

Requirements for allergen-induced airway inflammation and hyperreactivity in CD4-deficient and CD4-sufficient HLA-DQ transgenic mice

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Background: Airway inflammation is central to the pathogenesis of allergic asthma, and molecules that mediate this process obviously represent targets for therapy.

Objective: To study the role of CD4⁺ T cells and/or HLA-DQ molecules in allergic asthma, we have generated and characterized models of short ragweed allergen (SRW)-induced inflammation using transgenic mice with HLA-DQ (DQ6 or DQ8), human CD4 (hCD4), or both on a genetic background that lacks mouse MHC II and CD4 (A β^0 /mCD4⁰).

Methods: Mice were actively sensitized and later challenged intranasally with SRW allergenic extract. Bronchoalveolar lavage fluid composition, airway inflammation and hyperresponsiveness, blood eosinophil levels, and cell proliferation were examined.

Results: In response to SRW treatment, both DQ6 and DQ8 transgenic mice expressing hCD4 developed pulmonary eosinophilia and associated lung tissue damage with increase in eosinophil peroxidase and T_H2 cytokines in bronchoalveolar lavage fluid, strong airway hyperreactivity, and persistent blood eosinophilia. The response was independent of mast cells/histamine pathway and was mediated by DQ-restricted hCD4⁺ T cells. Interestingly, lungs of CD4-deficient DQ6 transgenic mice showed an eosinophilic inflammation without local increase in cytokines and eosinophil peroxidase. The allergic reaction was absent in double-knockout mice and mice expressing either DQ8 or hCD4 alone.

Conclusions: DQ6 molecules are critical to SRW-induced allergy and can operate in the presence or absence of CD4. However, both DQ antigens and CD4 molecules are critical for full manifestation of allergen-induced asthma in transgenic mice. (*J Allergy Clin Immunol* 2001;108:764-71.)

Key words: MHC, HLA, human CD4, transgenic/knockout, short ragweed allergen, *in vivo* animal models, asthma

Asthma is a chronic inflammatory disease of the airways with a heterogeneous collection of conditions that includes complex cellular and molecular interactions and involves numerous mediators. Eosinophils are the major effector cells in asthma. Their production, activation, dif-

Abbreviations used

A β^0 /mCD4 ⁰ :	Lacking MHC II and mouse CD4
AHR:	Airway hyperreactivity
BAL:	Bronchoalveolar lavage
DN:	Double-negative
EPO:	Eosinophil peroxidase
hCD4:	Human CD4
mCD4:	Mouse CD4
MNC:	Mononuclear cell
NK:	Natural killer
SRW:	Short ragweed allergen
TCR:	T-cell receptor
tg:	Transgenic

ferentiation, recruitment, and specific accumulation are promoted by T_H2-type cytokines.¹⁻⁵ In addition, T_H2 cytokines coordinate the growth/differentiation of mast cells⁶ and regulate Ig class switching.⁷

In murine models of asthma, pulmonary eosinophilia and airway hyperreactivity (AHR) can be attenuated by (1) eliminating T cells with either anti-CD4⁸ or anti-Thy-1⁹ mAb, (2) administering a soluble IL-13R α_2 -Fc,¹⁰ (3) neutralizing antibodies to IL-4,¹¹ IL-5,¹² or their receptors,^{13,14} or (4) using cytokine-deficient mice.^{15,16} Studies in human beings showed that bronchial biopsy specimens/sputum samples from asthmatic individuals had increased concentrations of IL-4 and IL-5 in comparison with those from nonasthmatic individuals.^{17,18} Therefore, CD4⁺ T lymphocytes play an important role in regulating the immune response to allergen, in part through the secretion of T_H2-type cytokines.

There is clear evidence for the hereditary nature of allergic diseases, and a review of the literature indicates that 30% to 80% of the etiology can be explained by genetic factors.¹⁹ Because allergic diseases are polygenic, several genetic loci might control the allergic phenotype.²⁰⁻²² However, the first discovered association with HLA class II loci on chromosome 6p continues to be the strongest, especially the association of short ragweed allergen (SRW) hypersensitivity with the DR2/DQ6 haplotype.²³⁻²⁷

We generated several transgenic (tg) mouse lines that express: (1) DQ (DQ6 or DQ8) molecules in the absence of mouse MHC II and mouse CD4 (mCD4); (2) DQ with human CD4 (hCD4) in the absence of MHC II and mCD4; (3) hCD4 in the absence of MHC II and mCD4; and (4) double knockout mice lacking MHC II and

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mCD4. These novel genetically altered mouse strains allow us to study the role of DQ molecules and hCD4 molecules alone or in combinations, as well as to investigate other cells and factors in asthma.

METHODS

Mice

Transgenic mice expressing human DQ6 (HLA-DQA1*0103 and HLA-DQB1*0601) and DQ8 (HLA-DQA1*0301 and HLA-DQB1*0302) genes in mice deficient in endogenous class II molecules (H-2A β ⁰) have been described previously.^{28,29} These mice were mated to mCD4 knockout mice (kindly provided by Tak W. Mak of the University of Toronto)^{30,31} and then to hCD4 tg mice (kindly provided by Richard A. Flavell of Yale University).³² The expression of HLA-DQ, H-2A α , H-2A β , H-2E α/β , mCD4, and hCD4 was assessed by fluorescence-activated cell sorting through use of the corresponding mAbs (IVD12, 7-16-17, 25-5-16, Y-17, GK1.5, and OKT4, respectively).

Six groups of mice were used in each experiment:

1. Mice expressing DQ6 and hCD4 with absence of mouse MHC II and mCD4 (referred to as *DQ6⁺/hCD4⁺ mice*)
2. Mice expressing DQ8 and hCD4 with the same background (referred to as *DQ8⁺/hCD4⁺ mice*)
3. Mice expressing DQ6 with absence of both mouse and hCD4 (referred to as *DQ6⁺ mice*)
4. Mice expressing DQ8 with absence of both mouse and hCD4 (referred to as *DQ8⁺ mice*)
5. Mice expressing hCD4 in absence of MHC II, DQ, and mCD4 (referred to as *hCD4⁺ mice*)
6. Double-negative (DN) mice (referred to as *A β ⁰/mCD4⁰ mice*).

All of the mice were A β ⁰/mCD4⁰. Mice were bred and maintained in the pathogen-free Immunogenetics Mouse Colony at the Mayo Clinic (Rochester, Minn). All procedures performed on the mice were in accordance with the guidelines of the Mayo Institutional Animal Care and Use Committee.

Antigen preparation and immunization protocol

Mice were injected intraperitoneally with 40 mg of short ragweed (*Ambrosia artemisiifolia*) extract (Antigen Laboratories) adsorbed to 1 mg of alum (Sigma) in 0.5 mL PBS (Gibco BRL) on days 0 and 7.³³ On day 14, mice were challenged intranasally with 120 μ g/50 μ L of extract 2 times 6 hours apart. For anesthesia, 4 μ g/0.2 mL or 6 μ g/0.3 mL per mouse of avertin solution was used.³³ Control mice were injected with PBS/alum and challenged intranasally with PBS. Each mouse was euthanized with 20 mg/mL of avertin 48 hours after challenge, and bronchoalveolar lavage (BAL) fluids were withdrawn.

Bronchoalveolar lavage characterization

BAL fluids were collected and total leukocyte numbers and differential cell counts determined as described previously.³³ IL-4, IL-5, IL-13, IFN- γ , and TNF- α contents in BAL fluids were measured by ELISA through use of mini-kits from Genzyme and Endogen.³³ Eosinophil peroxidase (EPO) levels were measured colorimetrically.³⁴ Human EPO (kindly provided by James Checkel and Gerald J. Gleich of the Allergic Diseases Research Laboratory, Mayo Clinic) was used as a standard. Histamine levels were determined through use of a Histamine-Enzyme Immunoassay Kit (Immunotech).

Histochemistry

Formalin-fixed, paraffin-embedded lung sections were prepared and stained with hematoxylin-eosin. For visualization of mast cells, the sections were deparaffinized through use of a xylene/ethanol/water

gradient, and 2 distinct staining procedures were performed. In the toluidine blue method, sections were incubated with 0.5% toluidine blue at pH 4 for 45 seconds and dehydrated through 70%, 96%, and absolute ethanol to xylene; they were then mounted. Mast cells stain violet or purple, whereas cell nuclei stain blue. In the naphthol AS-D chloroacetate esterase procedure, an Esterase Kit (Sigma) was used. Mast cells were defined by bright red granulation.

Airway hyperresponsiveness

AHR in PBS- or SRW-sensitized mice was measured 48 hours after the last intranasal challenge by recording respiratory pressure curves through use of whole-body plethysmography (model PLY 3211, Buxco Electronics, Inc), as described previously.³⁵ Values of PenH were calculated by means of BioSystem XA software, averaged, and expressed for each methacholine concentration as the percentage of baseline PenH values following PBS exposure.

In vivo antibody administration

Anti-CD8 (53-6.72) mAb³⁶ and anti-natural killer cell 1.1 (NK1.1; PK136) mAb³⁷ were purified from supernatants of hybridoma cells. To deplete CD8⁺ or NK1.1⁺ cells, mice were injected intraperitoneally 1 day before priming with SRW and every 7 days thereafter with 200 mg of the corresponding mAb. Control mice were injected with rat (Control mAb I, clone R35-95; PharMingen) or mouse (Control mAb II, clone UPC10; Sigma) IgG. The efficacy of depletion was monitored by flow cytometric analysis of spleen mononuclear cells (MNCs). Anti-CD1d mAb (clone 1B1; PharMingen) was administered intravenously (50 mg/mouse/day) at the same time intervals. The isotype-matched antibody, rat IgG2b (Control mAb III, clone R35-38; PharMingen) was used as a control.

Blood eosinophil levels

Mice were bled on days 0, 6, 13, 16, and 22 of the sensitization protocol, and blood was collected into EDTA tubes. For eosinophil enumeration, the Unopette Test (Becton Dickinson) was used.

Cell proliferation assay

Single-cell suspensions of spleen or suspensions of cells mixed with an experimental group of peribronchial lymph node cells from PBS- or SRW-treated mice were prepared and incubated at 37°C in Roswell Park Memorial Institute medium with supplements containing medium alone, 10 μ g/mL of ConA, or 200 μ g/mL of SRW.^{33,38} Proliferation was assessed by [³H] thymidine incorporation.

For the in vitro mAb blocking study, supernatants from the cell lines producing mAbs specific for DQ α -chain (IVD 12), DQ6/8 (TB1), DR (L227; cross-reacts with DQ6 β), H-2A α ^b (7-16-17), H-2A β ^b (25-5-16), H-2E α/β ^b (Y-17), hCD4 (OKT4), mCD4 (GK1.5), and CD8 (53-6.72) were used.³⁸ A total of 10 μ g/20 μ L per well of anti-CD1d mAb was used to block the CD1-restricted response.

Serum total IgE levels

An IgE-specific ELISA with matching antibody pair, R35-72 and R35-92 (PharMingen) was used to measure total IgE. IgE levels were quantified by comparison to purified isotype standard monoclonal antidinitrophenyl mouse IgE isotype antibody (Sigma) through use of Microplate Manager software for the Macintosh computer (Bio-Rad, Inc). A serum pool of SRW-immunized BALB/c mice was used in each assay as an internal laboratory positive control.

RESULTS

Generation and characterization of tg mice

We generated mice tg for DQ6 or DQ8 in the absence of mouse class II, mated them to the mCD4 knockout

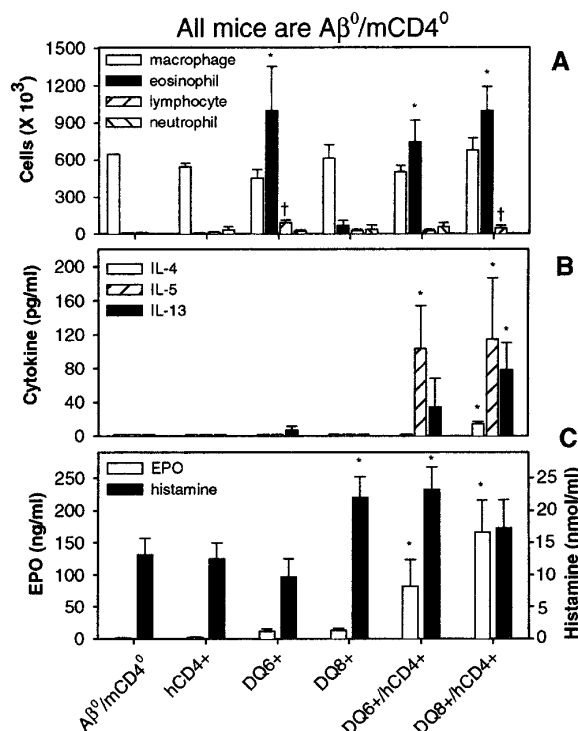


FIG 1. Cellular composition of BAL fluid (A) and cytokine (B) and EPO and histamine (C) levels in BAL fluid supernatants of tg mice. **A.** Eosinophils dominate the cellular infiltrate into BAL of DQ⁺/hCD4⁺ and DQ6⁺ mice. **P* < .03. †*P* < .05. **B.** SRW treatment resulted in the increase of IL-5 and IL-13 contents in DQ⁺/hCD4⁺ mice. **C.** EPO concentration was increased in DQ6⁺/hCD4⁺ and DQ8⁺/hCD4⁺ mice (**P* < .05). **P* < .05 in BAL histamine levels between either DQ8⁺ or DQ6⁺/hCD4⁺ mice and Aβ⁰/mCD4⁰, hCD4⁺, DQ6⁺ tg mice.

mice, and then mated them to hCD4 tg mice. None of the mice used in this study expressed either mouse MHC II or mCD4. HLA-DQ molecule expression was similar for CD4-deficient and CD4-sufficient DQ tg mice, amounting to 30% to 45% of the PBMC and spleen MNC populations. The DQ⁺/hCD4⁺ mice expressed from 34% to 64% hCD4⁺ cells in peripheral blood, whereas no detectable CD4 (<0.5%) was found in PBMCs from DQ⁺ mice. CD8 molecule was expressed on 10% to 20% of PBMCs and spleen MNCs in all groups of mice, except that there were low levels of its expression in CD4-deficient DQ mice (3% to 8%). Low numbers of DN CD4-CD8-NK1.1-CD3⁺ cells were found in Aβ⁰/mCD4⁰ and hCD4⁺ mice (1% to 3% PBMCs; 2% to 7% spleen MNCs). Their amount was increased in DQ tg mice with/without hCD4 (up to 11%).

Cellular composition and characterization of BAL fluids

Exposure to allergen induced an infiltration of eosinophils and lymphocytes into airways of DQ6⁺/hCD4⁺, DQ8⁺/hCD4⁺, and DQ6⁺ mice as assessed by BAL at 48 hours after intranasal challenge (Fig 1, A). Macrophages were the primary cell type in Aβ⁰/mCD4⁰,

hCD4⁺, and DQ8⁺ tg mice.

SRW treatment induced local lung IL-5 and IL-13 production in DQ6⁺/hCD4⁺ and DQ8⁺/hCD4⁺ tg mice (Fig 1, B). All DQ8⁺/hCD4⁺ mice showed minimal amounts of IL-4 (14.56 ± 2.72 pg/mL of BAL fluid). No IFN-γ was detected in any sample. TNF-α was detected in only 2 of 10 DQ8⁺ mice (198.44 ± 141.58 pg/mL of BAL fluid).

The DQ⁺/hCD4⁺ tg mice demonstrated substantial levels of EPO in BAL at 48 hours after SRW challenge (Fig 1, C). Despite an acute BAL eosinophilia, the EPO activity in DQ6⁺ mice was minimal and did not differ from that in DQ8⁺ mice. Histamine levels were increased in BAL samples obtained from SRW-exposed DQ8⁺ and DQ6⁺/hCD4⁺ mice (Fig 1, C) in comparison with the Aβ⁰/mCD4⁰, hCD4⁺, and DQ6⁺ experimental groups of animals.

Histologic assessment of lung cellular infiltration, eosinophilia, and mast cell determination

Examination of lung tissues revealed strong perivascular/peribronchial eosinophilia with significant epithelial damage, airway epithelial cell/goblet cell metaplasia, and thickening of the basement membrane in DQ6⁺/hCD4⁺ and DQ8⁺/hCD4⁺ mice (Fig 2, A). Lungs of SRW-challenged Aβ⁰/mCD4⁰ and hCD4⁺ mice were normal (Fig 2, B). Lungs of DQ6⁺ mice showed multiple inflammatory infiltrates in the form of severe eosinophilic perivascularitis or eosinophilic pneumonia (Fig 2, C) without considerable damage to the airway epithelium. In contrast, no inflammation was observed in DQ8⁺ mice (Fig 2, D). Lungs of PBS-treated mice were normal.

Light microscopy of toluidine blue- or naphthol AS-D chloroacetate-stained sections of the lungs revealed mast cells in the submucosa of large airway vessels in all experimental groups (Fig 3, A and B). Mast cells were fully granulated, and their granule release was observed in the lungs of all experimental mice.

Lung physiology studies

Analysis of airway responsiveness has shown the absence of hyperreactivity to methacholine in Aβ⁰/mCD4⁰ and hCD4⁺ mice (Fig 4, A). Methacholine challenge (50 mg/mL) induced a strong AHR in SRW-treated DQ6⁺/hCD4⁺ and DQ8⁺/hCD4⁺ mice, whereas it was significantly lower for DQ6⁺ mice despite the similar degree of BAL eosinophilia. Lung function in SRW-treated DQ8⁺ mice was equally strong; however, it was not enhanced in comparison with that in unimmunized littermates. The increase in lung function observed in DQ6⁺ and DQ8⁺ mice was not dependent on NK1.1⁺ and CD8⁺ cells, inasmuch as in vivo mAb depletion studies did not show any effect on AHR (Fig 4, B).

Blood eosinophil levels

Basal levels of eosinophils in the peripheral blood were similar for all tg mice (Fig 5). Eosinophil numbers were increased in all experimental groups on day 6 after the first intraperitoneal injection with allergen in com-

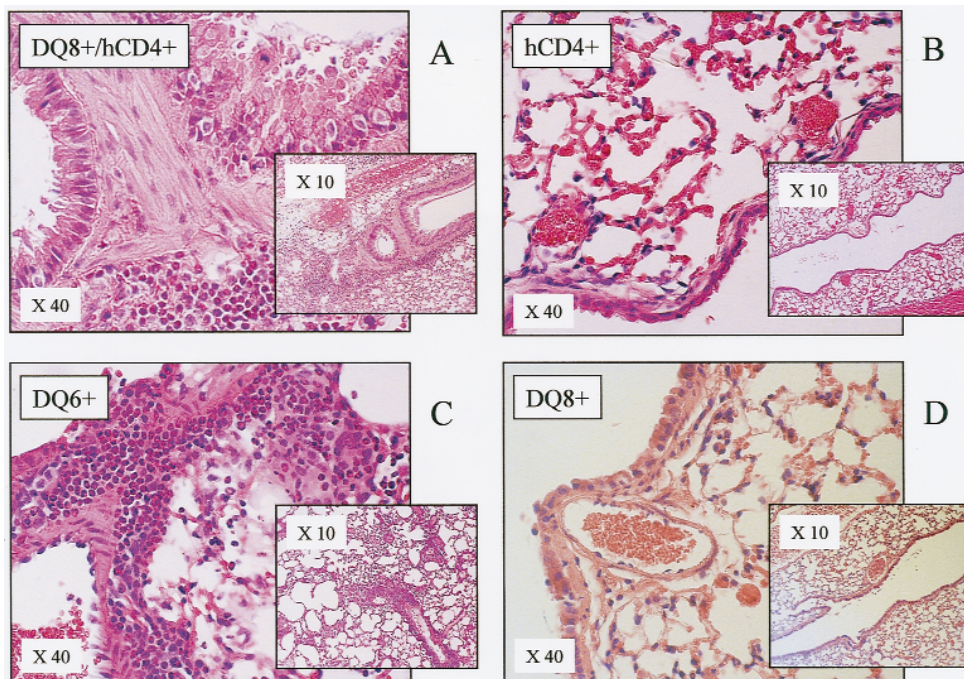


FIG 2. Histopathology of lung tissue. **A**, Lungs of DQ8⁺/hCD4⁺ mice showed a diffuse eosinophilic infiltration. **B**, There was no inflammation in lung tissue of A β ⁰/mCD4⁰ or hCD4⁺ mice. **C**, A perivascular eosinophil/MNC inflammation was observed in DQ6⁺ mice. **D**, Section of lung of DQ8⁺ mouse shows the absence of inflammatory cells.

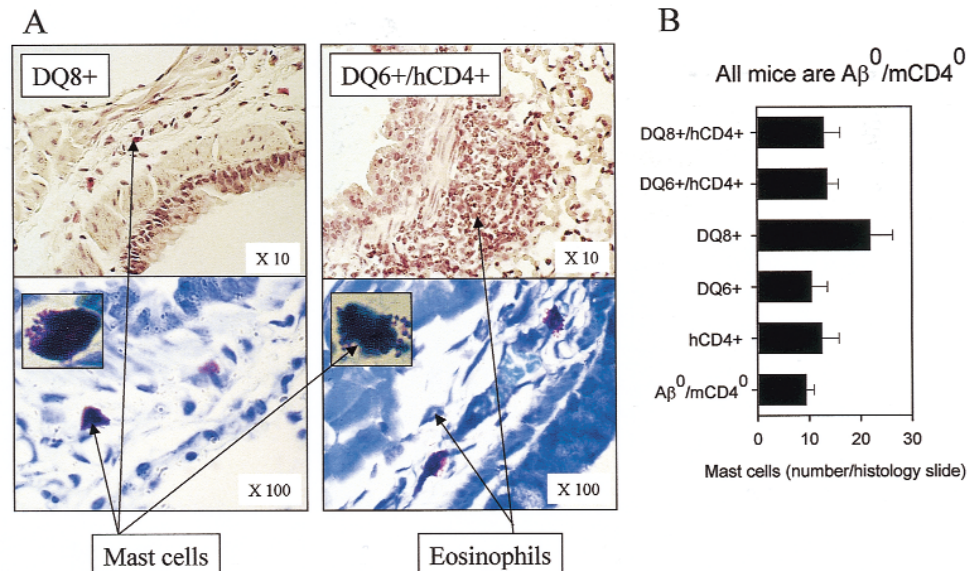


FIG 3. **A**, Histochemical staining with naphthol AS-D chloroacetate or toluidine blue reveals mast cells in the airway submucosa in either CD4-deficient or hCD4-sufficient DQ6⁺ and DQ8⁺ mice. **B**, Mast cells were also observed in sufficient numbers in other tg mice, the highest count being in DQ8⁺ mice.

parison with preimmune controls. A second injection with allergen strongly increased blood eosinophilia in DQ6⁺, DQ6⁺/hCD4⁺, and DQ8⁺/hCD4⁺ mice (day 13). Forty-eight hours after intranasal challenge with aller-

gen, eosinophil numbers decreased in DQ6⁺, DQ6⁺/hCD4⁺, and DQ8⁺/hCD4⁺ tg mice. On day 8 after challenge, strong blood eosinophilia was observed only in hCD4-sufficient DQ6⁺ and DQ8⁺ tg mice.

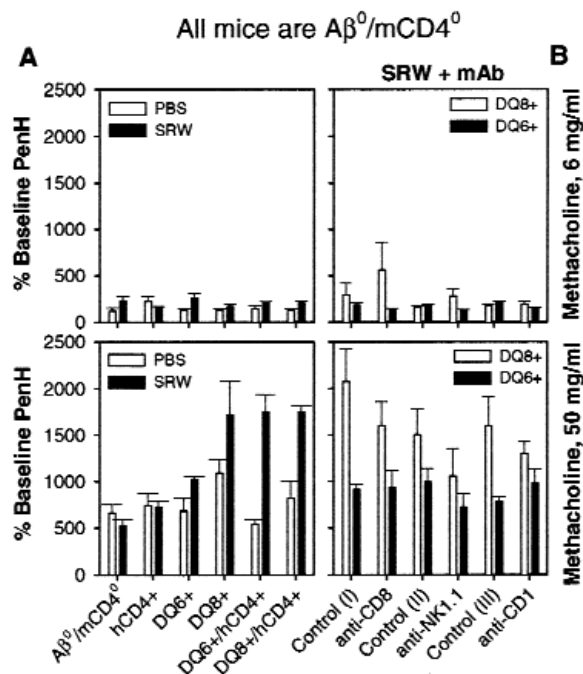


FIG 4. Respiratory system physiology of PBS-treated and SRW-sensitized and challenged tg mice (**A**) and DQ⁺ mice (**B**) treated with mAb during SRW treatment. **A**, Mean methacholine dose-response curves of 4 mice per control group and from 6 to 12 mice per experimental group ($\pm$ SEM) are shown. **B**, Treatment of mice with anti-CD1, anti-NK1.1, or anti-CD8 mAb did not significantly attenuate hyperresponsiveness in comparison with control mAb-treated groups.

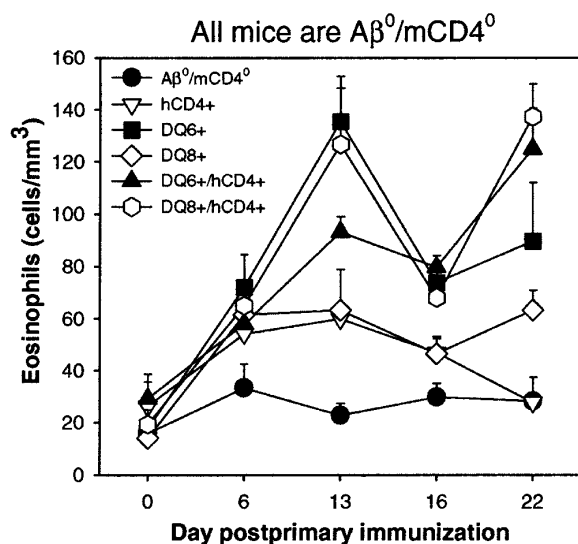


FIG 5. Changes in blood eosinophil numbers in tg mice during SRW treatment. Results are expressed as means $\pm$ SEMs.

Proliferation and mAb blocking assays

CD4-deficient or CD4-sufficient DQ6⁺ tg mice showed SRW-specific proliferation, though the response in DQ6⁺ mice was lower than that in DQ6⁺/hCD4⁺ litter-

mates (Fig 6). There was no response to SRW in Aβ⁰/mCD4⁰, hCD4⁺, and DQ8⁺ mice. No proliferation was observed when cells from PBS-treated mice were used. To identify the subpopulation of T cells necessary for the response to SRW in tg mice, different mAbs were added to the wells containing cultures of spleen MNCs with SRW. As can be seen in Fig 6, the proliferative response in both lines of DQ6 tg mice was blocked by DQ-specific mAb, showing that T cells recognize SRW in the context of DQ. Addition of mAb specific for the hCD4 molecule in DQ6⁺/hCD4⁺ mice inhibited the response by 57.41% $\pm$ 15.97%. It is clear that the DQ6⁺ mouse response was mediated by DN CD4-CD8⁺ T cells, because the use of mAbs with different specificities had no significant effect on cell proliferation.

Serum total IgE levels

Analysis of the effect of the various transgenes on total IgE levels in sera demonstrated increased levels of IgE in SRW-sensitized and challenged DQ6⁺, DQ6⁺/hCD4⁺, and DQ8⁺/hCD4⁺ mice in comparison with preimmune controls (Fig 7). In contrast, hCD4⁺ and DQ8⁺ mice did not exhibit a significant increase of total serum IgE during sensitization and challenge.

DISCUSSION

In the present study, we show that different mechanisms of airway inflammatory response to allergen in tg mice can be observed, depending on the presence or absence of CD4 and the particular HLA-DQ allele expressed. Both DQ6⁺/hCD4⁺ and DQ8⁺/hCD4⁺ mice display a full picture of allergen-induced asthma. In contrast, CD4-deficient DQ6⁺ tg mice did not show any of the characteristics of the asthmatic response despite an acute BAL and pulmonary eosinophilia. Mice lacking both CD4 and DQ molecules or expressing either DQ8 or hCD4 alone in the absence of mouse and human class II were normal. Interestingly, an increase in BAL histamine level was detected in CD4-deficient DQ8 mice. However, histamine concentration was also equally increased in DQ6⁺/hCD4⁺ mice developing a pulmonary eosinophilia. This suggests that mast cells' infiltration into the lung tissue and their subsequent activation and degranulation are processes dissected with eosinophilic inflammation.

The role of mast cells in the late allergic inflammatory response is not clearly defined.^{39,40} Mast cell-deficient and -sufficient mice demonstrated no differences in response to allergen,⁴¹ suggesting that neither mast cells nor the IgE-mast cell pathway is required for the development of asthma. Other studies have shown the important role of mast cells in asthma through production of eosinophil-regulating cytokines and prostaglandin D₂.⁴² Taken together, these seemingly controversial results suggest that the development of AHR could involve 2 different mechanisms, one IgE/mast cell-dependent and the other eosinophil/IL-5-dependent.^{39,43} Williams and Galli⁴⁴ have demonstrated that depending on the specific experimental model investigated, mast cells either have a

critical role or are not essential in asthma. Using a conventional model, we showed that the numbers of lung tissue mast cells were equal in tg mice with and without an asthmatic response. It did not depend on the presence of DQ or CD4 molecules, which suggests that there is another pathway leading to mast cell activation/degranulation in the absence of CD4⁺ T-cell help. One of the possibilities is stimulation of mast cell degranulation through a nonimmune mechanism by carbohydrate moieties or lectinlike factors.^{45,46}

Our previous observations^{33,38} and the results of the current study demonstrate that the requirement for species-matched CD4 might not be absolute in responses to allergen in some HLA class II tg mice. Previously, we showed a functional interaction between either DQ6 or DQ8 and mCD4, leading to the recognition of a unique set of SRW antigen 5 epitopes³⁸ and an allergen-induced airway inflammatory response³³ in tg mice. Substitution of mCD4 with hCD4 in DQ6 tg mice did not potentiate the allergic response. In contrast, DQ8 molecule showed a strong hCD4 dependence. The T-cell receptor (TCR)/DQ interaction is required for T-cell recognition of SRW antigens in DQ tg mice. In addition, interactions between the DQ/TCR/SRW-peptide complex and CD4 might be an important factor influencing its overall stability.⁴⁷

Interestingly, even in the absence of mCD4/hCD4, formation of the DQ6/TCR/SRW-peptide complex leads to T-cell activation. The expression of DQ6 molecules but not DQ8 molecules results in CD4-independent, allergen-induced lung eosinophilia mediated by DN CD4-CD8-NK1.1-TCR α/β ⁺ T cells. The DQ⁺/mCD4⁺ tg mouse response is dependent on DQ-restricted mCD4⁺ T cells.^{33,38} The response of DQ⁺/hCD4⁺ mice to SRW in general depends on DQ-restricted hCD4⁺ T cells. Therefore, the expanded population of DN TCR α/β ⁺ T cells in DQ6⁺ mice has partially taken over the role normally performed by the CD4⁺ helper T cells.

In this study, we clearly demonstrate not only that the presence of eosinophils in lung inflammatory infiltrates is sufficient for AHR but also that some other factors might be involved, as is the case in DQ8⁺ mice. Two CD4-deficient DQ⁺ lines displayed intrinsic differences in their pulmonary mechanics, in that reactivity to methacholine was higher in unimmunized, PBS-treated, and SRW-treated DQ8⁺ mice than in DQ6⁺ mice. The use of anti-NK1.1, anti-CD1, or anti-CD8 mAb had no effect on the SRW-induced AHR observed in both CD4-deficient DQ6⁺ and DQ8⁺ tg mice, eliminating the involvement of NK1.1⁺ and CD8⁺ cells in the observed disease outcome. We also found that after SRW treatment, DQ6⁺/hCD4⁺ tg mice demonstrated a significantly greater airway responsiveness than was seen in their DQ6⁺ tg counterparts despite similar degrees of eosinophilia in these 2 lines. Therefore, a pulmonary eosinophilia by itself is not enough for strong hyperreactivity, though the activation stage is sufficient. The discordance between airway eosinophilia and AHR has been noted in several other animal models.^{14,48,49}

We show here that CD4-deficient and CD4-sufficient

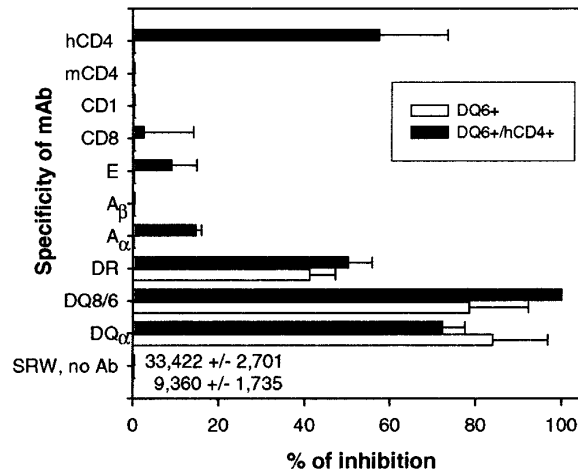


FIG 6. Inhibition of SRW-specific proliferative response of spleen MNCs with mAb. The data represent the percentage of inhibition of [³H]thymidine incorporation in cultures with SRW. Δ counts-per-minute values for cultures with SRW alone are indicated.

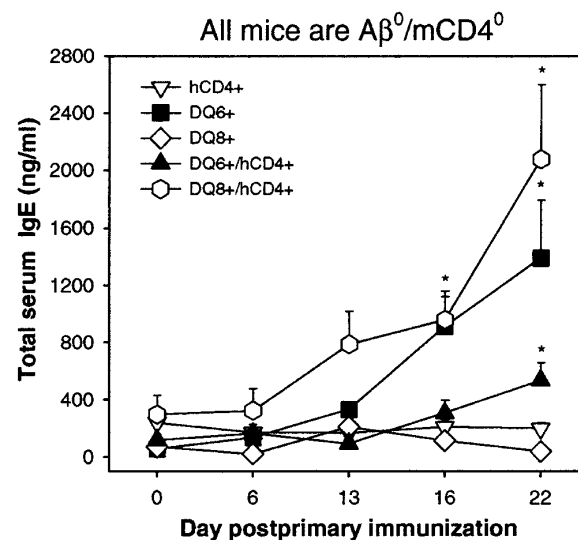


FIG 7. Serum total IgE levels in tg mice. **P* < .05 in comparison with IgE concentration in preimmune sera (day 0).

DQ tg mice have similar basal levels of eosinophils in the peripheral blood, suggesting that CD4 deficiency has no effect on eosinophil levels in these mice. Allergen priming resulted in the increase in this number in all mice used. Therefore, neither CD4 nor DQ molecules are affecting an initial allergen-induced eosinophil differentiation and influx to the peripheral blood. Persistence of blood eosinophilia with subsequent SRW treatment was dependent on DQ antigens and independent of CD4⁺ T cells. However, on day 22 (168 hours after intranasal challenge), when the inflammatory lung response resolved, a marked blood eosinophilia was observed only in hCD4-sufficient DQ⁺ mice, demonstrating the necessity of CD4 for the long-term persistence of circulating eosinophils in DQ tg mice.

Our experimental tg animal models of asthma are useful for elucidating the multiple mechanisms that contribute to disease and can be invaluable for studying the efficacy of potential therapies.

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