Identification of Antigenic Epitopes on Human Allergens: Studies with HLA Transgenic Mice

Svetlana P. Chapoval and Chella S. David

Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA

Environmental factors play an important role in the rise and manifestation of allergic conditions in genetically predisposed subjects. Increased exposure to indoor/outdoor allergens is a significant factor in the development of allergic sensitization and asthma. Recently, strong relationships between the immune response to several highly purified allergens and specific human leukocyte antigen (HLA)-DQ and -DR haplotypes have been reported. The major antigens from clinically important allergens have been cloned and sequenced. However, whether innate structural features of major allergens or peculiar immune recognition of these molecules contribute to the overly robust immune responses is not known. We generated and used transgenic (tg) mice expressing single HLA class II transgene(s) to characterize the allergen epitopes presented by particular HLA class II molecules. Next, we generated *in vivo* models for asthma in the HLA tg mice by intranasal challenge with allergenic extracts. Furthermore, we used a single epitope to induce an allergic lung inflammation. Our system offers a sophisticated technique for systematically identifying the genetic (individual human class II) and antigenic (individual allergenic epitopes) basis of asthma sensitivity and has important implications for new treatment strategies. *Keywords:* **antigens/peptides/epitopes, asthma, HLA, *in vivo* animal model, MHC, short ragweed allergen, transgenic/knockout.** *Environ Health Perspect* **111:245–250 (2003). [Online 21 January 2003] doi:10.1289/ehp.5706 available via *http://dx.doi.org/***

Allergy is the most widespread immunologic disorder in humans. It affects one in four individuals (HayGlass 1995). Today, 50 million Americans suffer from this disease and 15 million of them have asthma (Wasserman 1999). Allergy is defined as a T helper 2 (Th2)-driven hypersensitivity to innocuous antigens (allergens) of complex genetic and environmental origin and is acknowledged as a major risk factor for asthma (Holt et al. 1999). Allergens are derived from different sources. Both indoor allergens (house dust mite, cockroach, fungi, mold, animal-derived substances) and selected outdoor allergens (tree, weed, and grass pollens, fungal spores) have been implicated in causing asthma (Burge and Rogers 2000; Moore et al. 2001; Platts-Mills et al. 2000).

Despite much progress in understanding allergies—the dysfunction of the immune system (Campbell and Weiss 1999)—the molecular and genetic predisposition to allergy has not been identified. Identification of these factors is critical in managing atopic individuals (Cookson 1999). Recently, genome-wide searches and candidate gene approaches have been used to examine the possible involvement of several genes in the development of atopy and asthma. The regions of potential linkage to one or more asthma phenotypes were identified on chromosomes 5q, 6p, 11q, 12q, 13q, and 14q (Blumenthal 2000; Cookson 1999; Ono 2000).

Human leukocyte antigen (HLA) molecules are encoded by highly polymorphic gene families located on chromosome 6p. These molecules play a critical role in the initiation of immune responses to allergens because they bind allergen-derived peptides and present them to T lymphocytes, resulting in T cell activation and proliferation (Figure 1). $CD4^+$ Th2 cells are at the cellular epicenter of allergic disease. They produce an array of cytokines that directly or indirectly cause an acute and chronic allergic reaction in the airways (Figure 1). However, $CD4^+$ T-cell response to particular allergen/antigen critically depends on the genotype of HLA II, on the difference in their physicochemical characteristics of the molecular structure, and on binding and presentation of distinct peptides to $CD4^+$ T cells (Klein and Sato 2000; Little and Parham 1999).

The association of HLA haplotypes and short ragweed (SRW) allergy was the first human immune response (Ir) gene to be recognized (Levine et al. 1972), and HLA-DR2 and HLA-DQ6 restriction of immunoglobulin (Ig)E reactions to antigen 5 is well documented (Huang et al. 1991; van Neerven et al. 1996). However, SRW pollens contain 52 different antigens, suggesting that other HLA II molecules can be involved in their recognition (Marsh et al. 1987; Stewart et al. 1996; van Neerven et al. 1996). Furthermore, knowledge of the sets of determinants presented by each HLA molecule is not known, nor is the hierarchy of importance among HLA class II molecules in ragweed allergen presentation. In addition, specific HLA-DR and DQ molecules have been implicated in asthma (Gerbase-DeLima et al. 1997; Hizawa et al. 1998; Malo and Chan-Yeung 2001; Soriano et al. 1997). However, their role and contribution in the development of allergic asthma are difficult to assess in humans because of their genetic heterogeneity. Do particular HLA-DR or HLA-DQ alleles preferentially present peptides that elicit Th2-type cytokine production and promote allergic asthmatic conditions? Are there Th1-restricted determinants? Do some HLA class II molecules protect against allergic reactions? If so, how is protection from atopy maintained? To address these questions, it is necessary to analyze systematically the variables of the allergen, individual epitopes, and the presenting HLA molecule to a population of T cells. Such controlled *in vivo* studies are not possible in the human system. HLA class II transgenic (tg) mice provide an excellent model for studying the genetic and molecular basis of allergic response.

Production and Characterization of HLA Transgenic Mice

We have introduced HLA-DQ8 (HLA-DQB1*0302, HLADQA1*0301) and HLA-DQ6 (HLA-DQB1*0601, HLA-DQA1*0301) genes into H-2Aβ^0 mice (Bradley et al. 1997; Nabozny et al. 1996). H-2Aβ^0 mice have disrupted Aβ gene and therefore could not express H-2Aβ molecule. Because these mice were of the $H2^b$ haplotype and lacked the functional H2-E molecule, no mouse class II molecules were expressed on the cell surface. HLA tg mice express intact HLA molecules on cells of the lymphoid system and respond to several protein, peptide, and parasitic antigens (Abraham and David 2000; Geluk et al. 1998; Krco et al. 2000; Kudva et al. 2001; Pimtanothai et al. 2000; Raju et al. 2001). HLA-DQ molecules in H-2Aβ^0 mice are expressed on the lymph nodes, spleens, thymus, lungs, and kidneys of transgenic mice but not in liver, pancreas, or brain. Approximately 25–40% of peripheral blood lymphocytes express HLA-DQ molecules.

Address correspondence and reprint request to C.S. David, Department of Immunology, Mayo Clinic, Rochester, MN 55905 USA. Telephone: (507) 284-8182; Fax: (507) 284-1637; E-mail: david.chella@mayo.edu

This article is part of the mini-monograph "Animal Models to Detect Allergenicity to Foods and Genetically Modified Products."

This work was supported by National Institutes of Health grant AI 14764. S.P.C. is supported by NIH training grant CA09127.

Received 9 April 2002; accepted 26 November 2002.

Using a panel of monoclonal antibodies specific for Aα (clone 7-16-17), Aβ (clone 25-5-16), and Eα/β (clone Y-17) subunits, we detected no cell surface expression of hybrid class II. Thus, the HLA-DQ8 and HLA-DQ6 mice express intact human class II molecules in the absence of endogenous mouse or hybrid molecules.

Flow cytometry analysis of spleen mononuclear cells obtained from HLA-DQ6 and -DQ8 mice revealed that DQ transgene is expressed on 7.38–15.41% of $CD3^+$ cells, 2.71–8.15% of $Mac1\alpha^+$ cells, 25.68–32.51% of $B220^+$ cells, and 0.9–1.53% of $CD11c^+$ cells (Chapoval et al. 1999). In bronchoalveolar lavage (BAL), 80.66–92.92% of $Mac1\alpha^+$ cells and 79.68–90.33% of $B220^+$ cells coexpressed DQ antigen (Chapoval et al. 1999). There was no significant difference in transgene expression between HLA-DQ6 and HLA-DQ8 mice. Expression of the HLA-DQ molecule in H-2Aβ^0 mice induces the selection of $CD4^+$ Vβ TCR^+ cells and restores the $CD4^+$ T-cell population in the periphery to a substantial level (5.0–9.3%).

The specific staining of mouse lungs for HLA-DQ expression was performed and sections were examined using light microscopy. This study demonstrated that the expression of HLA class II molecules in normal lung tissue in tg mice is similar to that in humans (Chapoval et al. 1999). In addition to the professional APC in lungs, lung alveolar and bronchial epithelial cells and vascular endothelial cells expressed HLA-DQ molecules.

We have generated tg mice expressing HLA-DR2 (DRB1*1 502), DR3 (DRB1*0301), and DR4 (DRB1*0401) (Geluk et al. 1998; Pan et al. 1998, 1999). The DR molecules are also expressed on the cell surface, interact with $CD4^+$ T cells, and positively/negatively select various T-cell receptor-bearing subsets and present antigens. The DR4 mice can present type II collagen and derived peptides and develop mild arthritis (Pan et al. 1998, 1999). Thus, the DR molecules in tg mice are biologically functional.

Identification of HLA Class II Restricted Epitopes of Allergens in Transgenic Mice

Recent cloning and sequencing of cDNAs that encode major allergens (Gregoire and Chapman 2001; Ledford 1994; Stewart and Thompson 1996) has allowed T-cell epitope mapping in humans using long-term T-cell clones and lines (Bohle et al, 2000; de Silva et al. 2000; Dhillon et al. 1992; Katsuki et al. 1996). However, *in vitro* cloning procedure could bias toward certain T-cell response (van Neerven, 1996) rendering polyclonal T cells as better representatives of epitope diversity. Furthermore, although studies with T-cell clones from atopic patients might suggest potential epitopes, they do not provide a comprehensive picture of important epitopes for all class II molecules. Conventional mouse models do not fully reflect immune response to allergen seen in humans because major histocompatibility complex (MHC) II molecules are mouse derived. HLA II tg mice provide a powerful model for an exhaustive characterization and identification of the antigenic determinants on allergens.

In the past years, HLA tg mice have been used to identify epitopes on several autoimmune disease-related antigens such as collagen and cartilage glycoprotein, the autoantigens associated with arthritis (Cope et al. 1999; Krco et al. 1999), glutamic acid decarboxylase and insulin associated with type 1 diabetes (Abraham et al. 2000; Kudva et al. 2001; Raju et al. 1997), nicotinic acetylcholine receptor involved in myasthenia gravis (Raju et al. 2001), RO60 (SS-A) lupus-related antigen (Paisansinsup et al. 2000), myelin basic protein, myelin olygodendrocyte glycoprotein and proteolipid protein implicated in the development of multiple sclerosis (Das et al. 2000; Ito et al. 1996; Madsen et al. 1999; Khare M, David CS. Personal communication). In addition, the use of HLA II tg mice has proven to be a valuable tool for identification of T-cell epitopes on malarial parasites (Pimtanothai et al. 2000) and mycobacterial antigens (Geluk et al. 1998) that could be used to generate a new type of more effective subunit vaccines. Moreover, recent studies of HLA II tg mouse immune responses to tumor-associated proteins allow us to identify epitopes of potential clinical value for design of anticancer vaccines (Chapoval et al. 2001a; Wilson K, David CS. Personal communication).

We tested the ability of HLA-DQ tg mice to respond to house dust mite (Krco et al. 2000; Neeno et al. 1996), ragweed (Chapoval et al. 1998), and cockroach (Papouchado et al. 2000) allergens. The

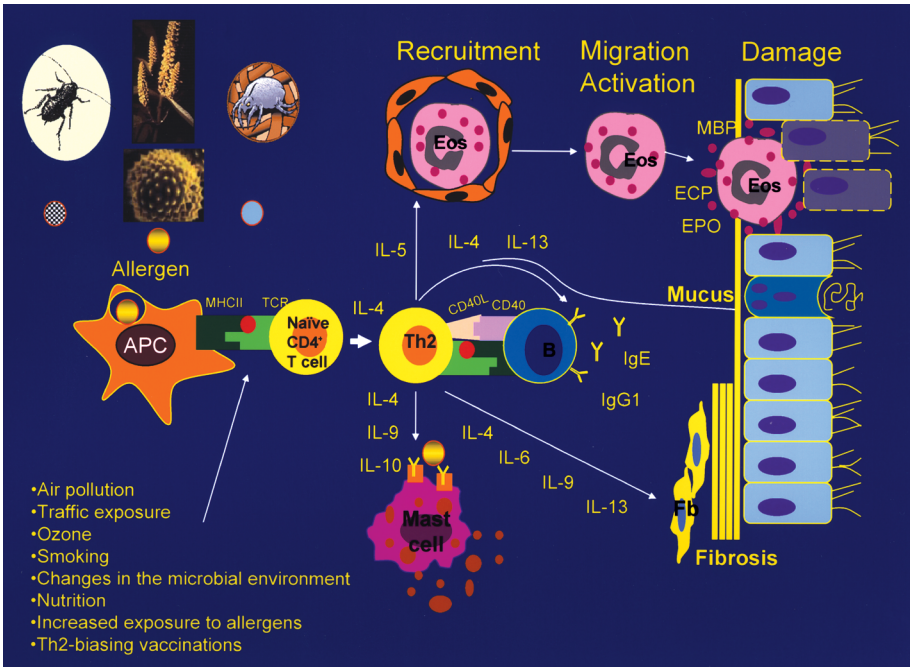

Figure 1. This diagram depicts schematically how the allergic response develops. Allergens are derived from different sources such as cockroaches, ragweed pollens, and house dust mites that are currently being investigated in our laboratory. Antigen-presenting cells (APC) capture, process, and present allergen as an allergen-derived peptide fragment in the contest of specific MHC II (HLA II) molecules. Recognition of this complex by T-cell receptor (TCR) on $CD4^+$ T cells leads to a cytokine (IL-4) mediated clonal expansion of the T helper 2 (Th2) type cells. Several factors bias the development of Th2 type of response (Romagnani 2000). Allergen also interacts with B-cell receptor and is internalized by B cells following antigen (Ag) processing and presentation to the TCR on expanded population of Ag-specific Th2 cells. This together with costimulatory factors and cytokines leads to clonal expansion and differentiation of the B cells involved. B cells switch to Ag-specific antibody (Ab) production, including IgE and IgG1 in mice (IgE and IgG4 in humans, correspondingly). The allergen-specific Ab bind to their receptors displayed on mast cells. Re-exposure to allergen and subsequent crosslinking of Ab molecules leads to mast cell activation, degranulation, and release of mediators. Th2 cells generate IL-5, which increases the production of eosinophils (Eos) in bone marrow, promotes their release into circulation, and regulates their migration into the lung tissue. Activated eosinophils release their toxic granule contents [major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO)] and cause serious tissue damage. Th2 cytokine can directly contribute to the tissue damage or airway remodeling in asthma (Elias et al. 1999) by inducing and promoting a mucus hypersecretion. They also act on fibroblasts (Fb) and favor the subepithelial fibrosis. Asthma and allergy involve a variety of steps and pathways, all of which are influenced by genetics as well as environmental factors.

response of HLA-DQ tg mice to the whole SRW extract was examined. Mice were immunized subcutaneously with SRW in complete Freund adjuvant. Seven days later draining lymph nodes were isolated and mechanically dispersed into a single-cell suspension. Lymph node cells (LNC) were incubated *in vitro* with various concentrations of SRW (Figure 2). Proliferation was assessed by ^{3}[H] thymidine incorporation and expressed as the change (Δ) in counts per minute (cpm) over the cell cultures without SRW. Stimulation of LNC with SRW resulted in strong T-cell proliferation in a dose-dependent manner giving peak Δ of 25,824 and 14,312 cpm for HLA-DQ6 and HLA-DQ8 mice, respectively. HLA-DQ6/DQ8/H-2Aβ^{0} mice were unresponsive to any dose of SRW at any point in time. Interleukin (IL)-5 and IL-10 were the primary cytokines produced by *in vitro* challenged LNC of SRW-primed transgenic mice, suggesting that Th2 response was generated in both transgenics (Chapoval et al. 1998). To identify the subpopulation of T cells necessary for responses to SRW in transgenic mice, monoclonal antibodies (mAb) with different specificity were added to the wells containing cultures of LNC with SRW. Proliferation was significantly inhibited or completely eliminated in cultures with either anti-CD4 or anti-DQ mAb. No significant effect was observed in cultures containing mAb specific for mouse CD8, H-2A^{b}, H-2Aβ^{b}, H-2E/β^{b}, or for human MHC class I molecules. Thus, the *in vitro* response to SRW extract in HLA-DQ6 and HLA-DQ8 transgenic mice is mediated by CD4^{+} HLA-DQ–restricted T cells.

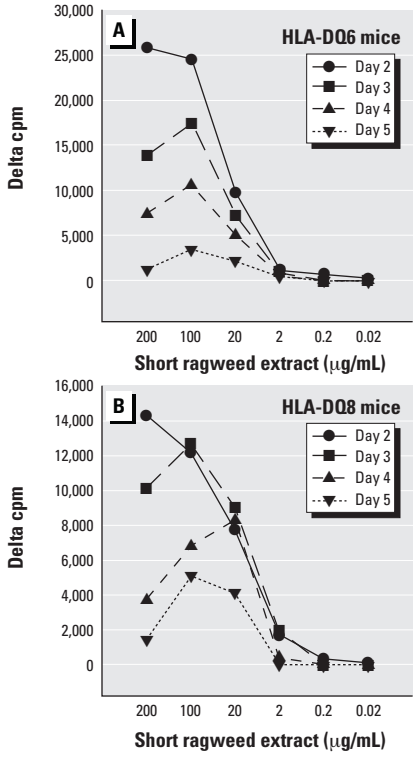

Figure 2. T-cell response to short ragweed allergenic extract in (*A*) HLA-DQ6 and (*B*) HLA-DQ8 tg mice. Seven days after *in vivo* priming with SRW, purified LNC were challenged *in vitro* with increasing doses of SRW. MHC II knockout mice were unresponsive to any dose of SRW at any point in time (data not shown).

Overlapping peptides spanning the entire amino acid sequence of antigen 5 from SRW were used to identify immunodominant and cryptic determinants in HLA-DQ6 and HLA-DQ8 tg mice (Chapoval et al. 1998). To identify immunodominant determinants, mice were immunized with individual overlapping peptide and LNC were challenged *in vitro* with relevant peptide. To identify cryptic epitopes, mice were immunized with crude allergenic SRW extract and LNC were challenged *in vitro* with either SRW or individual peptide representing antigen 5. SRW-immunized HLA-DQ6 mice respond to peptide 11-30 of *Amb a 5*, whereas HLA-DQ8 mice strongly recognize peptide 1-20 (Table 1). We concluded that a naturally processed epitope for HLA-DQ6 molecule resides within residue 10-20 or residue 18-30, whereas for HLA-DQ8 molecule it is residue 1-10 or 8-20. When immunized with peptides, HLA-DQ6–restricted T-cell responses were detected to two determinants (residues 1-20 and 11-30) on *Amb a 5*. In contrast, LNC of HLA-DQ8 mice recognized three *Amb a 5* determinants (residues 1-20, 11-30, and 21-40). Therefore, there is at least one HLA-DQ6–restricted determinant within the region 1-30 of antigen 5, and there are at least two HLA-DQ8–restricted determinants within residue 1-40. We are performing truncation analysis to precisely localize critical residues (Krco et al. 2000). Control H-2Aβ^{0} mice failed to show a proliferative response to any of the peptides. The mAb inhibition studies demonstrated that the immune response to individual allergen peptide in tg mice depended on CD4^{+} T cells and was HLA-DQ restricted (Chapoval et al. 1998). With knowledge of T-cell epitopes on allergens, we are exploring new strategies for immune intervention in allergic diseases by specifically interfering with the interaction among APC, allergen, and T lymphocyte.

HLA Transgenic Mice as Model for Allergen-Induced Asthma

The conventional murine models of antigen-induced experimental asthma have been extraordinarily helpful in addressing selected pathogenetic issues (Krinzman et al. 1996; Kung et al. 1994; Renz et al. 1992). Generation and use of transgenic and knockout mice for the multitude of mouse cytokines and their receptors (Borchers et al. 2001; Cohn et al. 2001; McMillan et al. 2002; Wang et al. 2001; Zhu et al. 2001), cells (Chapoval et al. 2001b; Corry et al. 1998; MacLean et al. 1999; Zuany-Amorim et al. 1998), chemokine receptors (Lukacs et al. 2001; Schuh et al. 2002), costimulatory (Gonzalo et al. 2001; Jember et al. 2001; Mehlhop et al. 2000), adhesion (Fiscus et al. 2001; Wolyniec et al. 1998), and signaling (Das et al. 2001) molecules demonstrate new evidence for the role and significance of the immune system in the mechanisms of allergic tissue inflammation and airway hyperreactivity.

To study the role and contribution of specific HLA class II molecules in the pathogenetic mechanism in atopic diseases and asthma, H-2Aβ^{0}, HLA-DQ6, and HLA-DQ8 mice were actively sensitized and later challenged intranasally with SRW (Chapoval et al. 1999, 2002). In a sensitization phase, SRW was applied intraperitoneally on day 0 and day 7 with aluminum hydroxide as an adjuvant. Seven days after the sensitization phase, airway inflammation was induced by application of dialyzed extract into the respiratory tract two times at 6 hr apart. Vehicle-sensitized control mice were injected with an equal volume of aluminum hydroxide suspension in phosphate-buffered saline (PBS), and challenged intranasally with PBS alone. The development of disease was evaluated both histologically and physiologically at 48 hr after challenge. After immunizations and challenge, HLA-DQ6 and HLA-DQ8 tg mice had an increase in cell accumulation in the BAL (Figure 3) and perivascular and peribronchial inflammatory infiltrates in the lung tissue (Figure 4). The severe inflammatory pulmonary reaction involved predominantly eosinophils (Figures 3 and 4). The *in vivo* response to SRW was mediated by HLA-DQ–restricted CD4^{+} T cells (Chapoval et al. 1999). H-2Aβ^{0} mice did not show any histopathologic features of

Table 1. HLA-DQ6 and HLA-DQ8 tg mice respond to peptides representing allergen 5 of short ragweed pollen.[a]

Mice immunized with SRW		Mice immunized with peptide	
HLA-DQ6	HLA-DQ8	HLA-DQ6	HLA-DQ8
	1-20	1-20	1-20
11-30		11-30	11-30
			21-40

[a]Amino acid sequence of allergen 5 (Chapoval et al. 1998):
1 10 20 30 40 45
LVPCAWAGNVCGEKRAYCCSDPGRYCPWQVVCYESSEICSKKCGK

disease (Figures 3 and 4) and the T-cell response to subsequent rechallenge with SRW was absent in these mice. HLA-DQ6 mice were treated with anti-DQ mAb by intraperitoneal injections 24 hr before, simultaneous with, and after each SRW application. This treatment significantly lowered the number of BAL total cells, eosinophils, and lymphocytes recovered from allergen-sensitized HLA-DQ6 mice, suggesting an important role for HLA molecules in the development of allergic inflammation in our model (Figure 3).

It has been firmly established that allergic inflammatory responses depend on the presence of Th2 cells (Krug and Frew 1997). As such, we examined BAL and spleen MNC cultures for cytokine expression. SRW treatment induced local lung IL-5 and IL-13 production in HLA-DQ mice with undetectable levels of interferon-γ (Chapoval et al. 1999, 2002) indicative for Th2 effector function. Similar to our observations in BAL, direct analysis of cytokine synthesis in spleen MNC cultures of HLA-DQ6 mice also demonstrated the production of Th2 cytokines (Chapoval et al. 2002). Thus, the immune system of HLA-DQ tg mice exhibits Th2 cell effector activity in local lymphoid and target tissues. None of Th2 cytokines was detected in BAL and culture supernatant preparations obtained from H-2Aβ^0 control animals, suggesting that their inability to receive CD4$^+$ T-cell stimulation from MHC II molecules leads to a complete failure to express Th2 type cytokines and allergen-specific Ab (Chapoval et al. 1999) or to develop allergic eosinophilic airway inflammation.

Human asthma is characterized and its severity is diagnosed by heightened airway reactivity to varying concentrations of nonspecific bronchoconstrictors or spasmogens such

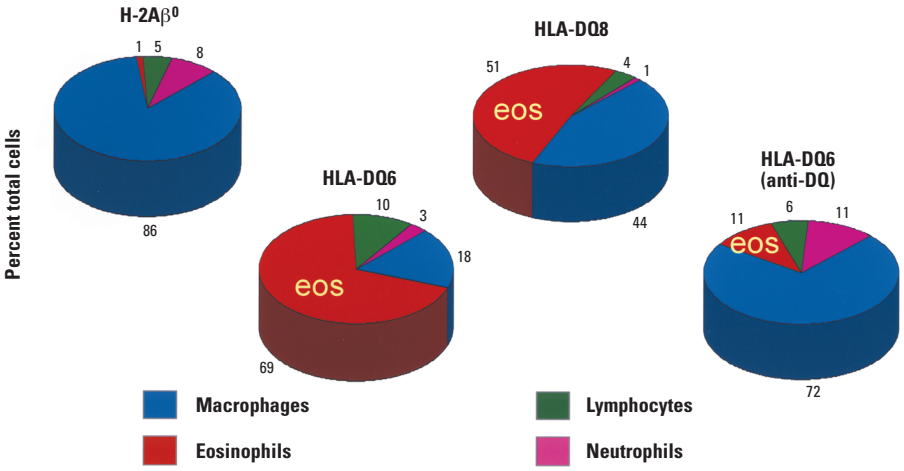

Figure 3. Cytospin analysis of BAL cells from SRW-sensitized and -challenged HLA-DQ tg mice. BAL fluids were collected at 48 hr after intranasal challenge, total leukocyte numbers were determined with standard hemocytometer, and differential cell counts were made using Giemsa-stained cytospin slides. Total BAL cell numbers: H-2Aβ^0, 674 x 10^3; HLA-DQ6, 1,460 x 10^3; HLA-DQ8, 1,048 x 10^3; HLA-DQ6, anti-DQ, 556 x 10^3, correspondingly.

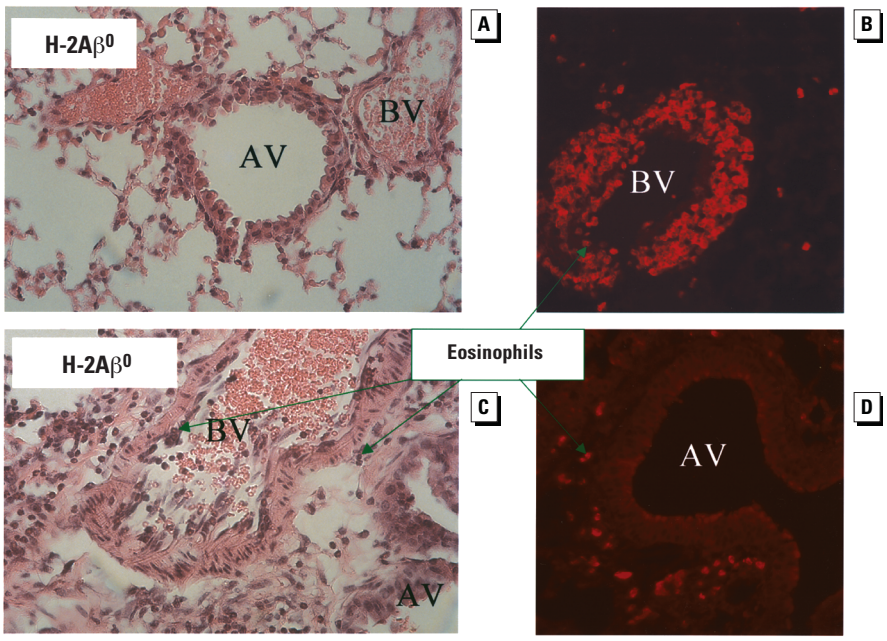

Figure 4. Histopathology or lung tissue stained with (*A,C*) hematoxylin/eosin (light microscopy) or with (*B,D*) anti-mouse eosinophil major basic protein mAb (confocal microscopy). (*A,C*) There was no inflammation in lung tissue of H-2Aβ^0 mice; a severe inflammation was observed in HLA-DQ6 tg mice. (*B,D*) Specific immunohistochemical staining for eosinophils revealed them as a majority of perivascular (*B*) and peribronchial (*D*) inflammatory cell infiltrates in HLA-DQ tg mice. Abbreviations: AV, airway vessel (bronchiole); BV, blood vessel.

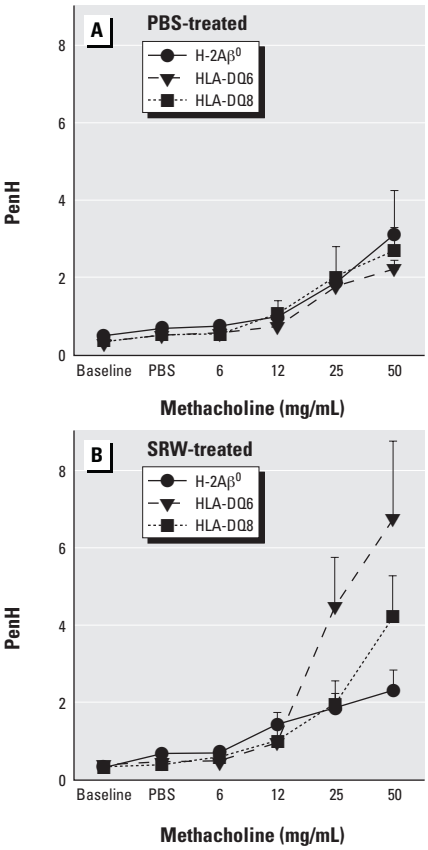

Figure 5. Respiratory system physiology of (*A*) PBS-treated and (*B*) SRW-sensitized and -challenged tg mice. Abbreviations: Te, expiration time; RT, relaxation time; Pef, peak expiratory flow; Pif, peak inspiratory flow. Airway reactivity was measured 48 hr after the last intranasal challenge by recording respiratory pressure curves by whole body plethysmography (Buxco Electronics Inc., Sharon, CT). After establishing a stable baseline of breathing in the plethysmograph, we exposed mice for 3 min to nebulized PBS and subsequently increasing concentrations of nebulized methacholine in PBS. After each nebulization, recordings were taken for 3 min. Values of PenH (index of airway obstruction) were calculated by BioSystem XA software with the following equation: PenH = (Te/RT-1) x (Pef/Pif).

as methacholine. To demonstrate these tendencies in our tg mice, we used a noninvasive barometric plethysmography, which allowed us to measure lung physiology in conscious, unrestrained mice (Hamelmann et al. 1997). When we challenged PBS-treated or SRW-treated mice with increasing concentrations of methacholine in a whole-body plethysmograph (Figure 5), we did not find an airway hyperreactivity in MHC II knockout mice. Allergen challenge induced strong hyperreactivity in HLA-DQ6 mice, but hyperreactivity was lower for HLA-DQ8 mice. The presence of specific HLA-DQ molecules was required for the induction of heightened airway reactivity *in vivo*. Overall, transgenic expression of two different HLA-DQ molecules in MHC II knockout mice produced different effects on several parameters of experimental allergen-induced asthma (levels of BAL eosinophilia, pulmonary inflammation, AHR, BAL cytokine, and protein concentrations). Thus, HLA class II transgenic mice offer the opportunity to investigate the role of distinct HLA molecules in allergen sensitivity and may be useful for devising new immune based therapies for allergic asthma.

References

Abraham RS, David CS. 2000. Identification of HLA-class-II-restricted epitopes of autoantigens in transgenic mice. Curr Opin Immunol 12:122–129.

Abraham RS, Kudva YC, Wilson SB, Strominger JL, David CS. 2000. Co-expression of HLA DR3 and DQ8 results in the development of spontaneous insulitis and loss of tolerance to GAD65 in transgenic mice. Diabetes 49:548–554.

Blumenthal MN. 2000. Genetics of asthma and allergy. Allergy Asthma Proc 21:55–59.

Bohle B, Wagner B, Vollmann U, Buck D, Niggemann B, Szepfalusi Z, et al. 2000. Characterization of T cell responses to Hev b 3, an allergen associated with latex allergy in spina bifida patients. J Immunol 164:4393–4398.

Borchers MT, Crosby J, Justice P, Farmer S, Hines E, Lee JJ, Lee NA. 2001. Intrinsic AHR in IL-5 transgenic mice is dependent on CD4(+) cells and CD49d-mediated signaling. Am J Physiol Lung Cell Mol Physiol 281:L653–L659.

Bradley DS, Nabozny GH, Cheng S, Zhou P, Griffiths MM, Luthra HS, David CS. 1997. HLA-DQB1 polymorphism determines incidence, onset, and severity of collagen-induced arthritis in transgenic mice: implications in human rheumatoid arthritis. J Clin Invest 100:2227–2234.

Burge HA, Rogers CA. 2000. Outdoor allergens. Environ Health Perspect 108(suppl 4):653–659.

Campbell P, Weiss U. 1999. Allergy and asthma. Nature 402:B1.

Chapoval SP, Fok C, Das P, Parker A, David CS. 2001a. Identification of HLA-DQ6 and HLA-DQ8 restricted p53 epitopes using transgenic mice [Abstract]. FASEB J 15:A1207.

Chapoval SP, Iijima K, Marietta EV, Smart MK, Chapoval AI, Andrews AG, David CS. 2002. Allergic inflammatory response to short ragweed allergenic extract in HLA-DQ transgenic mice lacking CD4 gene. J Immunol 168:890–899.

Chapoval SP, Marietta EV, Smart MK, David CS. 2001b. Requirements for allergen-induced airway inflammation and hyperreactivity in CD4-deficient and CD4-sufficient HLA-DQ transgenic mice. J Allergy Clin Immunol 108:764–771.

Chapoval SP, Nabozny GH, Marietta EV, Raymond EL, Krco CJ, Andrews AG, David CS. 1999. Short ragweed allergen induces eosinophilic lung disease in HLA-DQ transgenic mice. J Clin Invest 103:1707–1717.

Chapoval SP, Neeno T, Krco CJ, Marietta EV, Harders J, David CS. 1998. HLA-DQ6 and HLA-DQ8 transgenic mice respond to ragweed allergens and recognize a distinct set of epitopes on short and giant ragweed group 5 antigens. J Immunol 161:2032–2037.

Cohn L, Herrick C, Niu N, Homer R, Bottomly K. 2001. IL-4 promotes airway eosinophilia by suppressing IFN-gamma production: defining a novel role for IFN-gamma in the regulation of allergic airway inflammation. J Immunol 166:2760–2767.

Cookson W. 1999. The alliance of genes and environment in asthma and allergy. Nature 402:B5–B11.

Cope AP, Patel SD, Hall F, Congia M, Hubers HA, Verheijden GF, et al. 1999. T cell responses to a human cartilage autoantigen in the context of rheumatoid arthritis-associated and nonassociated HLA-DR4 alleles. Arthritis Rheum 42:1497–1507.

Corry DB, Grunig G, Hadeiba H, Kurup VP, Warnock ML, Sheppard D, et al. 1998. Requirements for allergen-induced airway hyperreactivity in T and B cell-deficient mice. Mol Med 4:344–355.

Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A. 2001. A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. Nat Immunol 2:45–50.

Das P, Drescher KM, Geluk A, Bradley DS, Rodriguez M, David CS. 2000. Complementation between specific HLA-DR and HLA-DQ genes in transgenic mice determines susceptibility to experimental autoimmune encephalomyelitis. Human Immunol 61:279–289.

de Silva HD, Sutherland MF, Suphioglu C, McLellan SC, Slater JE, Rolland JM, et al. 2000. Human T-cell epitopes of the latex allergen Hev b 5 in health care workers. J Allergy Clin Immunol 105:1017–1024.

Dhillon M, Roberts C, Nunn T, Kuo M. 1992. Mapping human T cell epitopes on phospholipase A2: the major bee-venom allergen. J Allergy Clin Immunol 90:42–51.

Elias JA, Zhu Z, Chupp G, Homer RJ. 1999. Airway remodeling in asthma. J Clin Invest 104:1001–1006.

Fiscus LC, Van Herpen J, Steeber DA, Tedder TF, Tang ML. 2001. L-Selectin is required for the development of airway hyperresponsiveness but not airway inflammation in a murine model of asthma. J Allergy Clin Immunol 107:1019–1024.

Geluk A, Taneja V, van Meijgaarden KE, Zanelli E, Abou-Zeid C, Thole JE, et al. 1998. Identification of HLA class II-restricted determinants of *Mycobacterium tuberculosis*-derived proteins by using HLA-transgenic, class II-deficient mice. Proc Natl Acad Sci USA 95:10797–10802.

Gerbase-DeLima M, Gallo CA, Daher S, Sole D, Naspitz CK. 1997. HLA antigens in asthmatic children. Pediatr Allergy Immunol 8:150–152.

Gonzalo JA, Tian J, Delaney T, Corcoran J, Rottman JB, Lora J, et al. 2001. ICOS is critical for T helper cell-mediated lung mucosal inflammatory responses. Nat Immunol 2:597–604.

Gregoire C, Chapman MD. 2001. Recombinant allergens. Clin Rev Allergy Immunol 21:215–227.

Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW. 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. Am J Respir Crit Care Med 156:766–775.

HayGlass KT. 1995. Allergy: who, why and what to do about it? Immunol Today 16:505–507.

Hizawa N, Collins G, Rafnar T, Huang S-K, Duffy DL, et al. 1998. Linkage analysis of *Dermatophagoides pteronyssinus*-specific IgE responsiveness with polymorphic markers on chromosome 6p21 (HLA-D region) in Caucasian families by the transmission/disequilibrium test. Collaborative Study on the Genetics of Asthma (CSGA). J Allergy Clin Immunol 102:443–448.

Holt PG, Macaubas C, Stumbles PA, Sly PD. 1999. The role of allergy in the development of asthma. Nature 402:B12–B17.

Huang S-K, Zwollo P, Marsh DG. 1991. Class II major histocompatibility complex restriction of human T cell responses to short ragweed allergen, *Amb a V*. Eur J Immunol 21:1469–1473.

Ito K, Bian HJ, Molina M, Han J, Magram J, Saar E, et al. 1996. HLA-DR4-IE chimeric class II transgenic, murine class II-deficient mice are susceptible to experimental allergic encephalomyelitis. J Exp Med 183:2635–2644.

Jember AG, Zuberi R, Liu FT, Croft M. 2001. Development of allergic inflammation in a murine model of asthma is dependent on the costimulatory receptor OX40. J Exp Med 193:387–392.

Katsuki T, Shimojo N, Honma K, Tsunoo H, Kohno Y, Niimi H. 1996. Establishment and characterization of ovalbumin-specific T cell lines from patients with egg allergy. Int Arch Allergy Immunol 109:344–351.

Klein J, Sato A. 2000. The HLA system. N Engl J Med 343:702–709.

Krco CJ, Harders J, Chapoval S, David CS. 2000. Immune response of HLA-DQ transgenic mice to house dust mite allergen p2: identification of HLA-DQ restricted minimal epitopes and critical residues. Clin Immunol 97:154–161.

Krco CJ, Watanabe S, Harders J, Griffths MM, Luthra H, David CS. 1999. Identification of T cell determinants on human type II collagen recognized by HLA-DQ8 and HLA-DQ6 transgenic mice. J Immunol 163:1661–1665.

Krinzman SJ, De Sanctis GT, Cernadas M, Kobzik L, Listman JA, Christiani DC, et al. 1996. T cell activation in a murine model of asthma. Am J Physiol 271:L476–L483.

Krug N, Frew AJ. 1997. The Th2 cell in asthma: initial expectations yet to be realized. Clin Exper Allergy 27:142–150.

Kudva YC, Deng YJ, Govindarajan R, Abraham RS, Marietta EV, Notkins AL, et al. 2001. HLA-DQ8 transgenic and NOD mice recognize different epitopes within the cytoplasmic region of the tyrosine phosphatase-like molecule, IA-2. Hum Immunol 62:1099–1105.

Kung TT, Jones H, Adams GK III, Umland SP, Kreutner W, Egan RW, et al. 1994. Characterization of a murine model of allergic pulmonary inflammation. Int Arch Allergy Immunol 105:83–90.

Ledford DK. 1994. Indoor allergens. J Allergy Clin Immunol 94:327–334.

Levine BB, Stember RH, Fontino M. 1972. Ragweed hayfever: genetic control and linkage to HLA haplotypes. Science 178:1201–1203.

Little AM, Parham P. 1999. Polymorphism and evolution of HLA class I and II genes and molecules. Rev Immunogenet 1:105–123.

Lukacs NW, Prosser DM, Wiekowski M, Lira SA, Cook DN. 2001. Requirement for the chemokine receptor CCR6 in allergic pulmonary inflammation. J Exp Med 194:551–555.

MacLean JA, Sauty A, Luster AD, Drazen JM, De Sanctis GT. 1999. Antigen-induced airway hyperresponsiveness, pulmonary eosinophilia, and chemokine expression in B cell-deficient mice. Am J Respir Cell Mol Biol 20:379–387.

Madsen LS, Andersson EC, Jansson L, Krogsgaard M, Andersen CB, Engberg J, et al. 1999. A humanized model for multiple sclerosis using HLA-DR2 and a human T-cell receptor. Nat Genet 23:343–347.

Malo JL, Chan-Yeung M. 2001. Occupational asthma. J Allergy Clin Immunol 108:317–328.

Marsh DG, Freidhoff LR, Ehrlich-Kautzky E, Bias WB, Roebber M. 1987. Immune responsiveness to Ambrosia artemisiifolia (short ragweed) pollen allergen Amb a VI (Ra6) is associated with HLA-DR5 in allergic humans. Immunogenetics 26:230–236.

McMillan SJ, Bishop B, Townsend MJ, McKenzie AN, Lloyd CM. 2002. The absence of interleukin 9 does not affect the development of allergen-induced pulmonary inflammation nor airway hyperreactivity. J Exp Med 195:51–57.

Mehlhop PD, van de Rijn M, Brewer JP, Kisselgof AB, Geha RS, Oettgen HC, et al. 2000. CD40L, but not CD40, is required for allergen-induced bronchial hyperresponsiveness in mice. Am J Respir Cell Mol Biol 23:646–651.

Moore WC, Hasday JD, Meltzer SS, Wisnewski PL, White B, Bleecker ER. 2001. Subjects with mild and moderate asthma respond to segmental allergen challenge with similar, reproducible, allergen-specific inflammation. J Allergy Clin Immunol 108:908–914.

Nabozny GH, Baisch JM, Cheng S, Cosgrove D, Griffiths MM, Luthra HS, et al. 1996. HLA-DQ8 transgenic mice are highly susceptible to collagen-induced arthritis: a novel model for human polyarthritis. J Exp Med 183:27–37.

Neeno T, Krco CJ, Harders J, Baisch J, Cheng S, David CS. 1996. HLA-DQ8 transgenic mice lacking endogenous class II molecules respond to house dust allergens: identification of antigenic epitopes. J Immunol 156:3191–3195.

Ono SJ. 2000. Molecular genetics of allergic diseases. Ann Rev Immunol 18:347–366.

Paisansinsup T, Deshmukh US, Poston S, Luthra H, Fu SM, David C. 2000. The role of HLA class II genes in immune response to human RO60 (SS-A) antigens: studies with transgenic mice. Arthritis Rheum 43:661.

Pan S, Taneja V, Griffiths MM, Luthra H, David CS. 1999. Complementation between HLA-DR4 (DRB1*0401) and specific H2-A molecule in transgenic mice leads to collagen-induced arthritis. Hum Immunol 60:816–825.

Pan S, Trejo T, Hansen J, Smart M, David CS. 1998. HLA-DR4 (DRB1*0401) transgenic mice expressing an altered CD4-binding site: specificity and magnitude of DR4-restricted T cell response. J Immunol 161:2925–2929.

Papouchado BG, Chapoval SP, Marietta EV, Weiler CR, David CS. 2000. HLA-DQ/human CD4-restricted immune response to cockroach allergens in transgenic mice. Tissue Antigens 55:303–311.

Pimtanothai N, Parra M, Johnson AH, David CS, Katovich Hurley C. 2000. Assessing the binding of four *Plasmodium falciparum* T helper cell epitopes to HLA-DQ and induction of T-cell responses in HLA-DQ transgenic mice. Infect Immun 68:1366–1373.

Platts-Mills TA, Rakes G, Heymann PW. 2000. The relevance of allergen exposure to the development of asthma in childhood. J Allergy Clin Immunol 105:S503–S508.

Raju R, Munn SR, David CS. 1997. T cell recognition of human pre-proinsulin peptides depends on the polymorphism at HLA-DQ locus: a study using HLA-DQ8 and DQ6 transgenic mice. Human Immunol 58:21–29.

Raju R, Spack EG, David CS. 2001. Acetylcholine receptor peptide recognition in HLA DR3-transgenic mice: *in vivo* responses correlate with MHC-peptide binding. J Immunol 167:1118–1124.

Renz H, Smith HR, Henson JE, Ray BS, Irvin CG, Gelfand EW. 1992. Aerosolized antigen exposure without adjuvant causes increased IgE production and increased airway responsiveness in the mouse. J. Allergy Clin Immunol 89:1127–1138.

Romagnani S. 2000. The role of lymphocytes in allergic disease. J Allergy Clin Immunol 105:399–408.

Schuh JM, Blease K, Hogaboam CM. 2002. CXCR2 is necessary for the development and persistence of chronic fungal asthma in mice. J Immunol 168:1447–1456.

Soriano JB, Ercilla G, Sunyer J, Real FX, Lazaro C, Rodrigo MJ, et al. 1997. HLA class II genes in soybean epidemic asthma patients. Am J Respir Crit Care Med 156:1394–1398.

Stewart GA, Thompson PJ. 1996. The biochemistry of common allergens. Clin Exp Allergy 26:1020–1044.

van Neerven JRJ, Ebner C, Yssel H, Kapsenberg ML, Lamb JR. 1996. T cell responses to allergens: epitope specificity and clinical relevance. Immunol Today 17:526–532.

Wang S, Fan Y, Han X, Yang J, Bilenki L, Yang X. 2001. IL-12-dependent vascular cell adhesion molecule-1 expression contributes to airway eosinophilic inflammation in a mouse model of asthma-like reaction. J Immunol 166:2741–2749.

Wasserman SI. 1999. The allergy and asthma report: discoveries in allergy and asthma. Discover. March: S-2.

Wolyniec WW, De Sanctis GT, Nabozny G, Torcellini C, Haynes N, Joetham A, et al. 1998. Reduction of antigen-induced airway hyperreactivity and eosinophilia in ICAM-1-deficient mice. Am J Respir Cell Mol Biol 18:777–785.

Zhu Z, Lee CG, Zheng T, Chupp G, Wang J, Homer RJ, et al. 2001. Airway inflammation and remodeling in asthma. Lessons from interleukin 11 and interleukin 13 transgenic mice. Am J Respir Crit Care Med 164:S67–S70.

Zuany-Amorim C, Ruffie C, Haile S, Vargaftig BB, Pereira P, Pretolani M. 1998. Requirement for gammadelta T cells in allergic airway inflammation. Science 280:1265–1267.