HLA-DQB1*03 in allergic fungal sinusitis and other chronic hypertrophic rhinosinusitis disorders

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Background: Many common chronic inflammatory disorders have strong HLA gene associations, particularly with MHC class II. Allergic fungal rhinosinusitis (AFS) and hypertrophic sinus disease (HSD) are chronic sinonasal mucosal inflammatory disorders. Allergic bronchopulmonary aspergillosis, a disorder analogous to AFS, was recently reported to have HLA–MHC class II associations.
Objective: We sought to determine whether MHC class II is also associated with AFS and HSD.
Methods: HLA DNA genotyping was obtained on 44 patients with AFS and 30 patients with HSD (of which 21 were atopic).
Results: Sixty-six percent of patients with AFS carried at least one HLA-DQB1*03 allele; DQB1*0301 and DQB1*0302 were the most frequent allelic variants (odds ratio [OR] vs healthy subjects = 8.22; 95% CI, 4.30-15.73; $P < .001$; OR vs all patients with HSD = 1.93; 95% CI, 1.09-3.41; $P < .01$; OR vs atopic patients with HSD = 2.57; 95% CI, 1.46-4.53; $P < .001$). Of the 31 patients with AFS and positive *Bipolaris spicifera* cultures, 68% had DQB1*03, with DQB1*0301 and DQB1*0302 being most frequent (OR vs healthy subjects = 8.93; 95% CI, 4.65-17.15; $P < .001$; OR vs patients with HSD = 2.10; 95% CI, 1.18-3.73; $P < .001$). Of the 30 patients with HSD, 50% carried DQB1*03 (OR vs healthy subjects = 4.25; 95% CI, 2.25-8.02; $P < .001$) but differed in frequencies of DQB1*03 allelic variants compared with patients with AFS ($P = .0004$). For HSD, nonatopic subjects had the highest DQB1*03 association (OR vs healthy subjects = 8.63; 95% CI, 4.50-16.54; $P < .001$). DQB1*03 allelic variants did not correlate with allergy skin test results, atopic status, total serum IgE levels, culture results, asthma, or aspirin–nonsteroidal anti-inflammatory drug hypersensitivity.
Conclusion: Patients with AFS and HSD have HLA-DQB1*03 alleles as a risk factor for disease, with AFS having the highest association. However, they differ in DQB1*03 allelic variant frequencies, suggesting several potential roles for MHC class II in their immunopathogenesis. (J Allergy Clin Immunol 2004;114:1376-83.)

Key words: *Chronic sinusitis, nasal polyps, allergic fungal sinusitis, fungal diseases, class II histocompatibility antigens, major histocompatibilty complex, HLA-DQB1*03, immediate hypersensitivity, aspirin hypersensitivity, allergic bronchopulmonary aspergillosis, superantigen, T lymphocytes, antigen-presenting cells*

Chronic hypertrophic–hyperplastic rhinosinusitis, or hypertrophic sinus disease (HSD), is a common form of chronic recurring sinusitis characterized (1) clinically by chronic rhinosinusitis with nasal polyps, often requiring repeated sinus surgeries, and (2) histopathologically by sinonasal mucosal hypertrophy-hyperplasia, with a chronic inflammatory infiltrate comprised of eosinophils, small lymphocytes, and plasma cells.[1-4] Sinus mucosal *in situ* cytokine analysis in HSD resembles that found in asthma.[5,6] HSD is often associated with asthma, inhalant atopy, and occasionally aspirin–nonsteroidal anti-inflammatory drug (NSAID) hypersensitivity.[2,7-10] Those patients with HSD, asthma, and aspirin-NSAID hypersensitivity are considered as having the *aspirin triad*.[11,12]

Allergic fungal rhinosinusitis (AFS) is a type of HSD that is additionally characterized by the presence of a peanut-buttery extramucosal sinus inspissate called allergic mucin that histopathologically contains masses of pyknotic eosinophils (eosinophil concretions), Charcot-Leyden crystals (lysophospholipase), and sparse numbers of fungal hyphae highlighted by fungal silver staining.[13-17] Most AFS cases involve dematiaceous fungi, such as *Bipolaris spicifera*.[17,18] AFS is a noninvasive form of fungal rhinosinusitis that represents an allergic hypersensitivity disorder analogous to allergic bronchopulmonary aspergillosis (ABPA), a fungal disease of the lung.[14,17] However, the classic inspissated allergic mucin that is grossly and histopathologically identical to that found in patients with AFS can also be seen in some patients with HSD but without the presence or involvement of fungi.[19-22] The term proposed for this category of HSD is *eosinophilic mucin rhinosinusitis* (EMRS).[20]

Polymorphic HLA MHC class II molecules are displayed on the surface of antigen-presenting cells (APCs). These molecules bind and present antigenic peptides to T cells bearing T-cell receptors that are then capable of recognizing the specific bound peptide within the context of the presenting MHC class II molecule, facilitating antigen-specific T-cell activation. Inherited differences in MHC class II α- and β-chain allelic polymorphisms place genetic constraints on the host's ability to bind and present specific antigenic peptides to T cells. Many inflammatory diseases have been linked to specific MHC class II DR and

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Abbreviations used
ABPA: Allergic bronchopulmonary aspergillosis
AFS: Allergic fungal rhinosinusitis
APC: Antigen-presenting cell
EMRS: Eosinophilic mucin rhinosinusitis
HERV: Human endogenous retrovirus
HSD: Hypertrophic sinus disease
LTR: Long terminal repeat
NSAID: Nonsteroidal anti-inflammatory drug
OR: Odds ratio

DQ alleles, including rheumatoid arthritis, type I diabetes, and multiple sclerosis.[23]

Patients with nasal polyposis were reported to express the MHC class I phenotype HLA-A74.[24] Another study suggested that patients with the aspirin triad have the MHC class II haplotype DR7-DQA1*0201-DQB1*0202.[25] It was also recently shown that ABPA is associated with the HLA-DR2 alleles DRB1*1503 and DRB1*1501 and the HLA-DR5 allele DRB1*1104.[26] HLA-DQ2 was found to be protective.[26] We hypothesized that specific MHC class II gene associations might also be found in AFS because it is an analogous disorder to ABPA. In this report we used high-resolution DNA HLA haplotyping to assess potential MHC class II gene associations with various forms of HSD, including AFS.

METHODS

Subjects

Patients were diagnosed and recruited from Phoenix, Arizona (author MSS). This study had institutional review board approval, and all patients provided informed consent. Patients with chronic recurring rhinosinusitis were recruited from 2 groups: (1) those with AFS and (2) those with HSD but without evidence of AFS. The following criteria were present to make a diagnosis of AFS[17]: (1) patients had chronic recurring rhinosinusitis with nasal polyps; (2) surgically obtained characteristic allergic mucin was seen histopathologically, grossly, or both; (3) fungal stain was positive for hyphae within the allergic mucin but not in the mucosa, or the surgical sinus fungal culture was positive in an otherwise characteristic patient; (4) the sinus mucosa demonstrated eosinophilic-lymphocytic inflammation without evidence of tissue necrosis, granulomas, or fungal invasion; and (5) other fungal diseases were excluded.

Patients given a diagnosis of HSD were those who had chronic recurring rhinosinusitis–nasal polyps but did not meet the aforementioned AFS criteria or the criteria for any other form of fungal rhinosinusitis.[18]

Laboratory and clinical evaluation

Laboratory evaluation included total serum IgE level, complete blood count with differential, metabolic panel of serum chemistries, erythrocyte sedimentation rate, quantitative immunoglobulin levels (IgG, IgA, and IgM), urinalysis, delayed hypersensitivity skin tests to a panel of T-cell recall antigens, chest radiography, and spirometry, as previously described.[17] An assessment of functional antibody status was performed when required, as previously described.[27] Patients with a history of asthma or reactive airways demonstrated a greater than 10% improvement in airway obstructive parameters with bronchodilator. Aspirin-NSAID hypersensitivity status was obtained on the basis of a clinical history of respiratory-allergic symptom exacerbation with ingestion. The co-occurrence of aspirin-NSAID hypersensitivity, HSD, and asthma defined those patients with the clinical syndrome of the aspirin triad.

Skin testing for type I immediate hypersensitivity

Allergy skin tests were performed by using the prick and intradermal methods on all patients, as previously described.[17] The standard testing panel of southwestern aeroallergens included southwestern grasses, weeds and trees, molds, house dust mite, cat hair, and dog epithelium–mixed breeds (Greer Labs, Lenoir, NC). *B spicifera* skin test material was obtained commercially (*Bipolaris/Curvularia spicifera*, Greer Labs) or prepared as previously described[17] but was not available for all patients. *B spicifera* skin tests were performed by using the prick method only. Skin tests were read at 20 minutes and rated on a scale of positivity compared with positive and negative controls. Patients were considered atopic if 2 or more skin tests in the standard testing panel were positive.

Surgical specimens

Hematoxylin & eosin and fungal stain histopathologies were performed according to standard protocols on surgical sinus specimens and were reviewed by one of the authors (MSS) for confirmation of diagnosis when required. Bacterial and fungal cultures were performed by using standard protocols.

DNA analysis

EDTA-anticoagulated whole blood from patients was stored at −17°C until analyzed. DNA from each patient was extracted by using a salting-out procedure.[28] HLA-DQB1 alleles–allelic variants were detected by means of PCR amplification of genomic DNA with high-resolution DQB1 allele sequence-specific primers for 45 possible alleles (One Lambda, Canoga Park, Calif). The presence or absence of an amplified product was visualized by means of UV light after electrophoresis for 20 minutes at 15 V/cm on ethidium bromide–prestained 2% agarose gels. HLA-DRB1 alleles were determined similarly.

Statistical analysis

Allele frequencies were compared between patient groups and with normal control allele frequencies taken from previously published data in western North America[29]; the normal control frequency of all HLA-DQB1*03 allelic variants combined totaled 19.04%. The odds ratio (OR) for HLA alleles was calculated by using the method of Svejgaard et al,[30] with P values calculated with the χ^2 test. A 2×5 contingency table analysis was used to analyze differences in allelic variant frequencies between groups. Of all patients in this study, there were 3 Hispanic patients in the AFS group and one in the HSD group; the remainder were white. There were no statistical differences in HLA genotypes found between these patients when compared with the whole population.

RESULTS

Demographics

There were 44 patients with AFS and 30 patients with HSD (Table I). All patients with AFS had positive skin test responses to at least 2 different inhalants in addition to the AFS organism and had a mean total serum IgE level of 1109 IU/mL. Patients with HSD had a mean total serum

TABLE I. Patient demographics

	Patients with AFS	Patients with HSD
Patient no.	44 (27 male, 17 female)	30 (16 male, 14 female)
Average age, y (range)	32 (8-65)	46 (12-65)
Average total serum IgE, IU/mL (range)	1109 (30-8132)	401 (16-2533)
Atopy (n)	100% (44)	70% (21)
EMRS (n)		33% (10)
Immunocompetent (n)	100% (44)	100% (30)

IgE level of 401 IU/mL and an incidence of inhalant atopy of 70%. Table II shows skin test results for both groups. All patients with AFS and HSD were clinically immunocompetent.

Various local otolaryngologic surgeons performed the operations on the study patients with AFS and HSD. Intraoperative surgical sinus fungal cultures were obtained in 86% of the patients with AFS (Table III). Of those sent for culture, *B spicifera* was grown in 82%, *Exserohilum rostratum* was grown in 8%, *Aspergillus* species was grown in 5%, and *Curvularia lunata* was grown in 3%; fungal cultures were negative in 16%. Twenty-three of the patients with AFS who had positive cultures for *B spicifera* were skin tested to *B spicifera*, and all had positive skin test responses. Of the remaining 8 patients with AFS with positive *B spicifera* cultures for whom skin tests to *B spicifera* were not available, 7 (88%) had positive skin test responses to the closely related dematiaceous fungus *Alternaria tenuis* (data not shown).

Patients with HSD had undergone an average of 3 sinus operations (range, 1-9; data not shown) and often had absence of extramucosal sinus material to culture or fungal stain. However, EMRS was found in 10 (33%) of the patients with HSD in at least one sinus surgery (Table I). These patients had negative results for the presence of fungi by means of both fungal staining and culture. Overall, 11 (37%) of the 30 patients with HSD had surgical sinus fungal cultures sent, all of which returned with negative results (Table III).

TABLE II. Type I immediate hypersensitivity skin tests in patients with AFS and HSD

Aeroallergen	Patients with AFS, % (n)*	Patients with HSD, % (n)†
Bermuda grass	98 (43)	81 (17)
Amaranth weed	84 (37)	71 (15)
Chenopod weed	75 (33)	52 (11)
Fransceria weed	66 (29)	62 (13)
Olive tree	93 (41)	86 (18)
Mulberry tree	84 (37)	71 (15)
Pine tree	61 (27)	52 (11)
Ash tree	23 (10)	43 (9)
House dust mite	84 (37)	100 (21)
Cat	58 (24)	86 (18)
Dog	48 (21)	57 (12)
Bipolaris spicifera	97 (32)‡	70 (7)§
Alternaria tenuis	84 (37)	71 (15)
Aspergillus fumigatus	66 (29)	57 (12)
Helminthosporium sativum	34 (15)	38 (8)

*All 44 patients with AFS were atopic.
†Expressed as the percentage of the 21 atopic patients with HSD.
‡*B spicifera* skin testing was available for 33 patients with AFS.
§*B spicifera* skin testing was available for 10 of 21 atopic patients with HSD.

TABLE III. Surgical sinus fungal cultures in patients with AFS and HSD

	Patients with AFS, % (n)*	Patients with HSD, % (n)
Fungal cultures sent	86 (38)	37 (11)
Bipolaris spicifera	82 (31)	0 (0)
Exserohilum rostratum	8 (3)	0 (0)
Curvularia lunata	3 (1)	0 (0)
Aspergillus species	5 (2)	0 (0)
Negative fungal culture	16 (6)	100 (11)
Positive *B spicifera* skin test response in patients with positive *B spicifera* cultures	100 (23)†	

*One patient grew *B spicifera* and *Aspergillus* species, and 3 patients grew *B spicifera* and *E rostratum*.
†*B spicifera* skin testing was available for 23 of the 31 study patients with positive *B spicifera* cultures.

DNA analysis

The results of DNA genotyping for HLA-DRB1 and HLA-DQB1 genes were obtained for all patients. HLA-DQB1*03 was the most frequent MHC class II allele found in both patients with AFS and patients with HSD. As shown in Table IV, 66% of all patients with AFS had at least one HLA-DQB1*03 allele, with the majority having either HLA-DQB1*0301 or *0302 (OR, 8.22; 95% CI, 4.30-15.73; $P < .001$; OR vs all patients with HSD = 1.93; 95% CI, 1.09-3.41; $P < .01$; OR vs atopic patients with HSD = 2.57; 95% CI, 1.46-4.53; $P < .001$). The similarity in the increased HLA-DQB1*03 allele positivity rate in patients with AFS and nonatopic patients with HSD (OR, 0.96; $P = .83$) supports the lack of a role for atopy itself as a cause for DQB1*03 positivity. However, the predominance of the HLA-DQB1*0301 and *0302 allelic variants in HLA-DQB1*03–positive patients with AFS was not seen in the corresponding HLA-DQB1*03-positive patients with HSD (P values ranging from < .0001 to < .03). Patients with AFS who had positive culture results for *B spicifera* had a 68% incidence of at least one HLA-DQB1*03 allele, also predominantly the HLA-DQB1*0301 or *0302 allelic variants. Subgroup analyses of those patients with AFS with asthma, aspirin-NSAID hypersensitivity, or with the full aspirin triad showed an increase in HLA-DQB1*03 allele frequencies similar to that found in the total AFS patient group; no significant differences in class II allelic frequencies between these

TABLE IV. HLA-DQB1*03 alleles in patients with AFS

Subgroup	With HLA-DQB1*03, % (n)¶	AFS vs regional controls, OR† (95% CI, *P* value)	AFS vs all HSD, OR‡ (95% CI, *P* value)	AFS vs atopic HSD, OR§ (95% CI, *P* value)	AFS vs nonatopic HSD, OR‖ (95% CI, *P* value)	HLA-DQB1*03XX allelic variants, % (n)#	*P* value**
All	66 (29/44)	8.22 (4.30-15.73, <.001)	1.93 (1.09-3.41, <.01)	2.57 (1.46-4.53, <.001)	0.96 (−1.88-1.73, .83 [NS])	01: 59 (17) 02: 52 (15) 03: 10 (3) 04: 3 (1) 05: 3 (1)	A: .0004 B: .0129 C: .0001
Bipolaris spicifera culture (+)	68 (21/31)	8.93 (4.65-17.15, <.001)	2.10†† (1.18-3.73, <.001)			01: 52 (11) 02: 48 (10) 03: 5 (1) 04: 0 (0) 05: 5 (1)	

NS, Not significant.
¶Expressed as the percentage of the subgroup with at least one DQB1*03 allele.
†OR compared with published regional healthy control subjects (see Methods).
‡OR compared with all patients with HSD.
§OR compared with atopic patients with HSD.
‖OR compared with nonatopic patients with HSD.
#Expressed as the percentage of each HLA-DQB1*03 allelic variant (HLA-DQB1*0301-*0305) found in patients with the DQB1*03 allele in the subgroup.
**Contingency table analysis was used to test for significant differences in HLA-DQB1*03 allelic variant frequencies between AFS and corresponding HSD subgroups: *A*, all patients with AFS versus all patients with HSD; *B*, all patients with AFS versus atopic patients with HSD; *C*, all patients with AFS vs nonatopic patients with HSD.
††OR compared with all patients with HSD.

patients and all other patients with AFS were found (data not shown). No correlation was found between specific HLA-DRB1 or HLA-DQB1 alleles and bacterial cultures, total serum IgE levels, or type I immediate hypersensitivity skin tests to common aeroallergens in patients with AFS (data not shown).

Table V shows the HLA-DQB1*03 results for patients with HSD and their subgroups. Of all 30 patients with HSD, 15 (50%) had at least one HLA-DQB1*03 allele (OR, 4.25; 95% CI, 2.25-8.02; $P < .001$). In contrast to patients with AFS, patients with HSD did not have a predominance of HLA-DQB1*0301 and *0302 allelic variants over that seen in healthy control subjects.[29] The frequency of having at least one HLA-DQB1*03 allele in the subgroups of patients with HSD was 43% in those with atopy (OR, 3.21; 95% CI, 1.70-6.07; $P < .001$) and was highest (67%) in those without atopy (OR, 8.63; 95% CI, 4.50-16.54; $P < .001$), further supporting the conclusion that the presence of atopy cannot be responsible for the increased incidence of the DQB1*03 allele in these patients. Patients with HSD with EMRS also had higher rates of having at least one HLA-DQB1*03 allele, and this was closer to the HLA-DQB1*03 incidence seen in patients with AFS; 6 (60%) of the 10 patients with EMRS had positive results for HLA-DQB1*03. However, the HLA-DQB1*0301 and *0302 allelic variant predominance found in patients with AFS was not found in this AFS-like subgroup of patients with HSD. Therefore AFS and other forms of HSD appeared similar in their increased association with HLA-DQB1*03 alleles but somewhat different in their frequency of DQB1*03 allelic variants. This suggests differences in MHC class II peptide-binding/antigen specificities (differences in DQB1*03 allelic variants) between AFS and HSD but also suggest the shared use (increased association with DQB1*03 alleles regardless of allelic variant) of another property associated with the DQB1*03 gene locus, the HLA-DQ3 molecule, or both, which are important for both AFS and HSD immunopathogenesis. Subgroup analyses of those patients with HSD with asthma, asthma and atopy, aspirin-NSAID hypersensitivity, or the full aspirin triad showed no additional significant differences in HLA class II allelic frequencies when compared with the total HSD patient group (data not shown).

B spicifera type I immediate hypersensitivity skin testing was available for 15 of the 30 patients with HSD; 7 had positive results (Table V). HLA-DQB1*03 alleles were found in only 2 (29%) of these patients, suggesting that the increased incidence of HLA-DQB1*03 alleles in patients with AFS and HSD is unlikely to be solely caused by *B spicifera* type I immediate hypersensitivity. As in AFS, microbial culture results, total serum IgE levels, and type I immediate hypersensitivity skin tests to common aeroallergens did not correlate in patients with HSD with HLA-DRB1 or DQB1 alleles (data not shown).

DISCUSSION

Polymorphic MHC class II molecules on the surface of APCs have been implicated in the immunopathogenesis of a number of chronic inflammatory diseases.[23] Several examples of strong MHC class II genetic associations with immunologic diseases include rheumatoid arthritis

TABLE V. HLA-DQB1*03 alleles in patients with HSD

Subgroup	With HLA-DQB1*03, % (n)¶	OR† (95% CI, *P* value)	HLA-DQB1*03XX allelic variants, % (n) ‡
All	50 (15/30)	4.25 (2.25-8.02, <.001)	01: 53 (8) 02: 33 (5) 03: 27 (4) 04: 13 (2) 05: 0 (0)
Atopy	43 (9/21)	3.21 (1.70-6.07, <.001)	01: 56 (5) 02: 44 (4) 03: 22 (2) 04: 11 (1) 05: 0 (0)
Nonatopic	67 (6/9)	8.63 (4.50-16.54, <.001)	01: 50 (3) 02: 20 (1) 03: 50 (3) 04: 20 (1) 05: 0 (0)
EMRS	60 (6/10)	6.38 (3.36-12.10, <.001)	01: 33 (2) 02: 50 (3) 03: 33 (2) 04: 17 (1) 05: 0 (0)
Positive *Bipolaris spicifera* skin test response	29 (2/7)§	1.74 (−2.90 to 3.37, <.05)	01: 100 (2) 02: 0 (0) 03: 50 (1) 04: 50 (1) 05: 0 (0)

¶Expressed as the percentage of the subgroup with at least one DQB1*03 allele.
†OR compared with published regional healthy control subjects (see Methods).
‡Expressed as the percentage of each HLA-DQB1*03 allelic variant (HLA-DQB1*0301-*0305) found in patients with the DQB1*03 allele in the subgroup.
§*B spicifera* skin testing was available for 15 patients with HSD.

(HLA-DRB1*0401, *0402, *0404, *0408, and *0101[31,32] and HLA-DQA1*03/DQB1*03[33,34]), type I diabetes mellitus (HLA-DQA1*0301/DQB1*0302),[35-37] multiple sclerosis (DRB1*0101 and DRB1*1501),[38] and celiac disease (DQA1*0501 with a DQB1*0201 or DRB1*0301 chain, DQA1*0301 with a DRB1*0401 or DQB1*0302 chain).[39] Recent data showing ABPA susceptibility associated with the HLA-DR2 alleles DRB1*1503 and *1501 and the HLA-DR5 allele DRB1*1104, with ABPA resistance linked to HLA-DQ2,[26] prompted us to look for MHC class II gene associations in the analogous disorder AFS. Patients with other forms of HSD were used as disease control subjects, and all patients were compared with published, regional healthy control MHC class II allele frequencies.

Many of the clinical, histopathologic, and immunologic features of AFS are analogous to those of ABPA, including the presence of an airway inspissate of allergic mucin containing fungal hyphae, the presence of inhalant atopy, increased total serum IgE levels, the presence of fungal-specific IgE and IgG, and favorable response to oral corticosteroids.[14,17] Although many patients with AFS and HSD had similar clinical and surgical presentations, including chronic recurring rhinosinusitis, nasal polyps, aspirin-NSAID hypersensitivity, aspirin triad, inhalant atopy, increased total serum IgE levels, and the presence of obstructing inspissated allergic mucin within the sinuses in some patients with HSD (EMRS), patients with AFS were differentiated from patients with other forms of HSD by using specific criteria for diagnosis, as previously published, including the demonstration of fungi.[15-18] Those patients with chronic recurring rhinosinusitis and nasal polyps who did not fulfill these diagnostic criteria for AFS were placed in the HSD group.

Patients with AFS and HSD also showed similarity in the increased prevalence of HLA-DQB1*03 alleles over background, where 66% of the patients with AFS and 50% of the patients with HSD had positive results for at least one allele; specific subgroups of patients had even higher gene frequencies, including nonatopic HSD and EMRS (Table V). This suggests an important role for MHC class II molecules, particularly HLA-DQ3, in virtually all forms of HSD, including AFS. This is strikingly reminiscent of the HLA-DQ3 associations seen with a number of other chronic inflammatory disorders[33-37] that differ from AFS and HSD in target organs and pathobiology.

All patients with AFS and positive *B spicifera* cultures for whom *B spicifera* skin testing was available had positive skin test responses, demonstrating a 100% incidence of *B spicifera* fungal-specific IgE *in vivo* (Table III). Furthermore, virtually all patients with *Bipolaris spicifera* who underwent skin tests had positive skin test responses (Table II). This is in agreement with our previous report that all patients with AFS have type I immediate hypersensitivity to the offending fungus in AFS[17] and also supports the notion that most patients with AFS who had negative fungal culture results actually had *B spicifera* as the causative mold. However, the incidence of HLA-DQB1*03 allele negativity in patients with AFS with positive *B spicifera* cultures was 32% (Table IV). Because not all of these patients carry HLA-DQB1*03 alleles, yet all produced fungal-specific IgE, this might indicate that the association of AFS with specific HLA-DQB1*03 alleles might also be due to factors in addition to HLA-DQB1*03–dependent *B spicifera* peptide antigen-binding specificity. This is supported by the low frequency (29%) of HLA-DQB1*03 alleles found in patients with HSD with positive *B spicifera* skin test responses but the high frequency of HLA-DQB1*03 alleles in patients with HSD as a whole (50%), including nonatopic subjects with uniformly negative skin test responses (67%; Table V). Further supporting the conclusion that the atopic diathesis could not be the cause for the increased incidence of HLA-DQB1*03 in patients with AFS and HSD was the finding that patients with AFS, who were uniformly atopic to multiple aeroallergens, and nonatopic patients with HSD had virtually identical HLA-

DQB1*03 positivity rates (66% vs 67%; OR, 0.96; $P = .83$; Tables IV and V).

The biologic role of the MHC class II genotype in the immunopathogenesis of inflammation has usually been believed to relate to the APC class II molecule peptide-binding cleft specificities with resultant peptide-specific presentation to the T-cell receptor. However, it is known that there are 2 additional potential explanations for MHC class II gene associations with some chronic inflammatory diseases. One is the association of the class II gene working in concert with a closely linked gene that is not identified. This linked gene might modify the inflammatory process either directly with its own gene product or indirectly through an effect on gene transcription. An example of this is the recent finding of the closely linked human endogenous retroviral (HERV) long terminal repeat (LTR) genes that significantly modify risk for various autoimmune diseases, including rheumatoid arthritis,[40,41] type I diabetes mellitus,[42] and Addison disease.[43] HERVs are inherited truncated retroviral gene inserts that make up approximately 1% of the human genome.[44] Some HERVs have been found to contain glucocorticoid response elements and other genetic regulatory motifs with either gene promoter or silencer activity that can potentially regulate transcription of nearby genes both upstream and downstream of the insert.[44-47] Most HERV LTRs in the HLA MHC class II region are located next to the HLA-DQB1 locus and likely influence both MHC class II gene transcription and recombination.[45,47]

Some HERVs are also known to contain truncated retroviral envelope glycoprotein genes that code for T-cell superantigens.[48-50] T-cell superantigens are microbial proteins that bind to the side of the MHC class II molecule on the APC and to the side of the T-cell receptor on the T cell simultaneously, causing inappropriate T-cell activation. Endogenous retroviral T-cell superantigens are common in mice, modifying susceptibility to mouse mammary tumor virus infection.[51] The normal life cycle of EBV might involve the induction and expression of a HERV T-cell superantigen.[50] HERV T-cell superantigens have also been implicated in the immunopathogenesis of both type I diabetes mellitus and multiple sclerosis.[48,52] It was also recently theorized that AFS, HSD, and other closely related chronic eosinophilic-lymphocytic respiratory mucosal inflammatory disorders, such as ABPA and chronic severe asthma, might be T-cell superantigen–amplified diseases.[53]

The other potential role for MHC class II gene associations with inflammatory disease involves the binding and presentation of T-cell superantigens themselves. Specific T-cell superantigens show a hierarchy of MHC class II molecule preference for binding,[54] preferring and therefore selecting some MHC class II phenotypes as more ''superantigenphilic'' than others. For example, it was recently reported that *Streptococcus pyogenes* T-cell superantigen–induced inflammation was associated with high cytokine responses and severe systemic disease if patients carried the MHC class II haplotype DRB1*14/DQB1*0503, whereas the DRB1*1501/DQB1*0602 haplotype was associated with lower cytokine responses and strong protection from severe systemic disease.[55] The basis for the observed differences in MHC class II modulation of superantigen-induced T-cell activation was shown to reside with differences in the efficiency of MHC class II–dependent presentation of superantigen to the responding T-cell receptor. Taken together, MHC class II gene associations with chronic inflammatory diseases might relate to a composite of the relative involvement of (1) class II molecule peptide binding and presentation; (2) exogenous or endogenous (or both) T-cell superantigen binding to preferential MHC class II molecule phenotypes; (3) preferential MHC class II gene linkage with superantigen-producing endogenous retroviral elements; and (4) active genetic regulation of MHC class II gene transcription by closely linked HERV LTRs that are, within the MHC class II genetic loci, mostly found within the DQB1 region, a ''composite model.''

We found that both AFS and HSD were strongly associated with HLA-DQB1*03 alleles but differ in their association with specific DQB1*03 allelic variants. AFS was more strongly associated with HLA-DQB1*0301 and *0302 than HSD. One potential explanation for these findings is that HSD is caused by many different disease-associated peptides, with a fewer number of different peptides being the cause of AFS. The HLA-DQB1*0301 and *0302 allelic variant associations in AFS might be due to peptide-binding specificity issues more specific for AFS pathogenesis than other forms of HSD. The other potential explanation is based on the composite model described above, in which both disorders are dependent on several MHC-associated components. These might include MHC class II peptide-binding specificities, MHC-linked HERV elements that influence HLA-DQB1*03 transcription, and T-cell superantigens (endogenous, exogenous, or both) that prefer the HLA-DQ3 molecule, regardless of HLA-DQ3 polymorphism-dependent peptide-binding specificity. It is also possible that the AFS association with specific DQB1*03 allelic variants is additionally associated with closely linked inherited endogenous retroviral genes that produce disease-stimulating T-cell superantigens under the inflammatory conditions present in AFS. This superantigen could even have a preference for the linked HLA-DQ3 molecule itself.

In conclusion, AFS and HSD share many clinical and immunologic attributes, including our finding of an association with HLA-DQB1*03 alleles, yet they also have distinctly different clinical (fungi only in AFS) and immunologic (differences in HLA-DQB1*03 allelic variants, inhalant atopy, and fungal-specific antibody responses) features. Both disorders share the genetic feature of an HLA MHC class II association that has also been seen with several other immunologic diseases, particularly the HLA-DQB1*03–associated autoimmune diseases rheumatoid arthritis and type I diabetes mellitus. Further research will be required to determine whether the MHC class II gene associations seen in all of these disorders demonstrate fundamental differences or similarities in their immunopathogenesis.

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