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The Association of HLA-DR Alleles and T Cell Activation with Allergic Bronchopulmonary Aspergillosis[1]

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Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease caused by the mold *Aspergillus fumigatus*. We previously reported that the majority of T cell clones (TCC) isolated from three ABPA patients, and specific for a dominant Ag of *A. fumigatus*, Asp f 1, were IL-4-producing CD4$^+$ Th2 cells capable of responding to Ag in association with the HLA-DR subtypes DRB1*1501, *1503, and *1601 for HLA-DR2, and DRB1*1101, *1104, and *1202 for HLA-DR5. In the present study we extended the previous findings to determine whether the observed restriction with the HLA-DR2/5 subtypes held importance in a larger patient population. Serotyping revealed that 16 of 18 ABPA patients were either HLA-DR2, HLA-DR5, or both. Compared with a normal control population, the frequencies of HLA-DR2 (50 vs 22.3%) and HLA-DR5 (44.4 vs 19.8%) were significantly increased in these ABPA patients. Genotype analyses of an additional 15 patients identified the same HLA-DR subtypes previously shown functional for Asp f 1 Ag presentation. The relative avidities of Asp f 1 peptides for the purified HLA-DR subtypes, DRB1*1501 (functional) and DRB1*1502 (nonfunctional), were examined to determine whether differential binding to the HLA-DR subtypes explains successful Ag presentation. Similar low binding avidities were detected for both HLA-DR subtypes, indicating that the functionality cannot be simply explained by differences in binding affinities. Thus, the limited number and their role in Ag presentation emphasizes the possibility that the six identified HLA-DR subtypes are important in the pathophysiology of ABPA. *The Journal of Immunology*, 1997, 159: 4072–4076.

Allergic bronchopulmonary aspergillosis (ABPA)[3] is a hypersensitivity lung disease that afflicts cystic fibrosis (CF) and asthmatic patients, and is caused by colonization of the airways by *Aspergillus fumigatus* (Af). Its diagnosis is based on clinical criteria including worsening asthma, recurrent febrile episodes, shifting pulmonary infiltrations, proximal bronchiectasis, and sputum containing plugs of Af. Laboratory criteria of disease includes peripheral blood eosinophilia, elevated total serum IgE, high titers of Af-specific IgG and IgE Abs, and immediate skin test reactivity to Af. All of these characteristics may not always be present in every patient. Differentiation between Af allergy and full-blown ABPA is often difficult, but of great clinical relevance, since untreated ABPA may lead to substantial lung damage.

We previously reported the characterization of CD4$^+$ T cell lines and clones (TCC) (1, 2) derived from ABPA subjects. Both the lines and clones were generated to an immunodominant Ag of Af, Asp f 1. TCC were analyzed for cytokine production, epitope specificities, and MHC restriction patterns. The results demonstrated the presence of two immunodominant epitopes on Asp f 1, since the majority of clones from three patients were directed to these two epitopes. Most of these clones produced significant amounts of IL-4, and with rare exception, minimal or no IFN-γ or IL-2, revealing a Th2 cytokine profile. Analysis of MHC class II restriction specificity of these TCCs indicated that Ag recognition was regulated by HLA-DR class II molecules, since in more than 90% of the cases TCC proliferation to Asp f 1 Ag was inhibited by anti-HLA-DR, and not by anti-DP or -DQ Abs. Panel studies using a number of matched and mismatched APC revealed that only HLA-DR2 or HLA-DR5 molecules were able to function as restriction elements for Asp f 1 Ag presentation. Furthermore, presentation of Asp f 1 with genotyped APCs revealed, surprisingly, that not all DRB1 chain variants of HLA-DR2/5 specificities were capable of Asp f 1 presentation.

Taken together, the above results prompted us in the present study to examine MHC class II restriction elements in a larger ABPA population to determine whether there was a significant association between the disease and the HLA-DR2/5 specificities. We also wanted to ascertain if this association further extended to only those HLA-DRB1 class II molecules (*1501, *1503, *1601, *1101, *1104, and *1202) found capable of Asp f 1 presentation (2). If Ag presentation by these allelic class II molecules is an important factor in ABPA, then a highly significant association should exist between the two. Our results show this is the case. Most notably, the HLA-DRB1 alleles that were identified in the larger ABPA population were the very same allelic class II molecules that had previously been shown in a limited sample to be capable of Asp f 1 presentation (2). This information provides not only insight into factors important to the pathophysiology of

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[3] Abbreviations used in this paper: ABPA, allergic bronchopulmonary aspergillosis; CF, cystic fibrosis; Af, *Aspergillus fumigatus*; TCC, T cell clones; SSP, sequence-specific primers.

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Table I. *Identification of DRB1 alleles in patients with allergic bronchopulmonary aspergillosis*

	Patient	HLA-DR Serotype[a]	Genotype[b] DRB1 Alleles
1	JR	4,5(11)	*1101
2	CM	5(11),13	*1101
3	RM	2(15),5(11)	*1101, *1501
4	DP	5(11),9	*1104
5	RS	5(11),7	*1104
6	ZS	5(11),■[c]	*1104
7	TH	1,5(11)	*1104
8	MA	1,5(12)	*1202
9	KL	2(15),7	*1501
10	NWE	2(15),1	*1501
11	MK	2(15),4	*1501
12	NC	2(15),13	*1501
13	WC	2(15),13	*1503
14	GH	2(15),■	*1503
15	EC	2(15),3	*1503
16	BM	2(16),7	*1601
17	NW	3,4	NT[d]
18	PN	4,9	NT

[a] HLA-DR antigen was identified by conventional serology.
[b] Genotyping (see *Materials and Methods*) was performed only in individuals bearing HLA-DR2/5 class II molecules.
[c] ■, patients homozygous for indicated serotypes.
[d] NT, not tested.

ABPA, but also calls attention to potential genetic markers that identify patients at risk for ABPA in CF and asthmatic populations.

Materials and Methods

Patients

Eighteen unrelated Caucasian patients with ABPA were examined. The group consisted of 10 male and eight female patients. All patients fulfilled criteria for diagnosis of ABPA as outlined previously (3). In these patients ABPA was diagnosed by the presence of recurrent wheezing, presence of new chest radiographic infiltrates, peripheral blood eosinophilia, immediate Af skin reactivity, positive precipitating Ab against Af Ags, elevated serum IgE greater than 1000 IU/ml, and elevated IgE and IgG anti-Af Abs by ELISA.

DNA extraction

B cells from the peripheral blood of the ABPA patients were transformed with the EBV and used as a source of genomic DNA for HLA-DRB1 genotyping. DNA extraction was performed as described by Miller, Dykes, and Polesky (4) with the following modifications. Briefly, 1 to 3 × 10^6 cells were placed in a 1.5-ml microcentrifuge tube and pelleted at 13,000 rpm for 30 s, and the cells washed with 1 ml of H_20 and centrifuged at 13,000 rpm for 1 min. The pellet was resuspended in 80 μl of 5× proteinase K buffer, 30 μl of proteinase K (10 mg/ml), 20 μl of 20% SDS, and 270 μl of H_20. Tubes were incubated at 55°C for 30 min, cooled to room temperature for 3 to 5 min, 100 μl of 6 M NaCl was added, and the tubes were shaken vigorously for 15 s. To remove the precipitated proteins, tubes were centrifuged for 3 min at 13,000 rpm. After transferring the supernatant into a 1.5-ml tube, DNA was precipitated by adding 1 ml of ethanol at room temperature. After centrifugation at 13,000 rpm for 2 min, supernatant was removed and the DNA pellet washed with 70% ethanol. DNA was dissolved in 50 to 100 μl of H_20 and the concentration was measured by spectroscopy.

PCR sequence-specific primer (SSP) HLA-DR typing

DRB1 alleles were determined by PCR amplification (Perkin-Elmer 9600 Cycler; Perkin-Elmer Corp., Norfolk, CT) with sequence-specific primers (PCR-SSP) using a kit from Dynal (Dynal Inc., Oslo, Norway). DNA from patients identified as DR2 or DR5 was amplified with allele and group-specific primers by which five alleles of DR2(15), six alleles of DR2(16), 19 alleles of DR5(11), and five alleles of DR5(12) could be identified. The presence or absence of amplified product was visualized by UV light after electrophoresis for 10 min at 15 V/cm on ethidium bromide-prestained 1.5% agarose gels.

Table II. *Distribution of HLA-DR antigen in ABPA and normal population*

HLA	% Frequency ABPA (n = 18)	% Frequency Controls[a]	Comparison of ABPA with Normal Controls Z[b]	P
DR2/DR5	88.3[c]	42.1	4.16	<0.0001
DR2 (15)	44.5	19.9	2.62	<0.005
DR2 (16)	5.5	2.4	–[d]	–
DR5 (11)	38.9	17.0	2.48	<0.01
DR5 (12)	5.5	2.8	–	–

[a] Values derived from Caucasian population within North America (7).
[b] Comparison of ABPA with normal controls using the Fisher exact test.
[c] Combined frequency of HLA-DR2, -DR5, or both in ABPA patients.
[d] (–), not determined due to sample size (n = 1).

Peptide binding competition in ELISA

The HLA-DRB1 *1501 and *1502 class II molecules were purified from human B lymphocytic cell lines as previously described (5, 6). The immunoaffinity-purified HLA-DRB1 *1501 and *1502 molecules (0.6 micrograms/well) were incubated overnight at 37°C with the biotinylated HA307–319 peptide (PKYVKQNTLKLAT) derived from the hemagglutinin molecule of the influenza virus (concentrations that gave 50% maximal binding to DRB1*1501 and DRB1*1502 were 3 nM for both), competitor peptide, and 1 mM Pefabloc SC in 96-well polypropylene microtiter plates (Costar, Cambridge, MA). All dilutions were made in PBS, pH 5.5, containing 1% octylglucoside (Boehringer Mannheim, Indianapolis, IN). LB3.1 Ab (anti-HLA-DR, 10 μg/ml) was immobilized onto 96-well polystyrene microtiter plates (Immulon 2; Dynatech, Chantilly, VA) overnight in 0.1 M $NaHCO_3$, pH 9.6, of coating buffer at 4°C. After each step described below, wells were washed with PBS containing 0.05% Tween-20 (Bio-Rad, Hercules, CA). LB3.1-coated microtiter plates were blocked with 1% Carnation nonfat milk for 1 h at 37°C. Extravidin-conjugated horseradish peroxidase (1:1000; Sigma Chemical Co., St. Louis, MO) was added to each well and incubated for 45 min at 37°C. *o*-Phenylenediamine dihydrochloride substrate and H_2O_2 (Sigma Chemical Co.) in a 0.5 M phosphate-citrate buffer, pH 5.0, were added and the absorbance of each well was read at 450 nm. Each experiment was performed three times.

Results

Prevalence of HLA-DR2/5 serotypes in ABPA

We have shown previously that DR2 or DR5 molecules in three ABPA patients are the main restriction elements for Asp f 1 Ag presentation. To assess the importance of the previous findings we examined an expanded ABPA population for the prevalence of the HLA-DR2/5 serotypes. HLA-DR specificities in an additional 15 ABPA patients were determined by serology as well as DNA typing using low resolution primers. As seen in Table I, 16 patients were found to be either HLA-DR2, HLA-DR5, or, in the case of one patient (RM), both. As seen in Table II, the association of HLA-DR2/5 with ABPA (88.8%) was found to be highly significant ($p < 0.0001$) when compared with the 42.1% frequency of HLA-DR2/5 found in the normal population (7). The frequency of the HLA-DR2(15) (44.4% vs 19.9%) and HLA-DR5(11) (38.8% vs 17.0%) subtypes in the ABPA population were both significantly increased in ABPA patients compared with normal controls ($p < 0.005$ and $p < 0.01$, respectively).

DRB1 allele distribution among ABPA

Since there are 11 DR2 and more than 20 DR5 alleles that can be detected at the DNA level but only 4 serologically, we extended our study to include high resolution DNA typing. DNA from patients identified as DR2 or DR5 was amplified with group-specific primers by PCR-SSP. The determination of DRB1 alleles for the 16 DR2/5 ABPA patients is shown in Table I. Shown in Figure 1

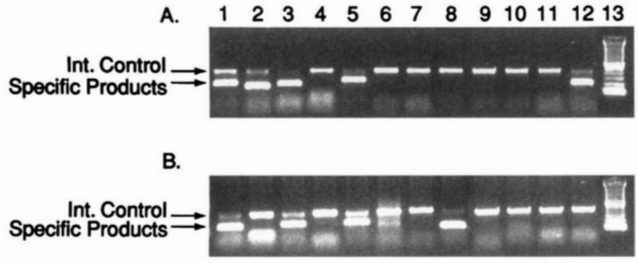

FIGURE 1. PCR-SSP HLA-DR typing. PCR-SSP typing of HLA-DRB1*1503 (*A*) and DRB1*1104 (*B*) using 12 PCR-SSP reaction mixtures for each allele. An internal positive control primer pair present in each reaction mixture amplifies a 429-bp segment of the human growth hormone gene. The positive reactions in *lanes 1, 2, 3, 5,* and *12* (*A*), and *lanes 1, 3, 5,* and *8* (*B*) identify DRB1*1503 and *1104, respectively. *Lane 13* contains m.w. markers.

are representative data (DRB1*1503 and DRB1*1104 in patients WC and TH, respectively) used to identify the DRB1 alleles in the ABPA population. Seen in Table III is the distribution of the alleles found within the ABPA patients. Most prevalent was the DRB1*1501 allele (5 patients), followed by DRB1*1104 (4 patients), *1503, and *1101 (3 patients each), and 1 patient each for DRB1*1601 and 1202. Only 2 of 5 DR2(15) (*1501, *1503), 1 of 6 DR2(16) (*1601), 2 of 19 DR5(11) (*1101, *1104)), and 1 of 5 DR5(12) (*1202) alleles identifiable by this kit were present in the patient population. Thus, it would appear that only 6 of 35 identifiable alleles were associated within this ABPA population.

We previously reported that a majority of TCC isolated from three ABPA patients were not restricted to a single HLA-DR subtype but cross-reacted with two or more class II molecules within their respective DR2 or DR5 subset. Interestingly, the cross-reactivity could not be predicted on the basis of their relatedness within a given serotype. For example, several TCC derived from a patient genotyped as DRB1*1202 did not respond to APC expressing the closely related HLA-DR DRB1*1201 molecules but were activated by lymphoblastoid B cell lines genotyped as the more distantly related DR5 DRB1*1101 or *1104. Based on this information, we wanted to establish whether the functional homologous as well as the cross-reactive class II alleles, i.e., HLA-DR (DRB1*1501, *1503, *1601, *1101, *1104, and *1202) were the prevalent subtypes in the extended ABPA population. As seen in Table IV, the inherited DRB1 alleles (six among a possible 35) were, without exception, the very same alleles whose products were involved in the activation of Asp f 1-specific TCC (2). Such a prevalence emphasizes the importance of these allelic subtypes in the disease process.

Binding of Asp f 1 peptides to purified HLA-DR2 molecules

Since we observed a significant association between genotype, T cell activation, and ABPA (2), we asked whether this linkage was based on the ability of immunogenic Asp f 1 peptides to differentially bind HLA-DR2/5 molecules. We previously reported that some TCCs derived from ABPA patients responded to the Asp f 1 peptide 106–125 (KFDSKKPKE DPGPARVIYTY) in context with HLA-DR (DRB1*1501) but not to the same peptide in association with HLA-DR (DRB1*1502). To test whether differential binding accounts for the contrasting function, both the DRB1*1501 and *1502 class II heterodimers were isolated and the relative avidities of the immunogenic peptide 106–125 for these proteins was assessed. The avidities were measured by the ability of peptide 106–125 to compete with an influenza hemagglutinin-derived peptide, amino acids 307–319, which binds to both DRB1

Table III. *Prevalence of HLA-DR2 and HLA-DR5 alleles in ABPA patients*

HLA	DRB1 Alleles	No. of ABPA Patients
HLA-DR2	DRB1*1501	5[a]
	DRB1*1503	3
	DRB1*1601	1
HLA-DR5	DRB1*1101	3
	DRB1*1104	4
	DRB1*1202	1

[a] Total number of alleles exceed 16 since 1 patient has both DRB1*1101 and *1501.

Table IV. *DRB1 alleles involved in ABPA and Asp f 1-specific T cell activation*

HLA-DR2		HLA-DR5	
DRB1 alleles[a]	T cell responses	DRB1 alleles	T cell responses
DRB1*1501[b]	+[c]	**DRB1*1101**	+
DRB1*1502	−	DRB1*1102	−
DRB1*1503	+	DRB1*1103	−
DRB1*1601	+	**DRB1*1104**	+
DRB1*1602	−	DRB1*1201	−
		DRB1*1202	+

[a] Genotypes of the tested APCs.
[b] Bold designation represents the alleles found in the ABPA population; of the 11 DRB1 alleles tested, these same 6 alleles were functional in T cell activation (2).
[c] +, the ability of the indicated APCs to stimulate T cell responses.

molecules with relatively high affinity (5, 6). The competition profiles of the peptide 106–125 generated with both allelic proteins appear comparable, with IC_{50} values at >85 μm, indicating extremely low affinities for both HLA-DR (DRB1*1501 and *1502) (Fig. 2). Similar low binding affinities were demonstrated for all of the Asp f 1 peptides containing T cell epitopes reactive with TCC derived from ABPA patients (data not shown). Also seen in Figure 2 are inhibition curves generated with the nonimmunogenic Asp f 1 peptide 16–35 (KWEDKRLLYNEAKAESNSHH) for both HLA-DR molecules (DRB1*1501 and *1502; IC_{50} at 0.8 and 0.3 μM, respectively). This peptide was used as a positive control to demonstrate higher affinity binding of Asp f 1 peptides in this assay. Interestingly, no TCC reacting to this peptide have been identified in the ABPA patients (2).

Shown in Figure 3 are T cell proliferation assays using either DRB1*1501 or *1502 genotyped APC. Despite the fact that peptide 106–125 binds with low similar affinities to both HLA-DR class II molecules (DRB1*1501 and *1502), it stimulates TCC only in association with HLA-DR (DRB1*1501). This is shown for two different TCC isolated from the same ABPA patient (MK, DRB1*1501). These results confirm our previous findings (2) and thus, the difference in Ag presentation between the *1501 (functional) and *1502 (nonfunctional) class II molecules cannot be simply explained by obvious binding differences.

Discussion

A major goal in the studies of immunologically mediated diseases is to identify, characterize, and eventually modulate determinants important to the disease process. The polymorphic MHC class II molecules on APC play a critical role in restricting Ag-specific T cell activation (8, 9). This activation is crucial in the induction of

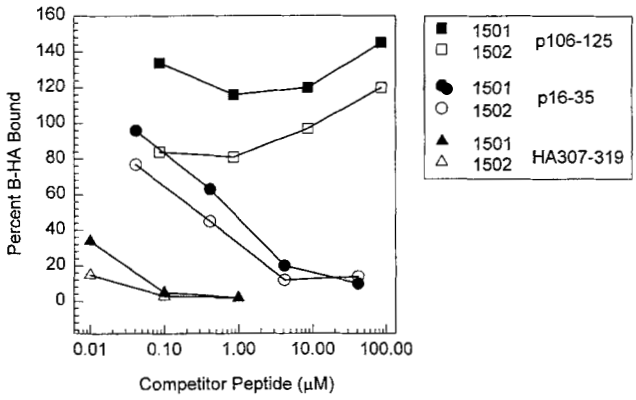

FIGURE 2. Determination of Asp f 1 peptides binding to purified HLA-DRB1 *1501 and *1502 molecules. Binding of the Asp f 1 peptides 106–125 and 16–35 was determined by ELISA (see *Materials and Methods*). The binding profiles were determined by the abilities of the various peptides to inhibit the binding of biotinylated HA307–319 peptide to the purified HLA-DRB1*1501 (closed symbols) and *1502 molecules (open symbols) at the designated concentrations. The IC_{50} values, or the concentration (μM) of peptide required to inhibit the binding of the biotinylated HA307–319 peptide by 50%, were determined by extrapolation from binding profiles: >85 μM for the Asp f 1 peptide 106–125 to both DRB1*1501 and *1502; ~0.8 and 0.3 μM for the Asp f 1 peptide 16–35 to DRB1*1501 and *1502, respectively; and ~0.003 μM for the homologous HA307–319 to both DRB1*1501 and *1502. Data are representative of one of three separate experiments.

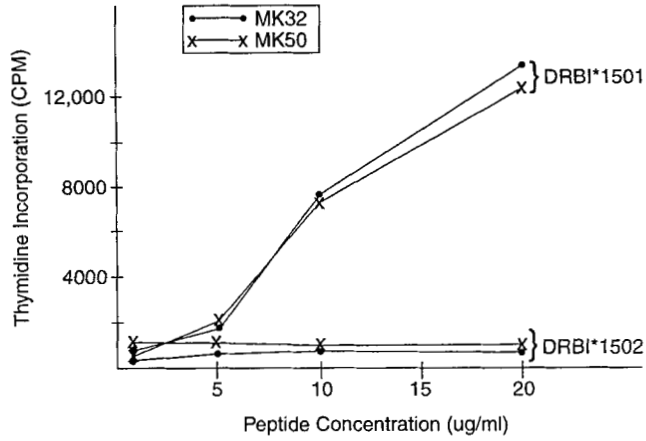

FIGURE 3. Influence of MHC alleles DRB1 *1501 and DRB1 *1502 on stimulation of TCC specific for the Asp f 1 peptide 106–125. Two TCC (MK 32 and MK 50) isolated from an ABPA patient (DR 2, DRB1*1501) were tested for their proliferative response in a dose-dependent manner with Asp f 1 in the presence of either DRB1*1501 or *1502 genotyped APC (see *Materials and Methods*).

immune responses that result in allergic and autoimmune diseases. In this report we have extended previous studies that showed that the activation of virtually all T cells to the Af Ag, Asp f 1, in ABPA patients was restricted by either the HLA-DR2 or HLA-DR5 specificities (2). These reported restrictions, found in a limited number of patients, appear to be important determinants since our current findings on a much larger population demonstrate a highly significant association ($p < 0.0001$) between HLA-DR2/5 and ABPA. Although T cell activity in response to Asp f 1 was not measured in the extended ABPA population, the HLA linkage to disease would make it highly suggestive that HLA-DR2/5 class II molecules play a major role in the pathophysiology of ABPA at the level of T cell activation. The linkage between allergic responses to house dust mite and ragweed Ag and the class II molecules HLA-DR2 and HLA-DR5 have been reported (10–13). In addition, genetic and epidemiologic studies support a strong association between the HLA-DR2/5 serotypes and allergic responses to ragweed and grass pollen (14–16).

In our previous studies (2) we tested a series of TCC derived from three ABPA patients (DRB1 *1503, *1601, and *1202) to determine whether these T cells responding to Asp f 1 were restricted solely by the homologous HLA-DR subtype, or whether closely related DR2 or DR5 subtypes were also functional in Asp f 1 presentation. Of the 11 genotyped APC tested, only three additional APC (DRB1*1101, *1104, and *1501) functioned to stimulate the Asp f 1-specific TCC. We reasoned that if Asp f 1 Ag in association with critical HLA-DR2/5 class II molecules were important to T cell activation, and subsequent development of ABPA, the six functional alleles should also be prevalent in the expanded ABPA population. Genotypic analysis, capable of distinguishing 35 HLA-DR2/5 genotypes, identified only those six alleles whose products were previously found capable of stimulating TCC. These findings appear highly significant given the fact that without exception the genotypes among the 15 additional patients were the very same identified with APC found to be functional for Asp f 1 presentation (2). Whether these represent "at risk" alleles will depend upon the frequency of these genotypes among the normal HLA-DR2/5 Caucasian population. The last published compilation (7) as well as more recent unpublished information (Dr. T. Fuller, University of Utah, unpublished observation) on the HLA-DRB1 genotype frequencies among the normal North American Caucasian population show that the HLA-DRB1 *1501 and *1101 genotypes are the most common of the HLA-DR2 and DR5 serotypes. Thus, these genotypes may not necessarily represent "at risk" alleles among HLA-DR2 or DR5 CF or asthmatic patients. However, the frequencies, or lack thereof, of the remaining HLA-DRB1 alleles among the ABPA population appear significantly skewed for some alleles when compared with the normal population. HLA-DRB1 *1501, *1502, and *1503 frequencies among a normal North American population (>1700 individuals) from the University of Utah database showed that *1501 is by far the most represented among these alleles at 12.8% of the total population, followed by *1502 and *1503 at 0.6% and 0.2%, respectively. Among the HLA-DR2(15) ABPA patients, only HLA-DRB1 *1501 (five of eight) and *1503 (three of eight) are represented (see Table III). Among the HLA-DR5(11) normal population, the DRB1 frequencies compiled from two sources (University of Utah and International Histocompatibility Workshop, 1991 (7)) are *1101, 4.2 to 5.6%; *1102, 0.4 to 1.1%; *1103, 0.7 to 1.4%; and *1104, 0.5 to 2.7%. Among the HLA-DR5(11) ABPA patients, only the DRB1 genotypes *1101 (three of seven) and *1104 (four of seven) are represented. Based on these differences from the normal distribution it would appear that the HLA-DRB1 *1503 and *1104 represent candidate "at risk" markers for ABPA in susceptible CF and asthmatic patients. However, more data among ABPA and particularly non-ABPA CF and asthmatic patients are needed to establish a linkage between certain HLA-DR alleles and ABPA.

To explore the molecular basis between functional and nonfunctional genotypes, competition ELISA assays were performed to measure the relative avidities of Asp f 1 peptides for both genotypes. Surprisingly, we found no apparent difference in the avidities of the Asp f 1 peptide between the functional (*1501) and nonfunctional (*1502) alleles, and that both the avidities appeared extremely low (>85 μM, see Fig. 2). This data, contrary to our

expectation, does not show a correlation between affinity and Ag presentation. In fact, we have data (partially depicted in Fig. 2) to show that a dominant immunogenic peptide 106–125 of Asp f 1 exhibits extremely low affinity in comparison to a nonimmunogenic peptide 16–35 from the same Asp f 1 molecule. Consequently, we cannot explain the difference in TCC responses to the various DR2/5 genotypes simply on the basis of differential binding of peptide for the class II molecule because functional DRB1*1501 does not bind peptide 106–125 at a higher affinity than nonfunctional DRB1*1502. Similar findings that have described T cell activation by peptides with low to nondetectable binding for class II molecules have been reported (17). Consequently, it would appear that the lack of T cell reactivity for at least some Asp f 1 peptides lies with the T cell repertoire and not the absence of binding to HLA-DR molecules.

The cellular and molecular basis for the association of particular HLA-DR genotypes, T cell reactivity, and ABPA still lack a clear understanding. T cell reactivity to Af Ags does not necessarily lead to ABPA. We and others have detected hypersensitivity to Af in asthmatics and CF patients who do not have ABPA (18). It will be necessary to determine the HLA genotypes as well as to study the nature of T cell reactivity to the Asp f 1 Ag in this non-ABPA population. Will the products of the HLA-DR2/5 alleles described in these studies restrict Asp f 1 T cell reactivity in the non-ABPA patients? If differences were found in the non-ABPA group, it would lend increased importance to the functional HLA-DR alleles operative in ABPA. We would predict that the majority of the Asp f 1-reactive T cells in the non-ABPA group will produce high ratios of IFN-γ and IL-2 over IL-4 (Th1 phenotype), in sharp contrast to Asp f 1-specific Th2 cells found in ABPA patients. These results would strongly suggest that Asp f 1 peptides in association with specific HLA-DR2/5 alleles contribute to a Th2 bias in the reactive T cell population. Although the eventual outcome of the number and nature of T cells (Th1 vs Th2) responding to Ag is complex (19), there is genetic evidence in mice to indicate that Ag in association with particular class II alleles influences the eventual outcome of the reactive T cell phenotypes (19). In addition, there is evidence in humans that indicates that HLA class II molecules are important to the outcome of the T cell phenotype and consequently to the pathophysiology of particular allergic diseases (20, 21). Studies are currently underway to study cytokine production and HLA restriction in Asp f 1-specific TCC from non-ABPA asthmatic and CF patients.

In summary, we have described a highly significant association between the HLA-DR2/5 serotypes and ABPA. In addition, we have shown that the DR2/5 genotypes identified in 16 ABPA patients match, without exception, the previously described HLA-DRB1 class II molecules (2) that functioned to activate Asp f 1-specific Th2 cells derived from three ABPA patients. The high correlation between HLA genotypes, T cell reactivity, and disease suggest that the HLA-DR2/5 genotypes, DRB1*1501, *1503, *1601, *1101, *1104, and *1202, potentially confer a risk for ABPA in CF and asthmatic patients. Furthermore, these findings provide newer information into the immunopathophysiology of this disease as well as insight into possible therapeutic intervention.

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