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MHC RESTRICTION IN ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Bela Chauhan [1], Patricia S. Hutcheson [1], Raymond G. Slavin [1] Clifford J. Bellone [2]

Department of [1]Internal Medicine, and [2] Molecular Microbiology and Immunology, Saint Louis University School of Medicine, St. Louis, MO 63104

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1. ABSTRACT

Allergic bronchopulmonary aspergillosis (ABPA) is a rare complication in patients with asthma but more common in patients with cystic fibrosis. In the presence of the fungus Aspergillus fumigatus (Af) in the lower respiratory tract, patients mount a heightened IgG and IgE humoral response specific for Af antigens. Studies on ABPA have suggested a pathogenic role for antigen specific CD4+ Th2 like T lymphocytes producing increased levels of IL-4 and IL-5. MHC class II genes coding for highly polymorphic HLA molecules have been shown to be the likely candidates for controlling immune responses to common allergens. However there has been a lack of information on the pathophysiological role of HLA genes in the development of ABPA.

This review describes an association between HLA- class II alleles and the specific responses to Af antigen (Asp f 1) in ABPA. These studies focused on MHC restriction and distribution of HLA- class II alleles in two groups of unrelated North American Caucasian patients with cystic fibrosis and/or asthma. One group consisted of patients with a confirmed diagnosis of ABPA and a second group of patients with Af sensitivity but no ABPA. HLA association studies revealed that the predisposition to develop ABPA is associated with HLA-DR2 and DR5, and possibly DR4 or DR7. A strong association of HLA-DR antigens with ABPA reflects that HLA-DR molecules may present disease-causing peptides. On the other hand a significant association of HLA-DQ2 with Af sensitive nonABPA indicates the involvement of HLA-DQ molecules in protection. A combination of these genetic factors determines the outcome of ABPA in patients with cystic fibrosis and asthma.

2. INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is a lung disease caused by an immunologic response to the mold Aspergillus fumigatus (Af). Pulmonary diseases caused by Af can be classified according to the site of the disease within the respiratory tract and the extent of mycelial colonization or invasion, both of which are influenced by the immunological status of the host (1). ABPA occurs only in asthmatic and cystic fibrosis patients. This disease, originally considered a rarity, is today diagnosed with increasing frequency as a result of improvement in serologic and radiologic diagnostic methods (2). The clinical diagnostic criteria for ABPA in asthmatic patients were proposed by Greenberger and Patterson (3). These criteria include peripheral blood eosinophilia, elevated total serum IgE, immediate cutaneous reactivity, serum precipitating antibodies to Af, elevated levels of Af specific serum IgE and IgG, chest roentgenographic infiltrates, and bronchietcasis. In stages of the disease during which most of the criteria are evident, ABPA can be readily diagnosed. However, all criteria are rarely present at the same time, even in patients with classic ABPA (3). The assessment of hypersensitivity to Af as a prerequisite for the diagnosis of ABPA is routinely investigated by skin testing and determination of fungal

specific IgE antibodies (4,5). The lack of standardized Af extracts for skin testing, and the fact that immune parameters related to fumigatus sensitization are not static and can spontaneously diminish over time complicates the diagnosis of ABPA (6). The incidence of ABPA is reported to range from 1-2% among asthmatics and 1-15% among cystic fibrosis patients sensitized to Af (7,8). Undetected ABPA has devastating consequences for patients because the untreated disease can lead to irreversible changes in lung function and to end stage pulmonary fibrosis. Therefore, unless there is either a formal screening program based on definite clinical criteria or genetic markers, many cases of aspergillus-induced complications may go unrecognized.

Population genetic studies have indicated that products of the major histocompatibility gene complex, particularly the class II HLA-DR molecules, influence susceptibility to allergic diseases. With the use of molecular genetic probes, several investigators have demonstrated the functional importance of both HLA-DRB1 and DQB1 gene products in T cell recognition of antigens (9) T lymphocytes recognize antigen when presented together with major histocompatibility complex (MHC) encoded class I and II molecules on the membrane of antigen presenting cells (APC). This is called MHC restriction and the parts of the MHC molecule that are recognized together with antigen are called restriction elements. Both class I and II molecules play a key role in antigen presentation to effector T lymphocytes. In order to be activated, T lymphocytes must recognize antigens presented on the surface of APC in association with their MHC products. Cytotoxic T lymphocytes recognize antigens predominantly in association with class I molecules while helper T lymphocytes do so predominantly in the context of class II molecules (10-12). Since immune components involved in MHC-restriction are highly polymorphic and represent a complex system of integrated structures this review highlights some of their structural and functional details.

3. MHC CLASS I AND II MOLECULES

3.1. Function of MHC Class I and Class II molecules

The role of MHC molecules in the immune system is primarily the presentation of antigens to T cells. The antigen receptor of T cells is unable to recognize antigen directly; it can only recognize foreign antigen in the form of short segments of peptide bound to MHC molecules. Thus both MHC class I and II molecules exert their function by physical interaction with antigens in the form of short linear peptides. This phenomenon of MHC-restricted recognition of antigens was first suggested by Zinkernagel and Doherty and can now be demonstrated directly by using purified MHC molecules and purified T cell receptors (TCR) (13). Neither peptide antigens nor MHC molecules independently can stimulate T cell responses; the formation of a peptide-MHC complex is clearly required (14).

3.2. Structure of MHC Class I and Class II molecules

The two distinct classes of MHC molecules that can be detected on the surface of cells differ biochemically on the basis of subunit structure. MHC class I molecules consist of two chains, an alpha or heavy chain associated non-covalently on the cell surface with the smaller β2-microglobulin protein. Only the class I α chain spans the membrane. MHC class II molecules consist of a complex of two chains, α and β, both of which span the membrane and are associated non-covalently on the cell surface. In the case of class I molecules, three domains, (α1, α2, and α3) are contained within the α chain transmembrane glycoprotein while the fourth domain is contributed by β2 microglobulin. In the case of the class II heterodimers, each chain contributes two domains. Generally, the overall structures of class II and I are very similar. In the membrane distal domains, the α1 and α2 domains of class I and α1 and β1 domains of class II fold together to form a long groove. The remaining extracellular domains adopt an immunoglobulin domain like structure. While the overall structures of class I and class II molecules are similar, there are a number of small differences that have an important effect on the way that these molecules interact with peptide antigens (15-17).

3.3. Antigen binding by MHC Class I and Class II molecules

The antigen that is bound by class I and class II MHC molecules exists in the form of relatively short peptide fragments. In both cases, the same general principle applies, although fine details of how the peptides are bound differ between the two classes of molecules (18). The precise topology of the MHC peptide-binding groove depends partly on the nature of the amino acids within the groove, and thus varies from one haplotype to another. Which peptide can bind to a particular MHC molecule depends on the nature of the peptide side chains and their complementarity with the MHC molecule's binding groove. In the case of MHC class I molecules, the peptides that are bound are predominantly short, usually octamers and nonamers, although longer peptides can be bound. The peptide is bound at both ends, with interactions between the MHC molecules and the N and C termini of the peptide (19). Peptide binding to class II molecules differs in a number of important respects from binding to class I. The majority of peptides bound to class II molecules are greater than 13 amino acids in length and there is, in principle, no upper limit to the length of peptide that can be bound. This allows the end of the peptide to protrude beyond the end of the peptide-binding groove.

3.4. MHC polymorphism on peptide binding

In humans the predominant class I antigens are HLA-A, B and C while class II antigens are HLA-DR, DP and DQ. The allelic diversity of the MHC is remarkably large, with most loci exhibiting the phenomenon of allelic polymorphism, meaning that the frequency of an allele is greater than that expected by the operation of chance mutations, i.e. they have undergone positive selection. All MHC products are polymorphic to a greater or lesser extent. With the exception of the HLA-DRα chain locus, both α and β chain loci of class II molecules HLA-DQ and HLA-DP contain polymorphic alleles. The DRα chain does not vary in sequence between different individuals and

is said to be monomorhpic. Individual alleles in each locus are referred to by a nomenclature that indicates locus and allele designation. Thus, polymorphic HLA-DR genes encode the DRβ polypeptide chain at a locus known as DRB1. Individual alleles carry a four digit designation that follows the locus names, i.e. DRB1* 0401. Many of the frequent alleles have historical names in common usage that are used as synonyms, thus, DW4 is often used synonymously with DRB1*0401. Individual MHC alleles can differ from one another by up to 20 amino acids, making each allele quite distinct. Most of these differences are concentrated on the exposed surfaces of the membrane-distal domains of the molecules and the peptide-binding groove, in particular. Different allele variants of MHC class II molecules bind different peptides; however the more open structure of the MHC class II peptide-binding groove, and the greater length of the peptides bound in it, allow greater flexibility in peptide binding. Since the role of the immune system is to protect against infection, the extensive polymorphism of the MHC protein provides a selective advantage within a population to deal with a variety of pathogens. For example different individuals in a population differ in the combination of MHC molecules they express and can present different sets of peptides from each pathogen. This makes it unlikely that all individuals in a population will be equally susceptible to any given pathogen, thereby limiting its spread.

3.5. Antigen presentation by MHC Class II molecules

Because peptide MHC interactions are critical to immunity, studies on antigen presentation by MHC classes I and II molecules will be important to the understanding of many MHC associated human diseases. For example, HLA-DR B1 *0401 and *0404 are strongly associated with rheumatoid arthritis (20;21). These alleles are positively charged or neutral at residues 70 and 71 in the peptide binding β1 domain of class II. These two sites have been shown to be critical to the function of HLA-DR in peptide presentation to T cells. In the case of insulin dependent diabetes mellitus (IDDM), aspartic acid at position 57 of HLA-DQ β alleles has been associated with protection (22;23). The amino acid at this position could alter the binding capacity and stability of the molecule and thereby alter the potential of the peptide-MHC complex to stimulate protective T cell immunity. Peptide binding motifs, based on critical amino acid residues have been predicted for alleles of both class I and II. Prediction of peptide epitopes has lead to a better understanding of ensuing immune responses to particular pathogens and the development of vaccines against microorganisms.

4. MHC AND DISEASE ASSOCIATIONS

A large number of diseases have been reported to show MHC associations. The extreme degree of MHC polymorphism and their function as restriction elements in generating specific responses to foreign molecules make HLA alleles key components in the immunopathogenesis and susceptibility to certain diseases. It has become apparent that there are strong associations between HLA antigens and a large number of specific diseases. Ankylosing spondylytis is one of the most striking examples of HLA and disease association 24. HLA-B27 occurs in 90% of patients compared to 9.4% of controls. Other strong associations include HLA-DR3 DQ2 with celiac disease (25), HLA-DR2 with multiple sclerosis (26;27) and HLA-DR1 or DR4 with rheumatoid arthritis (28-30). HLA population and family studies on susceptibility to IDDM have demonstrated genetic associations. The most striking association is noted with the class II DR3 and DR4 antigens (31;32). Increased risks in heterozygotes for HLA-DR3 and DR4 have been established unequivocally in studies of HLA allele frequencies in IDDM families. The strong association between DQ alleles such as DQ2 and DQ3 and susceptibility to IDDM has also been reported (33;34). Common to all of these HLA- disease associations is the influence of the particular DR or DQ and a combination of alleles from both loci on the host immune response and subsequent susceptibility.

4.1. Associations between MHC polymorphism and disease

In contrast to the recognition of conformational epitopes by B cells and antibody, T cells largely recognize peptides at the level of their primary structure. Polymorphic MHC molecules regulate this aspect of the T cell immune response by regulating antigen presentation. The amino acids that determine the particular polymorphism influence the preferential binding of certain classes of peptides for presentation to T cells (35;36). Subregions in the DR binding groove have been identified in the molecular structure of HLA-DR that likely influence the binding of an antigenic peptide and its subsequent recognition by T cells. These regions in different alleles form portions designated as pockets. This is also the basis of the phenomenon of determinant selection. Identification of polymorphic alleles within a particular disease is crucial because of fundamental role that MHC polymorphisms play in influencing the outcome of the T cell repertoire against an invading foreign antigen.

4.2. Allergic responses and HLA

MHC restriction patterns of antigen specific human T cells appear to be very diverse, as is the T cell epitope recognition. HLA-DRB1, HLA-DRB3, HLA-DP and HLA-DQ restriction has been demonstrated for several allergens (37-42). Early studies of HLA association with allergic disorders have described the presence of extended MHC haplotypes, which involve several alleles located at more than one locus within the MHC complex. Blumenthal, et al, demonstrated a significantly increased frequency of the extended MHC haplotype B7, Sc 31, DR2 in patients with asthma who had high titers of IgE anti-Amb a V, an important ragweed antigen (43-45). Conversely, these patients had a decreased frequency of HLA-DR3 46. It has been suggested that different extended haplotypes contain a different pattern of chromosomal deletions, some of which may influence the level of gene expression. In more recent studies certain alleles, especially DR/DQ combinations, are seen to be strongly associated with certain diseases (47;48). Among subjects with rye- grass pollen allergy, sensitization to both Lol p 2 and Lol p 3 was associated with HLA-DR3 (49).

Table 1. HLA-DR Restriction Determinants of Asp f 1 Specific TCC from ABPA Patients

Patients HLA-DR Haplotypes	DR2 DR6	DR3 DR4	TCC	DR6 DR11	DR5 DR13	DR1	DR2	DR7	DR4	DR2 DR7	DR5 DR1	DR2 DR4
(BM:	14.6	0.3	BM1	0.4	0.5	0.4	18.1	1.8	0.4	13.9	0.5	38.7
DR2(16),DR7)	17.4	0.2	BM2	0.3	0.2	0.2	14.3	0.2	0.3	21.2	0.3	19.5
	19.4	0.7	BM4	0.6	0.6	0.6	18.9	1.2	0.6	25.1	0.6	20.2
	2.0	0.2	BM8	0.3	0.3	0.2	17.7	0.2	0.3	12.9	0.3	28.7
	3.6	0.3	BM19	0.4	0.3	0.3	25.8	0.3	0.5	12.1	0.4	39.0
	11.1	0.2	BM21	0.3	0.2	0.2	17.1	0.2	0.2	14.5	0.2	21.2
(MA:	0.3	0.2	MA35	0.3	11.3	1.0	0.2	0.2	0.3	0.2	23.9	0.3
DR1,DR5(12))	0.5	1.1	MA38	0.8	20.2	1.2	0.7	0.5	0.5	0.4	12.3	0.5
	0.2	0.2	MA49	0.3	18.2	0.3	0.2	0.2	0.2	0.2	14.8	0.3
	0.2	0.2	MA52	0.3	24.1	0.4	0.3	0.3	0.2	0.3	15.8	0.4
(MK: DR2(15),DI	10.3	0.2	MK28	0.2	0.2	0.3	11.9	0.2	0.3	20.7	0.2	14.8
	14.7	0.6	MK32	0.6	0.6	0.5	11.6	0.6	0.5	5.8	0.6	10.5
	24.9	0.6	MK35	0.6	0.6	0.7	31.7	0.7	0.6	5.9	0.5	12.6
	10.2	0.4	MK38	0.5	0.7	0.4	20.1	0.5	0.5	9.2	0.5	8.7
	11.7	2.1	MK50	0.2	0.2	0.3	19.7	0.2	0.3	28.8	0.3	12.2

Analysis of HLA-DR restriction determinants using Asp f 1 specific TCC from three ABPA patients. Allelic restriction was determined by Asp f 1 induced proliferative responses of T-cell clones using DR-matched and DR-mismatched antigen presenting EBV-transformed B cells. a)HLA-DR types of patients. b)Heterozygous B cell lines; c)Homozygous B cell lines. d)[3H] TdR incorporated, CPM (1 ×10-3).

Unlike grass-pollen allergy, allergy to house dust mites (HDM) seems to be associated with the HLA-DQ gene. O'Brien et al (1995) found an association between in vitro T cell reactivity to a major HDM antigen, Der p2, derived peptides and the HLA-DQ7 antigen (50).

5. HLA CLASS II RESTRICTION SPECIFICITY OF T CELL RECOGNITION IN ABPA

The ability to isolate and propagate allergen reactive T cell clones (TCC) has encouraged many investigators, including our group, to study the contribution of individual MHC class II molecules in T cell recognition at the molecular levelCD4+ T lymphocytes initiate and regulate both the specific and non-specific effector mechanisms of allergic immune responses. The use of molecular genetic probes has facilitated studies demonstrating the functional importance of HLA-DR and DQ gene products in T cell recognition of allergens (51;52).

ABPA shares several features with atopic conditions leading to the hypothesis that T and B cell dysregulation is involved in its pathogenesis. In vitro an in vivo studies examining peripheral blood T and B cell function revealed a predominant Th2 CD4+ T cell response to aspergillus antigen in ABPA (44;53;54). There appears to be a quantitative and not a qualitative difference of the B cell IgE response in ABPA compared to aspergilllus-sensitized nonABPA asthmatic and cystic fibrosis patients (55). Increased levels of total and specific anti-aspergillus IgE antibody responses have been described by our group and others (55-57). In ABPA, there are also increased amounts of IgG and IgA anti-aspergillus antibodies, reflecting the dominance of Th2 humoral versus Th1 cellular responses to aspergillus antigens in these patients (54). To understand the cellular mechanism responsible for the high levels of IgE anti-Asp f1 antibodies, a panel of T cell lines and clones were generated from patients with ABPA with a single immunodominant aspergillus antigen, Asp f 1. Asp f 1 specific T cell lines were primarily of CD4+, CD25+, and HLA-DR restricted T cell of Th2 phenotypes and produced high IL-4, very low INFγ and IL-2. Thus the cell surface phenotypes and cytokine profiles of Asp f 1 specific T cell lines indicated that a Th2 T cell response in ABPA is specific to aspergillus antigens. Competition assays demonstrated that Asp f 1 has a low affinity of binding to HLA-DR, which is consistent with the Th2 T cell response previously reported. Strong HLA-DR-Ag-TCR affinity binding has been shown to favor a Th1 cellular response while a low affinity binding favors a Th2 humoral response (58;59).

The generation of Asp f 1 reactive T cell clones (TCC) from three patients with ABPA allowed us to investigate the contribution of individual MHC class II molecules in T cell recognition at the molecular level. Evidence that HLA-DR, as opposed to DP or DQ, functions as the major restriction element in the recognition of Asp f 1 were based on inhibition studies as restriction pattern of 19/21 clones showed HLA-DR mediated restriction. Antigen dependent T cell clones and lines blocked with anti HLA-DR, DP and DQ antibodies specific for framework determinants revealed HLA-DR to be the major restriction elements (Figure 1). Analysis of the clones demonstrated that out of 21 TCC tested, 19 clones were restricted by HLA-DR, 2 by HLA-DP and none by HLA-DQ. Since the majority of the TCC were restricted by the product of the HLA-DR locus, studies were focused on examining which of the HLA-DR broad specificities (serotypes) in each patient were responsible for antigen presentation. EBV transformed autologous and allogeneic B cell panels were used as antigen presenting cells to define restriction specificities. The restriction data on 15 TCC from three patients is shown in Table 1. As demonstrated Asp f 1 presentation was restricted to either HLA-DR2 or DR5 of an individual haplotype. In no case did the clones respond to an APC alternate nonpresenting DR molecule. Similar HLA-DR2 and DR5 restricted allergic responses to house dust mite and ragweed antigens have been reported (45;60).

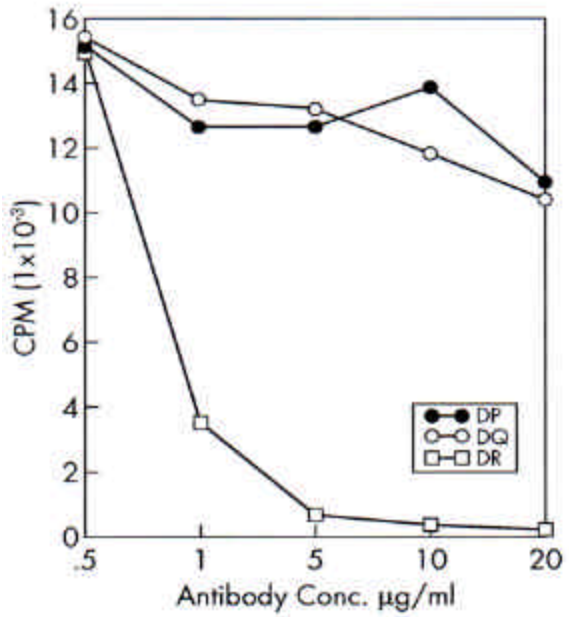

Figure 1. Class I MHC restriction of Asp f 1-specific T cell clones. The figure represents a typical blocking experiment using varying amounts of monoclonal anti-HLA-DR (?), anti-DP (?), and anti-DQ (?) antibodies added at the initiation of the cultures. T cell proliferation assays were performed according to standard method. A mouse anti-human antibody of isotype IgG2a was used with every blocking experiment as a control.

To identify the fine specificity of HLA, restriction studies with genotyped presenting cells were performed. DRB1 locus has been shown to demonstrate the highest polymorphism of all class II loci with more than 250 known allelic variants. Analysis of the restriction pattern of each TCC revealed that a single peptide could be presented by a number of allelic variants of each of the DR2 or DR5 antigens. Interestingly, activity by various genotyped APC could not be predicted on the basis of their relatedness within serotypes. For example several clones of the DRB1*1202 genotype responded to the more distinct DR5 subtypes DRB1*1101 or DRB1*1104. This same pattern of responsiveness also occurred with TCC to Asp f 1 in association with HLA-DR2. Thus using a large number of genotyped presenting cells, a restricted number of DRB1 alleles such as DRB1 *1501, *1503, *1601, *1101 and *1104, *1202 were demonstrated to be capable of presenting Asp f 1 peptides. Overall, two different restriction patterns were observed among Asp f 1-specific clones. Peptide recognition for some clones is restricted to a single DR allele while other clones recognize peptides in association with two or more alleles. Similar observations have been reported for peptide presentation to TCC derived from a DRB*0402 donor and specific for HIV gp120. In those studies the cross- reactive proliferation occurred with antigen presenting cells expressing DRB1*0403 but not with other closely related DR4 subtypes61. Future studies will establish the fine mechanisms between the DR2 and DR5 associated responsiveness and the development of ABPA.

Because of the importance of the HLA molecules in immunity and their association with disease, our laboratory has examined the role of HLA class II molecules in the development of ABPA. As described above the first indication of an association between the histocompatibility locus antigen and susceptibility to ABPA came from MHC restriction studies performed with T cell clones (TCC) generated against Asp f 1 (53). Based on this information from a small number of ABPA patients, we first described significantly higher frequencies of HLA-DR2 and DR5 specificity. The combined frequency of these two antigens was higher in ABPA as compared to normal North American caucasians. Because ABPA is a complicated disease confined to patients with cystic fibrosis and asthmatic patients, in subsequent studies Af sensitive patients with either CF or asthma but without ABPA were included as a critical control population along with normal control subjects. The nonABPA CF and asthmatic patients were important to these studies because only 1-15% of these patients contract ABPA. Any HLA- class II differences in these nonABPA patients emphasize the importance of the role that MHC molecules play in the ABPA disease process. As seen in Table 2 our studies confirmed a significant difference in the HLA-DR2/5 broad specificity frequencies between ABPA (72.7%) and normal controls (34.7%). Importantly, this difference was also seen in ABPA compared to Af sensitive nonABPA (72.7% vs. 35.3%). HLA-DR2 and DR5 specificities were each found at significantly higher frequencies in ABPA when compared to normal controls (45.4% vs.20.0 % and 31.8% vs. 14.0%, respectively). However comparisons between ABPA and control nonABPA groups reflected a significantly higher frequency of HLA-DR2 only in ABPA. The HLA-DR5 frequencies were elevated in ABPA but not significantly different from nonABPA. The remaining broad HLA-DR specificities showed no significant differences between the populations (62;63).

5.1. HLA-DRB1 allele distribution among ABPA, non-ABPA, and normal controls

Analysis of the DRB1 alleles provides a direct structural explanation for the ability of different individuals to bind different sets of antigenic peptides. Studies conducted to define the restriction elements in ABPA showed that presentation of Asp f 1, a major Af allergen, was largely confined to the HLA-DR2 and DR5 genotypes *1501, * 1503, *1101 and *1104 respectively. Based on this information HLA- DR2/DR5 (DR/DR indicates and/or e.g. DR2 or DR5 throughout the manuscript for convenience and should not be confused with heterozygous) gene frequencies were studied for the ABPA population and control nonABPA population. If these alleles are important to the disease process, than they should be present in significantly higher frequencies in ABPA patients compared to nonABPA patients or normal control subjects. The frequencies of DRB1 alleles in these populations are shown in Table 3. Of a total of thirty-five possible DR2/DR5 alleles that could have been detected by the HLA- typing procedures used, only four were detected among all ABPA subjects. The most striking data to emerge was the DRB1*1503 frequency seen in the ABPA group when compared to control nonABPA. (20.4% vs.

Table 2. Frequency of HLA-DR2 and DR5 in patients with ABPA, patients without ABPA, and normal control subjects

	ABPA (n=44)		NON-ABPA (n=65)		NORMAL (n=98)#	
HLA-DR antigen	No.	%	No.	%	No.	%
DR2/5	32	72.7	23	35.3	32	34.7
DR2	20	45.4	8	12.3	19	20
DR5	14	31.8	15	23	14	14

Numbers in bold indicate significant differences in allelic frequencies between patient and control groups.
Control population of unrelated healthy subjects from North America.

Table 3. HLA-DRB1 alleles in patients with ABPA, patients without ABPA, and normal control subjects

	ABPA (n=44)		NON-ABPA (n=65)		NORMALS*	
DRB1 alleles	No.	%	No.	%	No.	%
DR2						
DRB1*1501	11	25.0	5	7.7	226	7.1
DRB1*1503	9	20.4	0	0.0	1	0.7
DR5						
DRB1*1101	6	13.6	8	12.3	38	5.7
DRB1*1104	7	16.0	6	9.2	16	2.2

*Values derived from published data on the white population in North America: DRB*1501, n=3161; DRB1*1502, n=3044; DRB1*1503, n=151; DRB1*1601, n=448; DRB1*1101, n=661; DRB1*1104, n=717; DRB1*1103, n=256; and DRB1*1202, n=2500. Numbers in bold indicate significant differences in allelic frequencies between patients and control subjects.

0.0%, pc<0.005, RR=26.6). Comparison of DRB1*1503 frequency between ABPA and normal controls also demonstrated a striking difference in the frequencies between these two populations (20.4% vs. 0.7%, pc<0.005, RR=37.5). The DRB1 allele *1501 was also significantly higher in ABPA compared to nonABPA (25.0% vs. 7.7%, pc<0.05) and normal controls (25.0% vs. 7.1%, pc<0.005). Among the DR5 subtypes, DRB1*1104 was significantly higher in ABPA only when compared with normal population frequencies. As more patients are studied, DRB1*1101 may well approach significance compared to normals. However it is not certain that will be the case between ABPA and nonABPA. Further analysis of the HLA-D haplotypes in our ABPA population revealed that almost all ABPA patients lacking HLA-DR2 or DR5 carry either DR4 or DR7. However, the DR4 or DR7 frequency was not significantly different between ABPA and the control nonABPA group. Despite this, our current data suggests the possibility that the DR4 or DR7 allele may also contribute to ABPA susceptibility (62;63). In addition, these alleles have previously been shown to be associated with cystic fibrosis, general atopy and ABPA (47;64). Based on these observations, we suggest that HLA-DR4/7 alleles may also represent contributing factors in the development of ABPA.

5.2. HLA-DQ specificities in ABPA, non-ABPA and normal controls

In addition to HLA-DR class II molecules, recent studies have suggested that foreign peptides can also be presented in the context of HLA-DQ molecules. To investigate the involvement of HLA-DQ alleles in ABPA, low-resolution frequencies of DQ alleles were determined in ABPA and nonABPA, and normal control groups. Table 4 shows the frequencies of HLA-DQ types among the three groups. Interestingly, a highly significant increase in HLA-DQ2 frequency was observed in the nonABPA group (pc<0.005) when compared to both ABPA patients and normal controls. This was not due to the presence of an excess of DQ2 homozygotes in the nonABPA population. The high frequency of HLA-DQ2 in nonABPA patients thus appears to be associated with resistance to ABPA. The frequency of DQ6 was also significantly higher in ABPA patients compared to nonABPA and normal controls. However it is not likely that HLA-DQ6 contributes to ABPA susceptibility because 1) Asp f 1 specific T-cell do not proliferate in the presence of DQ6 antigen presenting cells and 2) HLA- DR2 is in strong linkage with HLA-DQ6. Therefore the observed increase in DQ6 may simply reflect the high frequency of HLA-DR2 in ABPA.

A significantly higher frequency of DQ2 (p<0.0001) in our nonABPA population suggests that DQ2 solely or in combination with other DQ or DR alleles might be conferring protection against ABPA. It will be important to determine which of the specific alleles within the DQ2 subtype account for DQ2 mediated protection. It is known that genetic predisposition to some diseases is the net result of a combination of HLA molecules. The typical example is IDDM, where susceptibility or protection seems to be determined by a particular combination of DR and DQ molecules (26;47). The notion that polymorphism within DQ molecules is important in determining disease susceptibility is supported by studies using transgenic mouse models (65;66). Given the complex pathophysiology of ABPA, it is not likely that the DR and DQ combinations are solely responsible for the presence or absence of this disease.

6. CONCLUSION

Although significant information regarding the genetic contribution of candidate genes (i.e. cytokines, TCR and MHC) to allergic diseases is emerging, the cellular and molecular basis for the association between particular HLA class II alleles, immune reactivity, and ABPA is still limited (53;62). Analysis of the HLA-DR and DQ alleles in ABPA and nonABPA patients indicates that susceptibility to ABPA is determined by the HLA-DR locus in which DRB1 allele diversity is critical. Our studies have also confirmed that allelic diversity in HLA-DR2/5 subtypes contribute susceptibility to ABPA, with DRB1* 1503 conferring the strongest risk, and HLA-DQ2

Table 4. Comparison of HLA-DQ frequencies among patients with ABPA, patients without ABPA, and normal subjects

		HLA-DQ antigen				
Subjects		DQ2	DQ4	DQ5	DQ6	DQ3
ABPA (n=43)	%	18.6	11.6	23.2	53.4	53.4
Non-ABPA (n=61)	%	67.2	6.5	22.9	22.9	44.2
Controls (n=98)	%	37.7	7.1	24.4	26.5	51.0

Numbers in bold designate significant differences in the frequencies of HLA- DQ alleles between groups. *Control population of unrelated health white subjects from North America.(67).

conferring resistance. Association data on DR4 and DR7, suggests that both these molecules might represent a common denominator for developing allergies as well as ABPA and that the HLA-DQ locus might primarily and dominantly confer resistance to ABPA. This information, as a whole, provides new insights that identify key genetic elements that put both CF and asthmatic patients at risk for ABPA. This information is crucial for experimentation that examines the role of MHC class II molecules in T cell differentiation into Th1 and Th2 phenotypes and ABPA development. Based on our HLA association and restriction studies described in this review, genetic determinants such as class II appear to play a major role in the development of ABPA within asthmatic and cystic fibrosis populations.

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Send correspondence to: Bela Chauhan, Ph.D., Department of Internal Medicine, Division of Allergy and Immunology, Saint Louis University School of Medicine, 1402 South Grand Blvd., R209, St. Louis, MO 63104, Tel:314-268-5954, Fax:314-577-8459, E-mail: chauhanb@slu.edu