

Cockroach Allergen-Induced Eosinophilic Airway Inflammation in HLA-DQ/Human CD4⁺ Transgenic Mice¹

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Airway eosinophilic inflammation is a characteristic feature of allergic asthma. Exposure to allergens produced by the German cockroach (*Blattella germanica*) is a risk factor for allergic disease in genetically predisposed individuals, and has been linked to an increase in asthma morbidity among cockroach-sensitive inner city children. To determine the role and contribution of specific HLA class II in the pathogenesis of allergic airway inflammation in cockroach-induced asthma, we generated double-transgenic, double-knockout mice expressing human HLA-DQ8, HLA-DQ6, and CD4 molecules in the absence of mouse class II and mouse CD4. Mice were actively immunized and later challenged intranasally with cockroach allergen extract. These mice developed bronchoalveolar lavage fluid (BALF) eosinophilia and pulmonary eosinophilia. This was accompanied by an increase in total protein levels, IL-5, and IL-13 in BALF. There were also elevated levels of cockroach-specific serum IgG1 and total serum IgE. Histological analysis revealed peribronchial and perivascular eosinophilic inflammation in cockroach-treated mice. Other pathologic changes in the airways were epithelial cell hypertrophy and mucus production. Treatment with anti-DQ mAb significantly reduced pulmonary and BALF eosinophilia in cockroach allergen-sensitized mice. A β^0 mice and transgenic mice expressing human CD4 molecule alone (without class II) or human HLA-DQ8 molecule (without CD4) treated in the same fashion showed no eosinophilia in bronchoalveolar fluid and no pulmonary parenchymal inflammation. Our results provide direct evidence that HLA-DQ molecules and CD4 T cells mediate cockroach-induced eosinophilic inflammation in the airways. *The Journal of Immunology*, 2001, 167: 4627–4634.

Asthma is a chronic inflammatory disease of airways with a multifactorial pathogenesis. Pulmonary inflammation is a central pathologic feature of allergic asthma (1). The severity of the disease is correlated with the degree of inflammation, particularly the degree of eosinophil and lymphocyte infiltration (1–3).

Both genetic and environmental factors contribute to the development of the disease. Genetic susceptibility to atopic asthma is thought to be polygenic. Although the gene(s) predisposing to asthma and atopy has not yet been identified, various genetic studies in human and other species have shown that specific HLA haplotypes are associated with various allergic responses (4).

The class II molecules of the MHC are highly polymorphic cell surface glycoprotein encoded by gene families located on chromosome 6p. Their primary function is to regulate the immune response by binding Ag-derived peptides and presenting them to T lymphocytes, which will result in T cell activation and initiation of immune response against that Ag (5).

Ragweed allergy (6–8), house dust mite asthma (9, 10), aspirin-induced asthma (11), soybean epidemic asthma (12), and occupational asthma (13) were found to be associated with specific HLA-DR and DQ haplotypes. Using experimental animal models,

it has been shown that HLA-DQ8 transgenic (tg)³ mice generate a DQ-restricted response to Der p Ags (14), and HLA-DQ6 and DQ8 tg mice recognize different epitopes of ragweed major Ag (15). Short ragweed allergen was found to induce eosinophilic lung disease in HLA-DQ6 and DQ8 tg (16). Besides these known allergens, cockroach allergen (CRa) has long been recognized as a major indoor allergen, particularly in inner city children with asthma. The rising morbidity and mortality of asthma in this population may be explained by the high levels of CRa exposure in their homes (17–19). In addition, cockroach allergy was found to be an important risk factor for emergency room visits for asthma and hospital admission (20). The most common domiciliary cockroach species are *Blattella germanica* (German cockroach), *Periplaneta americana* (American cockroach), and *Blatta orientalis* (Oriental cockroach) (21). *Blattella germanica* is prevalent, particularly in large, crowded cities in the southern United States and in tropical countries throughout the world (22). Inhalation of German CRa induces IgE Ab production and the development of asthma in genetically predisposed individuals. Extensive human linkage studies have not been performed to associate HLA and CRa in human allergic diseases. The only association reported was between HLA-DRB1*01 and allergy to *P. americana*/*B. germanica* (23).

To determine whether HLA genes are involved in inducing allergic airway inflammation in cockroach-associated asthma, tg mice expressing human HLA-DQ8 or HLA-DQ6 were used for in vivo analyses. We have previously shown that these mice respond to CRa extract in vitro, generating primarily Th2-type cytokines (24). The in vitro response was mediated by CD4⁺ T cells and was HLA-DQ restricted. In this study, we show that tg mice expressing

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³ Abbreviations used in this paper: tg, transgenic; AHR, airway hyperreactivity; BAL, bronchoalveolar lavage; BALF, BAL fluid; CRa, cockroach allergen; hCD4, human CD4; i.n., intranasal(ly); mCD4, murine CD4; PenH, enhanced pause; TP, total protein.

both human CD4 (hCD4) and either HLA-DQ8 or HLA-DQ6 developed bronchoalveolar lavage (BAL) eosinophilia and pulmonary parenchymal eosinophilia, in response to CRa immunization. This was accompanied by an increase in total protein (TP) levels, IL-5, and IL-13 in BAL fluid (BALF). There were also elevated levels of cockroach-specific serum IgG1 and total serum IgE. This phenomenon appeared to be due to modulation of the immune response by HLA-DQ-mediated Ag presentation, because treatment with Ab to DQ significantly reduced the pulmonary eosinophilia and lung tissue damage.

Materials and Methods

Mice

Tg mice expressing HLA-DQ8 (HLA-DQA1*0301 and HLA-DQB1*0302) and HLA-DQ6 (HLA-DQA1*0103 and HLA-DQB1*0601) were produced by our tg laboratory, as previously described (25, 26). The HLA-DQ tg mice were mated to class II-deficient $A\beta^0$ (27), CD4 knockout (28), and hCD4 tg mice (29) to generate double-tg/double-knockout mice. Mice were bred and maintained in the pathogen-free Immunogenetics Mouse Colony at the Mayo Clinic (Rochester, MN). All procedures performed on the mice were in accordance with the Mayo Institutional Animal Care and Use Committee.

Antigens

Cockroach, *B. germanica* (5.9 mg/ml TP), extract was purchased from Bayer (Elkhart, IL).

Anesthetic agent

Stock avertin solution: 10 g of 99% 2,2,2-tribromoethanol (Aldrich Chemical, Milwaukee, WI) was dissolved in 10 ml of *tert*-amyl alcohol (catalogue no. A730-1; Fisher, Pittsburgh, PA). One hundred microliters of stock solution were diluted in 5 ml of PBS. An i.p. injection of 1 ml/mouse was used for euthanizing, and 0.25 ml was administered for anesthesia.

Ag sensitization and challenge protocol

On the first day of the experiment (day 0) and day 7, each experimental group of mice was actively immunized by i.p. injection of 50 μ g of cockroach extract, adsorbed to 1 mg of aluminum hydroxide in 0.5 ml of sterile endotoxin-free PBS (Life Technologies, Grand Island, NY), pH 7.2. On day 14, mice were challenged intranasally (i.n.) twice with a 6-h interval by application of 200 μ g/100 μ l dialyzed extract in the nostrils under light anesthesia. Intranasal application was performed, as previously described (16). The provocation volume of allergen was selected on the basis of distribution studies in the respiratory tract of solutions administered i.n. (16). Control mice were injected with an equal volume of aluminum hydroxide suspension in PBS, and challenged i.n. with PBS alone.

Ab administration

Purified anti-HLA-DQ mAb IVD12 (obtained from cell line prepared in our laboratory) or isotype-matched IgG1 Ab MOPC-31c (Sigma, St. Louis, MO) was used. A total of 200 μ g of mAb was injected i.p. 24 h before and 24 h after each allergen application, including priming, sensitization, and i.n. challenge with allergen.

BAL

Mice were euthanized 48 h after the last i.n. challenge. Lung lavage was performed as previously described (16). The BALF was immediately centrifuged at $200 \times g$ for 5 min. The BAL cells were resuspended in 1 ml of PBS and counted using a hemacytometer. Differential counts were performed on cytospin cell preparations stained by Giemsa (Cytospin 2; Shandon, Pittsburgh, PA). Cells were identified as macrophages, eosinophils, neutrophils, and lymphocytes based on morphology and staining characteristics using light microscopy. The absolute number and percentage of each cell type were then calculated.

TP concentration in BALF

The level of TP concentration was determined using a commercial kit (Bio-Rad, Hercules, CA) using the Bradford method.

Cytokine levels in BALF

Quantification of IL-4, IL-5, IL-10, IL-12, IL-13, and IFN- γ was performed by sandwich ELISA, as previously described (24). Mini-kits for IL-4 and IFN- γ from Genzyme Diagnostics (Cambridge, MA) were used according to the recommendations provided. IL-5 content was measured using mini-kit from Endogen (Cambridge, MA). The minimum detectable level of cytokine in each of the ELISAs was 3 pg/ml (IL-4), 10 pg/ml (IL-5), 30 pg/ml (IL-9, IFN- γ , IL-13), 20 pg/ml (IL-10), and 9 pg/ml (IL-12p70).

Lung histology

In separate experiments, the lungs were excised, fixed with 10% Formalin, and embedded in paraffin. The Pathology Department of the Mayo Clinic performed the histological sectioning of the lung and staining with H&E solution (16). To characterize the intensity of the inflammatory infiltrate in the lung, a previously described grading scheme was used (30). Grade 0 showed no focal inflammation or peribronchial or perivascular inflammatory infiltrates. Grade 1 had one or two centrally located foci of inflammatory infiltrate. Grade 2 had a dense inflammatory infiltrate in a perivascular and peribronchial distribution originating in the center of the lung and extending along the vessels and bronchi into the middle third of the lung parenchyma. Grade 3 showed extension of the inflammatory cells to the periphery of the lungs approaching the visceral pleura. Grading was performed blinded on unidentified slides examined by light microscopy.

Lung physiology

Airway hyperreactivity (AHR) in PBS- or CRa-sensitized mice was measured 48 h after the last i.n. challenge by recording respiratory pressure curves by whole body plethysmography (model PLY 3211; Buxo Electronics, Sharon, CT), as described previously (31). Values of enhanced pause (PenH) were calculated by BioSystem XA software, averaged, and expressed for each methacholine concentration as percentage of baseline PenH values following PBS exposure.

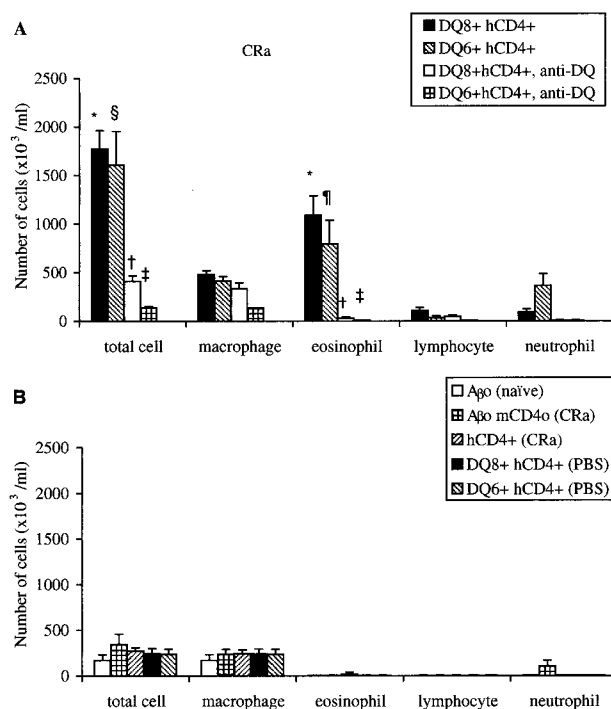


FIGURE 1. Inflammatory cells in lung lavage fluid. BALF were obtained 48 h after last CRa (A) or PBS i.n. challenge. The columns represent the absolute numbers of total BAL cells, macrophages, eosinophils, lymphocytes, and neutrophils. Data represent the mean $\pm$ SD of counts of assays involving four to six mice. *, $p < 0.001$ compared with unimmunized $A\beta^0$, PBS-treated HLA-DQ8/hCD4 $^+$, and CRa-treated $A\beta^0$ mCD4 ^{0} and hCD4 $^+$ tg mice (B); §, $p < 0.007$ and ¶, $p < 0.01$ compared with unimmunized $A\beta^0$, PBS-treated HLA-DQ6/hCD4 $^+$, and CRa-treated hCD4 $^+$ tg mice (B). †, $p < 0.001$; ‡, $p < 0.007$ compared with mAb-untreated counterparts.

Allergen-specific Ab levels and serum total IgE

Mice were bled before the first immunization with CRa, bled on days 6 and 13 during the sensitization protocol, and bled at 48 and 96 h after i.n. challenge. Blood was centrifuged ($1500 \times g$ for 10 min), and serum was separated and stored at -70°C until analysis. The levels of cockroach-specific Ab were measured by ELISA, as previously described (16). Briefly, plates (Immulon 1; Dynatech Laboratories, Chantilly, VA) were coated with $10 \mu\text{g/ml}$ cockroach extract in PBS. After incubating with sera, avidin-conjugated alkaline-phosphatase (Southern Biotechnology Associates, Birmingham, AL) was added to the wells. Optimal concentration of phosphatase substrate, *p*-nitrophenyl phosphate (Southern Biotechnology Associates) was added, and plates were read at 415 nm in a microtiter autoreader (Bio-Rad, Hercules, CA). Serum Ab concentrations were determined by comparison with serially diluted purified isotype standards (Southern Biotechnology Associates), using the Microplate Manager software for the Macintosh computer (Bio-Rad).

Statistics

Data are expressed as the mean $\pm$ SD. Student's *t* test was used to calculate significance levels between experimental groups. Values of $p < 0.05$ were considered to be significant.

Results

Cellular constituents of lung lavage fluid

The effects of in vivo CRa sensitization and challenge on airway inflammation in tg mice were examined by analyzing the cellular

composition of BALF at 48 h after the second i.n. challenge. The values for total leukocyte numbers and differential leukocyte counts in BALF are shown in Fig. 1. BALF taken from unimmunized $\text{A}\beta^0$ and HLA-DQ8/hCD4 mice yielded $170,000 \pm 62,556$ and $296,000 \pm 30,594$ cells/ml, respectively. The majority of cells were macrophages (Fig. 1B). Tg mice expressing human HLA-DQ8, HLA-DQ6, and CD4 molecules showed significant increases in both the total cell numbers ($p < 0.001$ and $p < 0.007$, respectively) and eosinophils (representing the primary cell type) ($p < 0.001$ and $p < 0.01$, respectively) after immunization and challenge with CRa extract (Figs. 1A and 2, B and C). These mice showed a decrease in the percentage of macrophages in BALF when compared with PBS-treated control mice (Fig. 2A), CRa-treated $\text{A}\beta^0$ murine CD4⁰ (mCD4⁰) mice, and tg mice expressing human HLA-DQ8 molecule (without CD4) or hCD4 molecule alone (without class II) (Fig. 2D), in which the majority of cells were macrophages (Fig. 1B). Of note, BAL total cell counts and eosinophil counts from tg mice expressing human HLA-DQ6 molecule (without CD4) did not differ significantly from those obtained in HLA-DQ6/hCD4⁺ tg mice (data not shown). Lymphocytes and neutrophils were also present in both HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice, but in a mild degree.

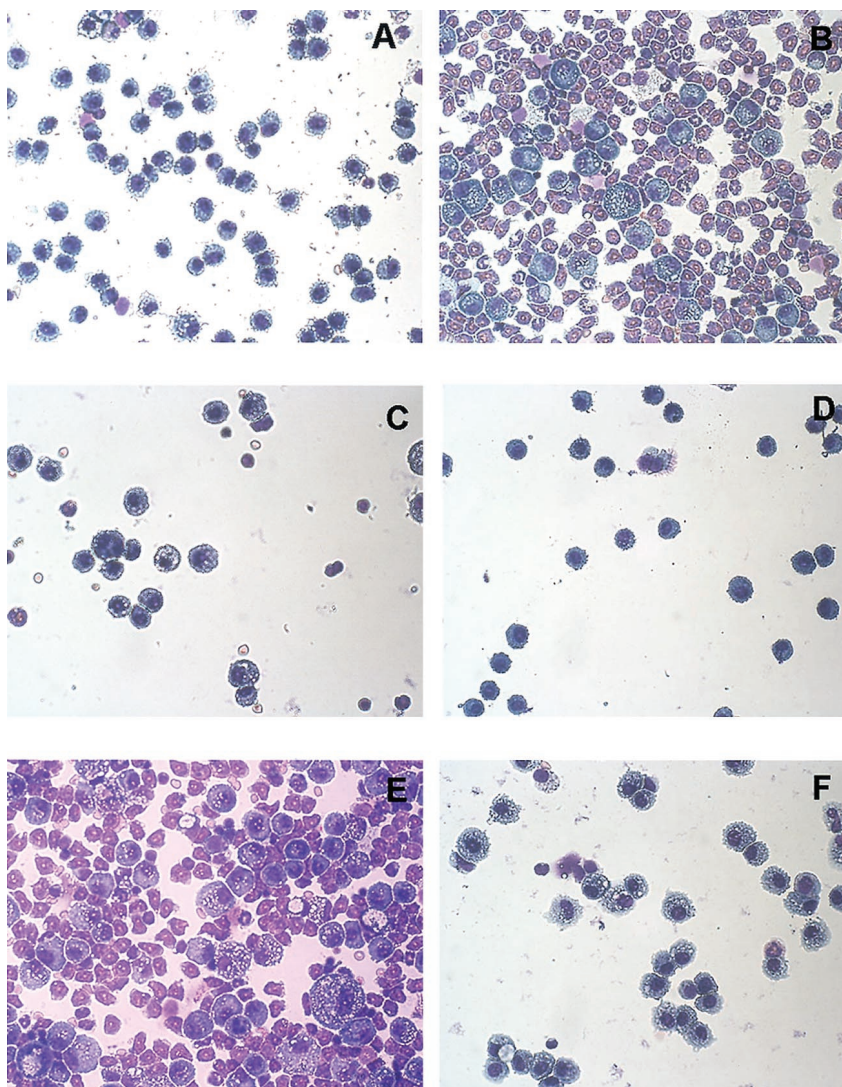


FIGURE 2. Representative examples of BALF cell composition. No eosinophils were recovered in BALF from PBS + Al-treated HLA-DQ8/hCD4⁺ tg mice, macrophages being the only cell type found (A). Eosinophils were the dominant cell type in BALF of CRa-treated HLA-DQ8/hCD4⁺ (B). Lower levels of total number of cells, being primarily macrophages, were detected in anti-DQ-treated CRa-immunized HLA-DQ8/hCD4⁺ (C) as compared with mAb-untreated counterpart. Macrophages were the primary cell type in tg mice expressing hCD4 molecule alone (without class II) (D). CRa-treated HLA-DQ6/hCD4⁺ tg mice (E) showed greater numbers of eosinophils as compared with anti-DQ-treated CRa-immunized counterpart (F), macrophages being the predominant cell type.

Effect of anti-DQ mAbs

To demonstrate the role and contribution of HLA-DQ molecules in the development of allergic airway inflammation, HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice were injected i.p. with anti-DQ mAb (IVD12) or isotype-matched IgG1, as described in *Materials and Methods*. The pretreatment of CRa-primed tg mice with anti-DQ mAb was associated with a significant reduction in the total leukocytes number and eosinophil counts on lung lavage fluid elicited by CRa airway challenges (Fig. 1A). Eosinophils represented only 6–8% of the total cells recovered, with macrophages being the major cell type (>80%) (Fig. 2, C and F). There were also fewer macrophages, lymphocytes, and neutrophils in BALF of anti-DQ mAb-treated tg mice (Fig. 1A). No significant differences in either total number of cells or differential counts on lung lavages were observed in HLA-DQ6⁺/hCD4⁺ and HLA-DQ8⁺/hCD4⁺ tg mice treated with control isotype Ab (mouse IgG1) compared with CRa-treated counterparts (data not shown). These results suggest that HLA-DQ molecules mediate CRa-induced eosinophilic inflammation in the airways.

TP contents in BALF

To determine whether lung permeability was altered by CRa inoculation, TP concentration was measured in BALF at 48 h after the i.n. challenge (Fig. 3). Immunized and challenged with CRa extract, tg mice expressing human HLA-DQ8 or DQ6 and CD4 molecules showed significantly higher levels of TP concentration compared with PBS-treated counterparts, or compared with CRa-treated tg mice expressing human HLA-DQ8 molecule (without CD4) ($p < 0.001$) or hCD4 molecule alone (without class II) ($p < 0.006$). Pretreatment with anti DQ mAb significantly decreased the TP level by 67% in HLA-DQ8 and by 77% in HLA-DQ6 tg mice ($p < 0.001$). Of note, high level of TP was detected in BALF taken from tg mice expressing human HLA-DQ6 molecule alone (without human or mouse CD4).

Cytokine levels in BALF

To determine the emerging response (Th1 and/or Th2) in the lungs of these animals after CRa exposure, we measured cytokines in BALF taken 48 h after last i.n. administration. High levels of IL-5 and IL-13 were detected in HLA-DQ8/hCD4⁺ tg mice in response

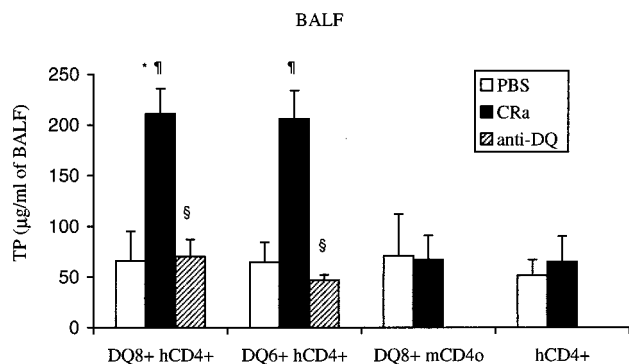


FIGURE 3. TP levels in BALF supernatants taken 48 h after last PBS or CRa i.n. challenge. TP levels were measured by the Bradford method. High levels of TP were detected in both CRa-treated HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice, compared with CRa-treated HLA-DQ8⁺ mCD4⁰ and hCD4⁺ tg mice. A significant decrease in TP was present in both HLA-DQ8 and HLA-DQ6 mice after treatment with anti-DQ mAb. *, $p < 0.001$ compared with CRa-treated DQ8⁺ mCD4⁰; §, $p < 0.006$ compared with CRa-treated hCD4⁺ tg mice; §, $p < 0.001$ compared with mAb-untreated CRa-immunized counterparts.

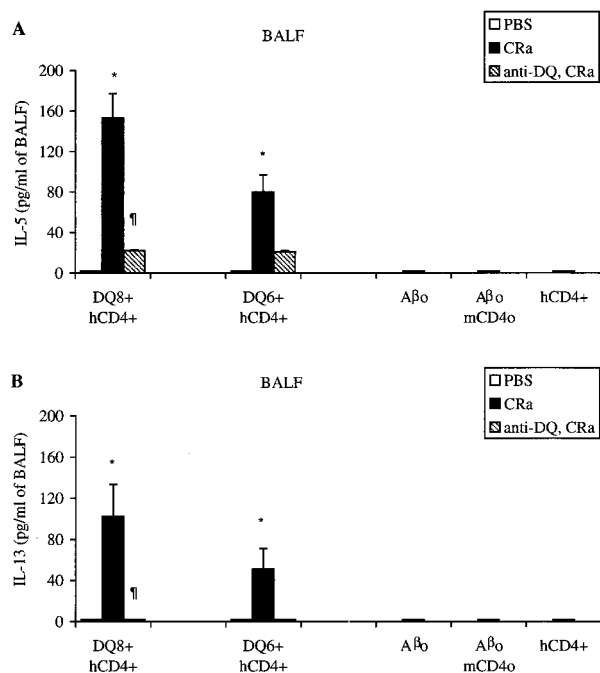


FIGURE 4. Assessment of IL-5 and IL-13 levels in BALF supernatants. BALF were obtained from each group of mice ($n = 4-8$) at 48 h after last i.n. challenge with PBS or CRa. Cytokine levels were measured by ELISA. An increase in IL-5 (A) and IL-13 (B) was present in both HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice compared with PBS-treated counterparts. No detectable levels of IL-13 and minimal amounts of IL-5 were found in anti-DQ-treated CRa-sensitized HLA-DQ8 and HLA-DQ6 tg mice. No measurable levels of IL-5 (A) and IL-13 (B) were present in CRa-treated Aβ⁰ mice, Aβ⁰ mCD4⁰ mice, or tg mice expressing hCD4⁺ molecule alone (without class II). *, $p < 0.001$ (DQ8/hCD4⁺ mice) and $p < 0.02$ (DQ6/hCD4⁺ mice) compared with PBS-treated counterparts and CRa-treated Aβ⁰, Aβ⁰ mCD4⁰, and hCD4⁺ tg mice; §, $p < 0.001$ compared with mAb-untreated CRa-immunized counterpart.

to CRa sensitization (153 ± 24 and 102.6 ± 30.7 pg/ml, respectively) (Fig. 4). The levels of IL-5 and IL-13 in BALF taken from HLA-DQ6/hCD4⁺ tg mice were lower than those obtained in

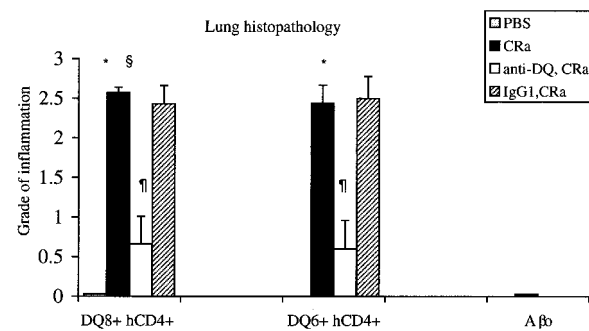


FIGURE 5. Grade of pulmonary inflammation based on a grading scheme, as described in *Materials and Methods*. CRa-treated Aβ⁰ mice and PBS-treated HLA-DQ8/hCD4⁺ tg mice showed no inflammatory infiltrates. High grade of inflammation was noted in both CRa-treated HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice. Significant reduction in the degree of inflammation in both HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice was observed after treatment with anti-DQ mAb compared with CRa-treated counterparts. No differences in the grade of inflammation were noted in control isotype Ab (mouse IgG1) compared with CRa-treated counterparts. *, $p < 0.001$ compared with CRa-treated Aβ⁰ mice; §, $p < 0.001$ compared with PBS-treated counterpart; §, $p < 0.001$ compared with mAb-untreated CRa-immunized counterparts.

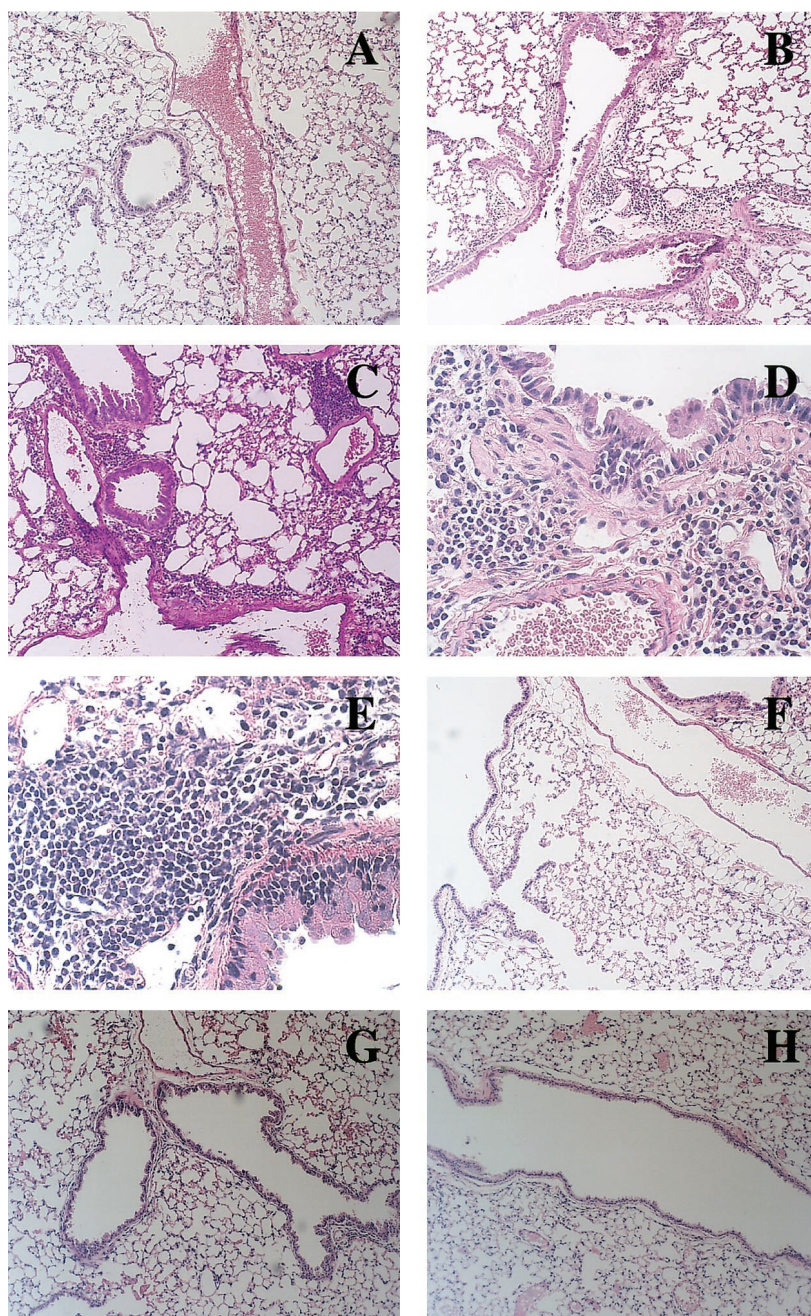
HLA-DQ8/hCD4⁺ tg mice (80 ± 17 and 51 ± 20 pg/ml, respectively) (Fig. 4). Minimal amounts of IL-10 were found only in HLA-DQ8/hCD4⁺ tg mice (data not shown). No IL-4, IL-9, IL-12 p70, or IFN- γ was detected in BALF in any tg mice (data not shown). There were no detectable levels of any of the cytokines in BALF obtained from unimmunized or PBS-treated DQ8/hCD4⁺ and DQ6/hCD4⁺ mice, CRa-treated A β ⁰ mice, A β ⁰ mCD4⁰ mice, and tg mice expressing human HLA-DQ8 molecule (without CD4) or hCD4 molecule alone (without class II). Mice pretreated with anti-DQ mAb showed a significant reduction in IL-5 and IL-13 levels in BALF. The data suggest that the decrease in local allergen-driven IL-5 and IL-13 production may be the basis for the reduction of mucus production and eosinophilic airway inflammation in HLA-DQ tg mice.

Histologic assessment of lung tissue

Examination of the lungs taken from CRa-treated A β ⁰ (Fig. 5) or unimmunized HLA-DQ/hCD4⁺ tg mice showed no inflammatory

infiltrates and were uniformly scored as grade 0. The lungs of HLA-DQ8/hCD4⁺ tg mice sensitized with aluminum hydroxide in PBS and challenged i.n. with PBS displayed no inflammatory responses (Fig. 6A). In contrast, inflammatory cell infiltrates consisting predominantly of eosinophils were observed in CRa-treated HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice (Figs. 5 and 6, B–E). These infiltrates were present in a peribronchial and perivascular distribution (grade 2), extending to the periphery of the lungs and approaching the visceral pleura (grade 3). Epithelial alterations were also noted in larger airways of both HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice. These changes included airway epithelial cell hypertrophy and mucus production. Tg mice expressing human HLA-DQ8 alone (without CD4) showed minimal areas of inflammation (grade 0.53), consisting primarily of mononuclear cells (Fig. 6F). The pretreatment of both CRa-primed HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice with anti-DQ mAb significantly decreased the magnitude of the lung tissue eosinophilic infiltration induced by CRa airway challenges ($p < 0.002$ and $p <$

FIGURE 6. Histologic examination of lung tissue. Sections of Formalin-fixed lung tissue were stained with H&E before examination by light microscopy. The lungs taken from PBS-treated HLA-DQ8/hCD4⁺ mice (A, $\times 10$) represented normal lung histology. In contrast, lungs taken from CRa-treated HLA-DQ8/hCD4⁺ (B, $\times 10$) and HLA-DQ6/hCD4⁺ (C, $\times 10$) tg mice showed a dense peribronchial and perivascular inflammatory infiltrate. Higher magnification ($\times 40$) revealed that the infiltrate consisted predominantly of eosinophils, and there were epithelial cell hypertrophy and mucus hyperproduction (D and E). Minimal inflammation consisting primarily of mononuclear cells was detected in tg mice expressing only HLA-DQ8 molecule (without CD4) (F, $\times 10$). A considerable reduction in the degree of inflammation in both HLA-DQ8/hCD4⁺ (G, $\times 10$) and HLA-DQ6/hCD4⁺ (H, $\times 10$) is observed after treatment with anti-DQ mAb.



0.005, respectively) (Figs. 5 and 6, *G* and *H*). No significant differences in the grade and type of inflammation were observed in control isotype Ab (mouse IgG1)-treated HLA-DQ6⁺/hCD4⁺ and HLA-DQ8⁺/hCD4⁺ tg mice compared with CRA-treated counterparts (Fig. 5). The marked reduction in the decrease of eosinophilic inflammation as a result of pretreatment of anti-DQ mAb suggests that specific HLA-DQ molecules are essential for the development of allergen-induced eosinophilic airway inflammation.

Airway reactivity in cockroach-treated tg mice

To study whether HLA-DQ and hCD4 molecules contribute to the development of airway hyperresponsiveness, we challenged CRA-treated tg mice with increasing concentrations of methacholine in a whole-body plethysmograph. Fig. 7 shows that CRA-immunized HLA-DQ8 tg mice with or without CD4 demonstrated a strong AHR 48 h after last i.n. challenge with allergenic extract compared with the Aβ⁰ mCD4⁰ mice. Thus, specific HLA-DQ molecules are required for the induction of AHR in vivo.

Systemic cockroach-specific IgG Ab responses

To determine the peripheral immune response associated with CRA-induced airway eosinophilic inflammation, we measured CRA-specific IgG1, IgG2a, IgG2b, and IgG3 levels in sera obtained at various times during CRA sensitization. High levels of CRA-specific IgG1 isotype in the sera after a booster injection and i.n. challenge with CRA were detected in both HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice (Fig. 8). Of note, the levels of IgG1 Abs at 96 h after i.n. challenge were considerably higher in DQ8/hCD4⁺ as compared with DQ6/hCD4⁺ tg mice. Mice expressing HLA-DQ8 molecule alone (without CD4) showed minimal amounts of IgG1, while levels in mice expressing only hCD4 molecule alone (without class II) were undetectable. Only minimal amounts of IgG2a, IgGb, and IgG3 were encountered in both HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice (data not shown).

Measurement of total IgE

Because allergic disorders are characterized pathophysiologically by enhanced IgE production, we measured total IgE levels at different time points during CRA sensitization. Total serum IgE levels in HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice were significantly increased at 96 h after the i.n. administration of CRA compared with those obtained at preimmunization ($p < 0.005$ and $p < 0.05$, respectively) (Fig. 9). In contrast, tg mice expressing HLA-DQ8 molecule alone (without CD4) showed a slight increase in

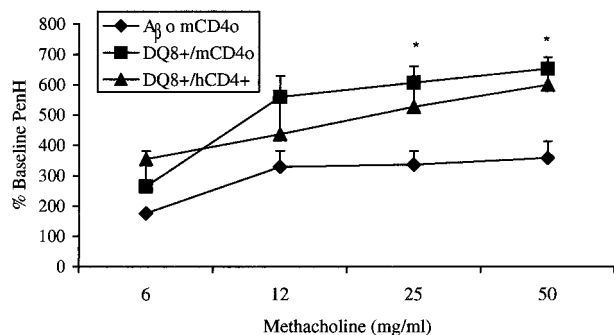


FIGURE 7. Airway responsiveness in cockroach-sensitized mice. Results are expressed for each methacholine concentration as a percentage of PenH (index of airway obstruction) values after PBS exposure ($n = 4$ for each experimental group). *, $p < 0.04$ HLA-DQ8 mice with or without hCD4 vs Aβ⁰ mCD4⁰ mice.

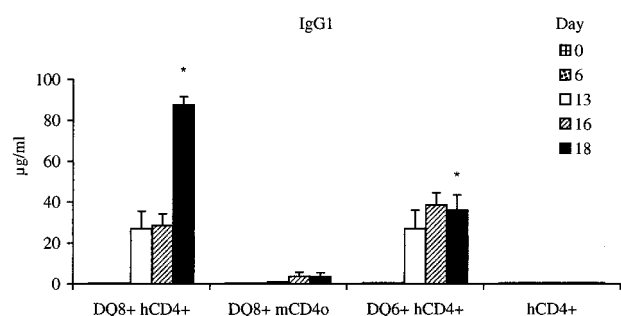


FIGURE 8. CRA-specific IgG1 isotype determined by ELISA. Mice were bled at different times during the sensitization protocol, as described in *Materials and Methods*. Significantly higher levels of CR-specific IgG1 isotype were detected in the sera of HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice at 96 h after i.n. challenged with CRA compared with those obtained before immunization (*, $p < 0.05$). HLA-DQ8 mCD4⁰ mice showed minimal amounts of IgG1, whereas levels in mice expressing only hCD4 molecule alone (without class II) were undetectable.

total serum IgE after i.n. challenge as compared with preimmune sera (the difference was not significant). Total IgE was not detected in the sera of tg mice expressing hCD4 only (without class II) before or after exposure to CRA extract.

Discussion

The presence of eosinophils within airway secretions and recruitment of eosinophils into lung tissue are considered a hallmark of allergic airway diseases, including asthma (32). To investigate the role and contribution of HLA genes to airway inflammation in cockroach-induced asthma, tg mice expressing hCD4 and either DQ6 or DQ8 were used for in vivo analysis. Our results strongly support a major role for HLA class II molecules in the pathogenesis of eosinophilic airway inflammation. We have previously shown that these mice respond to CRA extract in vitro, generating primarily Th2-type cytokines (24). The in vitro response was mediated by CD4⁺ T cells and was HLA-DQ restricted. In this study, we demonstrate that HLA-DQ tg mice display many features characteristic of an asthmatic phenotype. First, these animals showed eosinophilic inflammation of the airways in vivo. This was characterized by dense peribronchial and perivascular accumulation of eosinophils extending deep into the lungs. These findings were corroborated by BAL analysis verifying an increase in eosinophil

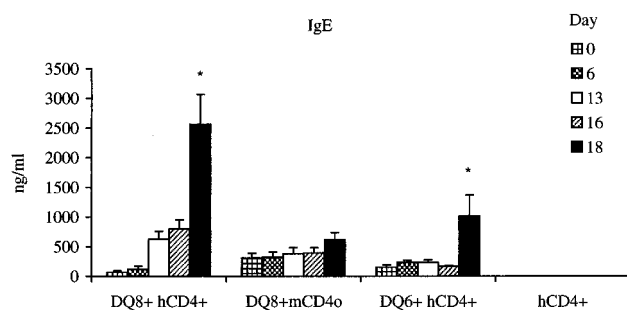


FIGURE 9. Total serum levels of IgE measured by ELISA. Significantly higher levels of total serum IgE were found in HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice at 96 h after the i.n. administration of CRA compared with those obtained at preimmunization (*, DQ8⁺hCD4⁺, $p < 0.005$; *, DQ6⁺hCD4⁺, $p < 0.05$, respectively). In contrast, tg mice expressing HLA-DQ8 molecule alone (without CD4) showed a slight increase in total serum IgE after i.n. challenged as compared with preimmune sera. Total IgE was not detected in the sera of tg mice expressing hCD4 only (without class II) before or after exposure to CRA.

counts. Another important finding is the presence of mucus production and airway epithelial hypertrophy. Pretreatment with anti-DQ mAb significantly reduced airway eosinophilic inflammation. MHC class II knockout mice expressing only hCD4 were normal; and minimal inflammatory infiltrates consisting mainly of macrophages were found in tg mice expressing human HLA-DQ8 molecule without CD4. Second, CRa-immunized and -challenged HLA-DQ8/hCD4⁺ and HLA-DQ6/hCD4⁺ tg mice show an increase in TP concentration, eosinophil peroxidase levels, IL-5, and IL-13 in BALF. Recent studies have clearly demonstrated the importance of Th2 cell-derived cytokines in mediating airway inflammation in allergic asthma (33–35). The production of IL-4, IL-5, and IL-13 by these cells is considered to play a central role in the initiation and maintenance of allergic inflammation. IL-4 is essential for generating and sustaining a Th2-type immune response, directing IgE isotype switching in B cells, and inducing endothelial cell VCAM-1 expression, which directs migration of eosinophils to sites of allergic inflammation (36–38). Although we were unable to detect measurable levels of IL-4 in BALF, high amounts of IL-5 and IL-13 were identified. IL-5 production appears to have a central role in the development of eosinophilic inflammation observed both in human and animal models (39–41). IL-5 has a major role in regulating activation, differentiation, proliferation, and chemotaxis of eosinophils (42–45). Several studies in murine systems have provided evidence for a primary role for IL-13 in the pathogenesis of the inflammation in the asthmatic airways, including the ability to induce IgE production, VCAM-1 expression on endothelial cells, and the induction of chemokine production (46–48). In addition, it was shown to induce mucus hyperproduction and alter muscle contractility, increasing AHR (47, 49). Third, these mice demonstrate an induction of CR-specific IgG1, and an increase in total levels of IgE. Both IgE and IgG1 (Th2 response) as opposed to IgG2a (Th1 response) are good markers for the induction of an allergic response in mice. IgE has long been recognized to have a critical role in the allergic inflammatory responses, including asthma. IgE activates mast cells, resulting in the production of cytokines, which are involved in eosinophil recruitment and survival. It has been shown that treatment of mice with anti-IgE Ab could result in an attenuated response to airway challenge, supporting the important role of IgE in the induction of lung eosinophilic inflammation and Th2 cell cytokine production in this mouse model (50). We identified that the IgG1 isotype, which like IgE is capable of sensitizing mast cells (51), was the predominant IgG subtype.

In conclusion, this study has shown that eosinophilic airway inflammation and related manifestations of allergic airway disease are significantly reduced after treatment with anti-DQ mAb. The present data together with our previous findings strongly suggest that HLA-class II molecules play an essential role in the development of allergic diseases. Recent studies from our laboratory using HLA-DQ⁺/mCD4⁺, HLA-DR⁺/mCD4⁺, and HLA-DR⁺/DQ⁺/mCD4⁺ tg mice for autoimmune diseases and asthma research have demonstrated that different HLA subtypes have different effect on the Ag/allergen recognition and the disease outcome (52–55). The examination of the in vitro and in vivo responses of DQ6, DQ8, DR2, DR3, and DR3/DQ6 tg mice to short ragweed allergen demonstrated that DQ and DR mice differ in the set of allergen epitopes recognized in vitro and in some characteristics of the in vivo response (levels of eosinophilia, cytokines, AHR) (15, 16, 53). Therefore, our tg mice offer the opportunity to investigate the role of distinct HLA molecules in allergen sensitivity and may be useful for the generation of therapeutic strategies against allergic diseases, including asthma.

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