fig. 2A). These infiltrates were present mainly in the peribronchiolar and perivascular areas. In contrast, lungs from CD81−/− mice showed much reduced peribronchiolar and perivascular inflammatory infiltrates (Fig. 2B). PBS-challenged wild-type and CD81-deficient mice showed normal lung morphology (Fig. 2, C and D).

The majority of infiltrating cells in the lung of OVA/OVA-treated CD81+/+ mice were eosinophils (Fig. 2E). In contrast, there were only very few eosinophils in lungs of OVA/OVA-treated CD81−/− mice (Fig. 2F). Eosinophils were not present in lungs of PBS-challenged wild-type or CD81-deficient mice (data not shown). It was also observed that many airway epithelial cells of OVA/OVA-treated CD81−/− mice were enlarged and filled with homogenous materials, which stained positively for mucin with PAS stain (Fig. 2G). Airway epithelia from OVA-sensitized and -challenged CD81−/− mice showed no sign of hypertrophy and stained negative for mucin (Fig. 2H).

Analysis of BAL fluid of OVA/OVA-treated wild-type mice revealed large numbers of inflammatory cells, the majority of which were eosinophils (Fig. 3). In contrast, BAL fluid of OVA/OVA-treated CD81−/− mice contained fewer total cells and a trend toward fewer eosinophils, even though their difference was not statistically significant (Fig. 3).

Similar IgE titers in CD81+/+ and CD81−/− mice

Because IgE is thought to be important in the pathogenesis of allergic responses (34), we measured total and OVA-specific IgE levels in OVA/OVA-treated wild-type and CD81−/− mice. In both groups of mice, OVA sensitization by i.p. immunization was associated with significant increases in total and OVA-specific serum IgE. CD81−/− mice produced comparable levels of total and OVA-specific IgE (Table I). Unimmunized mice of both groups produced negligible amounts of IgE (data not shown). These results indicate that the reduction of AHR in CD81−/− mice is not due to reduced OVA-specific IgE production.

Ab isotype levels have been used to assess the relative influence of Th1 cells vs Th2 cells. The Th1 cytokine IFN-γ augments mouse IgG2a production and inhibits IgG1 production, whereas the Th2 cytokine IL-4 has the reverse effect (35). Both wild-type and CD81−/− mice had no detectable level of OVA-specific IgG1 and IgG2a before immunization. Wild-type mice responded to OVA immunization and challenge with significant increases of OVA-specific IgG1 and IgG2a with IgG1 being predominant. Although CD81−/− mice responded in a similar manner, their specific IgG1 response was generally decreased (Table I). However, because of large variations in OVA-specific Ab levels within the same group of mice, the difference in OVA-specific Ig levels between OVA/OVA-treated CD81+/+ and CD81−/− mice was not significant. The total IgG levels between the two strains were not significantly different before and post OVA immunization (prior: CD81+/+ 2367 ± 619 μg/ml; CD81−/− 1655 ± 392 μg/ml; post: CD81+/+ 5841 ± 2363 μg/ml; CD81−/− 4503 ± 1688 μg/ml). These results suggest that the reduced AHR and lung inflammation in CD81-deficient mice are not associated with reduced OVA-specific humoral immune responses.

Comparable Ag-specific lymphocyte proliferation in CD81+/+ and CD81−/− mice

To determine whether the difference in AHR was associated with a difference in cellular immune responses, peribronchial LN lymphocytes and splenocytes from OVA-sensitized and -challenged (OVA/OVA) CD81+/+ and CD81−/− mice were cultured in the presence or absence of 100 μg/ml of OVA for 4 days. During the last 20 h of culture, cells were pulsed with [3H]thymidine and cell proliferation was measured. Both CD81+/+ and CD81−/− lymphocytes responded to OVA with a similar rate of cell proliferation (Fig. 4), indicating that both the draining LNs and the spleens of CD81−/− mice contained OVA-specific T cells, which were capable of responding to the Ag in vitro.

Reduced Th2 cytokine production in CD81−/− mice

Given the correlation between allergic airway disease and increased expression of Th2 cytokines by lung T cells, it was of interest to determine whether the reduced AHR and lung inflammation in CD81−/− mice reflected a change in their OVA-specific cytokine responses. As expected, splenocytes and peribronchial LN lymphocytes of wild-type mice produced considerable amounts of IL-4, IL-5, and IL-13 in response to OVA (Fig. 5, A–C, respectively). In contrast, the production of Th2 cytokine by CD81−/− peribronchial LN cells was reduced 15-fold (IL-4), 2-fold (IL-5), or was undetectable (IL-13) (Fig. 5, A, C, respectively). The effect on local cytokine production was less pronounced systemically as CD81−/− splenocytes produced about half the amounts of IL-4 and IL-13 as compared with CD81+/+ splenocytes (Fig. 5, A and C) and comparable low levels of IL-5 (Fig. 5B).

Although OVA-stimulated splenocytes produced IFN-γ, peribronchial LN cells secreted negligible levels of this cytokine. However, IFN-γ levels were reduced in both splenocytes and peribronchial LNs derived from CD81−/− mice (Fig. 5D).

**FIGURE 1.** Reduced AHR in CD81−/− mice. CD81−/− mice and their wild-type littermates were immunized i.p. with OVA in aluminum potassium sulfate (alum) on days 0 and 14 and challenged intranasally with OVA on days 14, 25, 26, and 27. Control groups were immunized i.p. with OVA but were challenged intranasally with PBS. AHR was measured on day 28. AHR was expressed as enhanced pulse (Penh). Values are expressed as mean ± SD. **p < 0.01; *p < 0.05 comparing OVA/OVA-treated wild-type and CD81−/− mice. Results are representative of three different experiments (n = 4–6 per group).
Reduced numbers of B cells in peribronchial LN and spleen of immunized CD81−/− mice

The reduced cytokine profiles secreted in vitro by OVA-stimulated CD81−/− T cells could be due to an imbalance of lymphoid subsets in these organs. To test this notion, the composition of lymphoid cells in spleen and peribronchial LNs was analyzed using T (FITC-conjugated anti-CD3 mAb) and B (PE-conjugated anti-CD19 mAb) cell markers after immunization and challenge with OVA. The proportion of B and T cells were identical in unimmunized CD81−/− and CD81+/+ mice (Refs. 28–30 and data not shown). However, in response to immunization the percentage of B cells was reduced considerably in peribronchial LN and to a lesser degree in spleens of CD81−/− mice in comparison with CD81+/+ mice (Table II). Conversely, the T cell percentage of CD81−/− mice was slightly but consistently higher than that seen in CD81+/+ mice (Table II). Because Ag-specific B cells function as potent APC in secondary immune responses (36), the reduction of B cells in CD81−/− mice might have contributed to reduced cytokine production.

Naive CD81−/− T cells can proliferate and produce normal levels of Th1 and Th2 cytokines when stimulated in vitro

Having shown that the reduced cytokine production in CD81−/− mice was not due to a reduction in T cell numbers, it was important to determine whether CD81−/− T cells were intrinsically deficient in cytokine production. Therefore, we tested whether cytokine responses of T cells from naive CD81−/− differed from those of CD81+/+ mice when induced to secrete Th1 and Th2 cytokines under polarizing stimulation conditions. T cells from all experimental conditions showed identical high levels of proliferation (Fig. 6A). IL-12 induced the polarization of CD81+/+ and CD81−/− T cells to the Th1 phenotype, characterized by high level of IFN-γ and very little IL-4 production. Conversely, IL-4 induced the Th2 phenotype characterized by a high level of IL-4 and a very low level of IFN-γ production. CD81−/− and CD81+/+ T cells produced comparable levels of IFN-γ and IL-4 in response to Th1 or Th2 stimuli, respectively (Fig. 6B). These results suggest that the reduced cytokine production in immunized CD81−/− mice was not due to an intrinsic defect in their T cells.
In this study, we demonstrate that CD81 is essential for the development of allergen-induced AHR. CD81−/− mice, unlike their wild-type littermates, do not develop AHR to an intranasal challenge with the OVA allergen (Fig. 1). In addition, allergen-induced lung inflammation, eosinophilia, and mucous cell hyperplasia are also reduced in CD81−/− mice (Figs. 2 and 3). These reduced responses are associated with a marked decrease in cytokine production by peribronchial LN cells (Fig. 5). Interestingly, reduced AHR in CD81−/− mice is not due to deficient production of Ag-specific IgE because levels of anti-OVA IgE in these mice are comparable to those of wild-type mice (Table I). These results provide the first evidence that CD81 is necessary for the development of allergen-induced AHR.

Because CD4+ Th2 cells play a crucial role in initiating and sustaining allergen-induced airway responses (2, 3, 8) and because CD81 is necessary for proper Th2 responses, we examined the role of CD81 in local and systemic cytokine production in an allergen-induced AHR model. Our results demonstrate that CD81 is important for allergen-induced cytokine production, especially for local Th2 cytokine production by peribronchial LN (Fig. 5). Production of IFN-γ, the Th1 cytokine, although low to begin with, was reduced in the CD81−/− mice compared with their wild-type littermates. The impairment in cytokine secretion was not due to

![Table I](https://via.placeholder.com/150)

**Table I. OVA-specific Ig (μg/ml) levels in OVA/OVA-immunized mice**

<table>
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<tr>
<th>Mice</th>
<th>OVA-IgG1</th>
<th>OVA-IgG2a</th>
<th>OVA-IgE</th>
<th>Total IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD81+/+</td>
<td>306 ± 136</td>
<td>16 ± 8.8</td>
<td>2.2 ± 0.8</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>CD81−/−</td>
<td>75 ± 71</td>
<td>15 ± 8.7</td>
<td>3.3 ± 2.7</td>
<td>6.0 ± 5.4</td>
</tr>
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CD81−/− mice and their wild-type littermates were immunized and challenged with OVA as described in Fig. 1. Serum samples were collected before OVA immunization and 1 day after AHR measurement. Serum anti-OVA IgG1, IgG2a, IgE, and total IgE were determined by ELISA. OVA-specific Abs were not detectable before OVA immunization. Data are expressed as mean ± SE and are representative of three experiments (n = 4–6 per group in each experiment).

![Figure 3](https://via.placeholder.com/150)

**FIGURE 3.** Reduced inflammatory cells in BAL fluid of CD81−/− mice. On day 29, 1 day after AHR measurement, BAL was performed as described in Materials and Methods. Total cells and relative number of the indicated type of leukocytes were determined with Wright-Giemsa stain and expressed as mean ± SE.

![Figure 4](https://via.placeholder.com/150)

**FIGURE 4.** Ag-specific cell proliferation is similar in CD81+/+ and CD81−/− mice. One day after AHR measurement, lymphocytes from spleens and peribronchial LNs of OVA-challenged (OVA/OVA) CD81+/+ and CD81−/− mice were cultured in the presence or absence of OVA (100 μg/ml) for 4 days in 96-well plates. During the last 20 h of culture, cells were pulsed with [3H]thymidine (1 μCi/well), and its incorporation is expressed as mean ± SD of triplicate wells. Splenocytes and peribronchial LN cells of OVA/OVA-treated mice from both groups showed high levels of proliferation in response to OVA. Results are representative of three different experiments (n = 6 per group).

![Figure 5](https://via.placeholder.com/150)

**FIGURE 5.** Th2 type cytokines are considerably reduced in CD81−/− mice. Peribronchial LN and spleen lymphocytes from OVA/OVA-treated CD81−/− and CD81+/+ mice were cultured for 4 days as described in Fig. 4. Levels of IL-4 (A), IL-5 (B), IL-13 (C), and IFN-γ (D) secreted to the culture medium were measured by cytokine ELISA. IL-4 and IFN-γ were expressed as mean ± SD of triplicate cultures. IL-5 and IL-13 were measured in pooled medium from triplicate wells. Results presented in (A) and (D) are representative of three different experiments and in (B) and (C) of two different experiments (n = 6 per group).
reduced OVA-specific T cell response because a similar proliferative response was seen in CD81−/− and wild-type mice (Fig. 4). Nor was it due to decreased T cell numbers (Table II) or to an innate ability of CD81−/− T cells to produce cytokines. This was supported by evidence that when naïve CD81−/− T cells were stimulated in vitro with anti-CD3 and anti-CD28 in the presence of either IL-12 or IL-4, they could polarize into Th1 and Th2 phenotypes, respectively, and were able to produce normal levels of IFNγ and IL-4 (Fig. 6).

It is likely that the reduced B cell number in OVA-immunized and -challenged CD81−/− mice (Table II) contributes to reduced cytokine production because Ag-specific B cells function as potent APCs in response to cognate Ag (36). In contrast, we have demonstrated that T cells of CD81−/− mice have the ability to respond to the Ag and the innate ability to produce cytokines. Thus it is possible that the deficiency in their interactions with B cells and other APCs is the cause for the impaired Th2 immune response in vivo.

Reduced local Th2 cytokine production in OVA/OVA-treated mice is most likely the cause of reduced lung eosinophil infiltration (Figs. 2 and 3). IL-4, IL-5, and IL-13 are all involved directly or indirectly in the development of eosinophilic lung inflammation in murine asthma models. IL-5 has been considered a major player in promoting the activation and maintenance of eosinophils in tissues (37, 38). IL-4 may contribute to pulmonary eosinophilia directly by inducing endothelial VCAM-1 expression, which together with integrin αvβ3 expression on eosinophils increases eosinophil adherence to the vessels (39, 40). IL-4 may also contribute to pulmonary eosinophilia indirectly via its effect on Th2 response and IL-5 synthesis. IL-13 not only activates eosinophils and promotes their differentiation (41, 42), it also enhances eotaxin expression by airway epithelial cells (43). Because these Th2 cytokines were reduced in CD81−/− mice, it is possible that CD81 may affect allergen-induced pulmonary eosinophilia through regulation of local Th2 cytokine production. However, CD81 may also directly influence eosinophil maturation and function because CD81 expression is increased when human eosinophils are induced to mature by helminth or by exposure to cytokines (IL-13 and GM-CSF) (44). In addition, stimulation of human eosinophils with anti-CD81 mAb causes down-regulation of L-selectin, a sign of eosinophil activation (45).

Lack of CD81 was also associated with reduced mucous cell hyperplasia and mucous secretion (Fig. 2), which may also be due to reduced Th2 cytokine production. Several recent studies have demonstrated that IL-4 plays an important role in airway goblet cell differentiation and mucous secretion. For example, blockade of IL-4 receptor prevented Ag-induced mucous-containing cells (46), and addition of IL-4 to the culture medium induces MUC2 gene expression and mucous glycoconjugate production in a cultured epithelial cell line (47). In addition, neutralization of IL-13 prevents allergen-induced increase in mucous-containing cells in the airway (16). Thus, the reduced mucous secretion in CD81−/− mice was most likely a secondary event following reduced IL-4 and IL-13 production.

Epidemiological data have demonstrated an association between elevated IgE levels and bronchial asthma (48). It is believed that
increased IgE levels contribute to AHR by binding the IgE-Fc receptor, thereby inducing mast cell degranulation. Upon degranulation, mast cells release a variety of mediators such as bronchoconstrictors, which may contribute to the development of AHR (49, 50). Here we show that CD81<sup>−/−</sup> mice display no AHR after OVA sensitization and challenge even though they have normal levels of total and allergen-specific IgE (Table I). It should be noted that CD81 has been previously implicated in mast cell degranulation. Fleming et al. have used an assay aimed at identifying molecules that will inhibit IgE-induced mast cell degranulation and have identified an anti-CD81 mAb as such an inhibitor (51). Thus, it is possible that the reduced AHR in the presence of comparable IgE levels, as seen in CD81<sup>−/−</sup> mice, could be due to a failure of mast cells to degranulate in response to IgE-cross-linking.

In summary, our results demonstrate that CD81 is essential for the development of allergen-induced AHR. It is likely that disruption of CD81 negatively affects AHR mainly by reducing local Th2 cytokine response, which, in turn, regulates lung inflammation and mucus production in the airway. In addition, CD81 may affect mast cell degranulation and eosinophil functions. Further dissection of the immunological and physiological defect of these mice may provide targets for the development of new antiasthma therapies.

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