

Association of class II sequences encoding DR1 and DQ5 specificities with hypersensitivity to chironomid allergen *Chi t I*

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Abstract

A panel of 188 unrelated Caucasian subjects who were exposed to the larvae of *Chironomus thummi* (Diptera, nonbiting midges) was HLA-typed by polymerase chain reaction amplification of the second exons of the *DRB*, *DQA1*, and *DQB1* genes followed by dot-blot hybridization with sequence-specific oligonucleotide probes. Type I sensitization to the allergen *Chi t I* and a large number of other inhalant allergens was determined by RAST and skin testing. Sixty-one individuals were found to be sensitized to *Chi t I*, of whom 24 were sensitive to this allergen and to no other allergens tested. Statistical analyses showed that only in the latter group were the *HLA-D* genes *DRB1*0101*, *DQA1*0101*, and *DQB1*0501* associated with IgE-responsiveness to *Chi t I*. These results suggest that HLA associations with responsiveness to certain allergens may be more striking in monosensitized subjects. (J ALLERGY CLIN IMMUNOL 1994;93:918-25.)

<i>Chi t I</i>	Group I allergen from <i>Chironomus thummi</i> (nonbiting midges)
GM	Geometric mean
SSO	Sequence-specific oligonucleotide

Key words: [Allergy](#), [HLA-D typing](#), [polymerase chain reaction](#), [sequence-specific oligonucleotides](#)

Usually, in genetic studies of disease susceptibility (e.g., for autoimmune disorders), no clear information exists about the antigen involved. This is not the case for human IgE-mediated atopic allergy in which the antigens or allergens implicated in causing the disease can be identified. Studies with several highly purified allergens have shown significant associations between IgE-mediated immune responsiveness and specific HLA-D type.¹⁻⁵ Such studies can serve as models for understanding the mechanisms of specific immune responsiveness in human beings. In certain of these studies, the level of overall IgE responsiveness (as reflected in the total serum IgE level) influences the strength of the observed HLA-D association.⁴ In the present study we investigate the relationships between sequence polymorphisms of *HLA-DRB*, *DQA1*, and *DQB1* genes of persons exposed to the *Chi t I* *Chironomus* hemoglobin, which is the major allergen of *Chironomus thummi*, a nonbiting midge of the family Diptera.^{6,7} The hemoglobins (*Chi t I*) of *C. thummi* are well characterized in their primary and tertiary structures, and B- and T-cell epitopes have been identified for the 12 homologous components.⁸⁻¹² Allergens from this species have been shown to be important in type I hypersensitivity reactions in about 20% of individuals exposed naturally to the insects or the larvae, which are commonly used as fish food.^{8, 13-16}

Methods

Subjects

For this study, 188 individuals (135 male and 53 female subjects) aged 15 to 69 years were investigated, including 31 workers in a fish food factory and 157 aquarists from Munich who were willing to participate in a fish food allergy study. The duration of exposure to *Chi t I* was between 0.5 and 25 years. All subjects were German Caucasians. Case histories were recorded with a standardized questionnaire that included questions on general health, smoking habits, complaints caused by exposure to ubiquitous allergens and *C. thummi* larvae, and family history of IgE-mediated atopic allergy.

Antibody determination

Total IgE in the serum of each individual was determined with the Phadebas IgE PRIST system (Pharmacia, Uppsala, Sweden). Specific IgE responses to a set of common inhalant allergens were measured by RAST with paper discs coupled to extracts from *Dermatophagoides pteronyssinus*, mixed molds (*Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria alternata*), mixed animal danders (cat, horse, cattle, dog), wheat flour (Pharmacia), mixed tree pollens (B3: *Betula*, *Fagus*, *Quercus*, *Alnus*, *Fraxinus*, *Corylus*, *Populus*, *Platanus*, *Ulmus*, and *Salix* spp.), mixed grass pollens (B2: *Arrhenatherum elatius*, *Holcus lanatus*, *Cynosurus cristatus*, *Dactylis glomerata*, *Lolium* spp., *Poa* spp., *Anthoxanthum odoratum*, *Festuca pratensis*, *Agrostis tenuis*, *Bromus* spp., *Alopecurus pratensis*, *Phleum pratense*), and mixed weed pollens (B5: *Artemisia* spp., *Chenopodium album*, *Calluna vulgaris*, *Atriplex* spp., *Parietaria* spp., *Plantago* spp.) (Bencard, Neuss, Germany). In cases of relevant exposure (e.g., through domestic animals or fish food ingredients), the allergen spectrum was broadened with extracts of cat, dog, *D. farinae* (*Der f*), bird feathers (*Gallus* spp., *Serinus canaria*, *Columba* spp., *Melopsittacus* spp.), rodent (hamster, mouse, rat, guinea pig) and fish food ingredients. For *C. thummi* specific IgE determination by RAST, a crude extract of *C. thummi* larvae and the purified antigen *Chi t I* were coupled to cyanogen-bromide-activated paper discs in our laboratory.⁶

All of the IgE antibody measurements by the RAST, including *Chi t I*-specific IgE antibody, were standardized against the Pharmacia system as described elsewhere⁶ and expressed in units per milliliter. Values less than or equal to 0.35 U/ml were indistinguishable from those obtained from sera on nonexposed healthy control subjects and are therefore regarded as negative.⁶

Allergen preparation and skin testing

Chi t I was prepared from *C. thummi* larvae as previously described.⁶ Skin prick tests were performed in 69 exposed persons with the purified *Chi t I* (concentration = 0.1 mg/ml). Other allergens used for skin testing were commercially available standardized extracts of house dust, *D. pteronyssinus* (*Der p*), *D. farinae* (*Der*

f), horse dander, dog, cat, bird feathers (see above), *Alternaria alternata* (Alt a), *Aspergillus fumigatus* (Asp f), *Cladosporium herbarum* (Cla h), *Penicillium notatum* (Pen n), *Secale cereale* pollen, food (egg, cheese, cow's milk) (Pharmacia), and from Bencard the allergen mixes animal dander (A2: epithelium from rabbit, cattle, and human beings and sheep wool), mixed molds (M10: *Chaetomium globosum*, *Epicoccum purpurascens*, *Fusarium* spp.; M11: *Phoma betae*, *Paecilomyces marquandii*, *Sporobolomyces roseus*), tree pollens (B3 see above), grass pollens (B2 see above), weed pollen (B5 see above) were obtained. Wheal-and-flare reactions equal to or greater than 50% of those induced by 0.1% histamine were regarded as positive.

DNA isolation and HLA typing

Genomic DNA was extracted from whole blood according to the method described by Marcadet et al.¹⁷ The second exon-specific primers, PZ2A1 and PZ5A2 for *DRB* genes and primers DGM 4 and DGM 7b for *DQB1* genes were used for the polymerase chain reaction amplification,^{18, 19} as described previously.²⁰ The polymerase chain reaction–amplified DNA was dot-blotted onto Pall Biodyne B nylon membranes (Pall, Dreich, Germany) and allowed to hybridize with radioactively labeled sequence-specific oligonucleotides (SSOs) for the hypervariable regions of the second exons.²¹ These SSOs defined most of the Caucasian *HLA-DRB* alleles (including all broad specificities) and *HLA-DQB1* alleles known at the time.²²

For amplification of the *DQA1* second exons, the primer pair DQAAMP-A/DQAAMP-B from the 11th Histocompatibility Workshop was used.²³ Labeling of the 17 *DQA1*–specific SSOs obtained from the 11th Histocompatibility Workshop, which discriminate among nine *DQA1* alleles and dot-blot hybridization, was performed with a modified nonradioactive procedure.²⁴

DNA cloning and sequencing

Purified polymerase chain reaction–amplified second-exon DNA fragments of *HLA-DRB* genes from 13 subjects were cloned into the bacteriophage vector M13mp19 as described.²⁰ *Escherichia coli* XL1 Blue (Stratagene, La Jolla, Calif.) clones containing DR1-associated *DRB1* sequences were screened using the phosphorous 32–labeled SSO, CT1.²⁵ TGGCAGCTTAAGTTTGAA⁴² (5′-3′). Single-stranded DNA was isolated by standard techniques, and sequencing with the universal M13 primer was carried out with the dideoxy method.²⁵

Data analyses

Responsiveness to *Chi t I* was evaluated according to the presence of detectable serum IgE antibodies; responsiveness to other allergens was determined according to the RAST and skin test results. After the study subjects were screened with allergen extracts by RAST and skin testing, the individuals were assigned to four groups according to their specific IgE response.

- **Group 1:** no IgE antibody to any tested allergen ($n= 93$)
- **Group 2:** IgE antibodies only to *Chi t I* ($n= 24$)
- **Group 3:** no IgE antibodies to *Chi t I* but IgE antibodies to at least one complex allergen ($n= 34$)
- **Group 4:** IgE antibodies to *Chi t I* and other allergens ($n= 37$)

The proportion of different HLA alleles in the four groups of subjects were compared in 2×2 tables by nonparametric analyses with the Chi square test with Yates' correction. No correction was made for the number of tests performed.

For estimations of relative risk odds ratios were calculated.²⁶ Differences in the mean log[total IgE] of the studied subgroups were compared with Students' *t* test (two-tailed).

Stepwise multiple regression analyses²⁷ were used to investigate further the significance of the associations found by the nonparametric analyses for each of the groups of *DRB1*, *DQA1*, and *DQB1* alleles. Each of the HLA specificities, age (in years), log[total IgE], and sex were used as independent variables in these analyses. With log [*Chi t I* IgE antibody] as the dependent variable, the following sets of subjects were

analyzed: groups 1 + 2 + 3 + 4, groups 1 + 2, and groups 3 + 4. For these analyses some *DRB* and *DQB1* specificities were grouped: *DRB1*0101/02*, *DRB1*0103*, *DRB1*1501/02*, *DRB1*16*, *DRB1*03*, *DRB1*04*, *DRB1*11*, *DRB1*1301/02*, *DRB1*1303/14*, *DRB1*07*, *DRB1*0801*, *DRB1*0803*, *DRB1*1001*, *DRB1*1201*, *DRB3*0101*, *DRB3*02*, *DRB3*0301*, *DRB4*0101*, *DRB5*0101*, *DRB5*0102/02*, *DQB1*02*, *DQB1*0301/04*, *DQB1*0302/03*, *DQB1*04*, *DQB1*0501/02*, *DQB1*0503*, *DQB1*0601*, *DQB1*0602/03*, *DQB1*0604/05*.

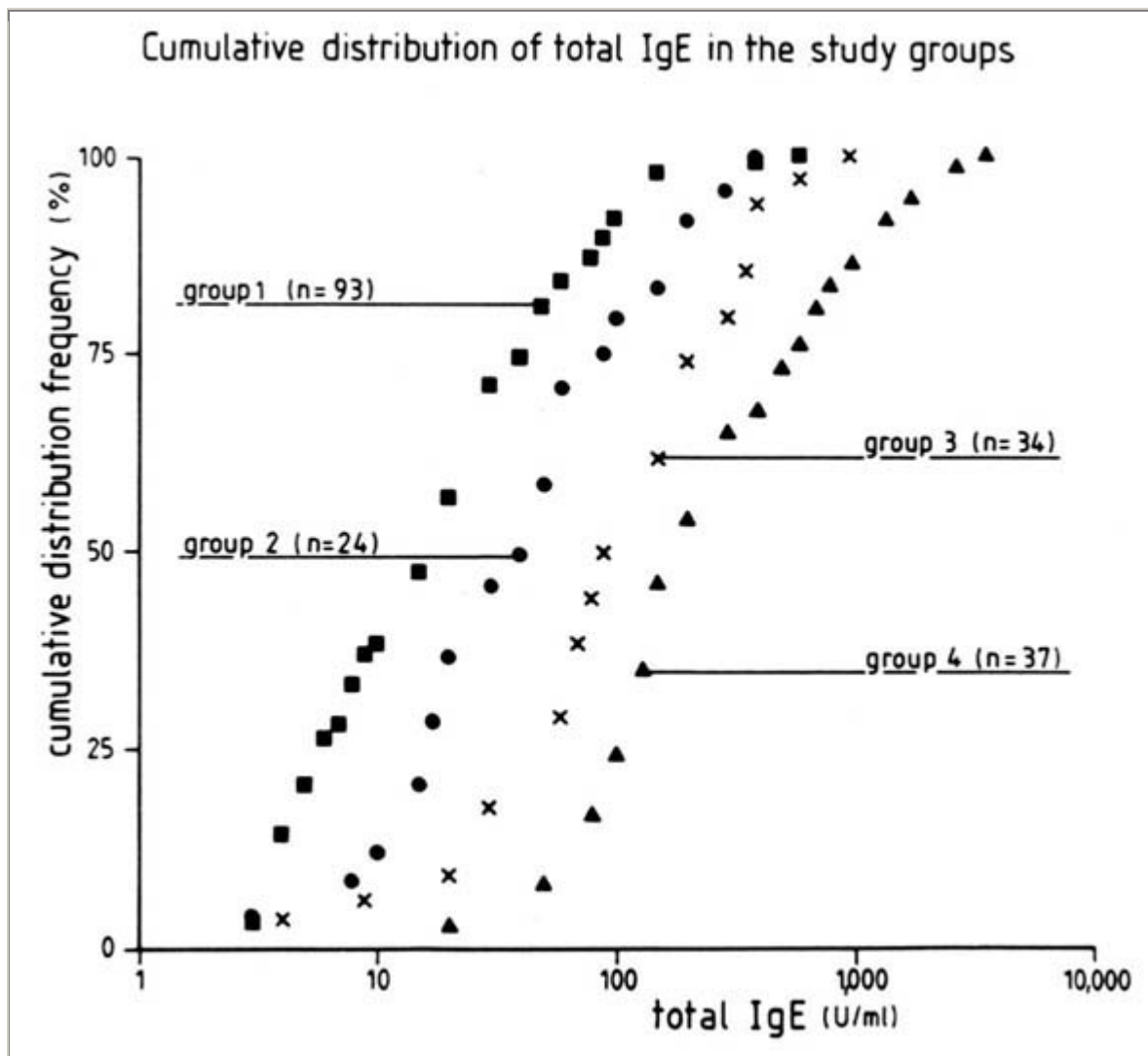
Results

Sensitization to various allergens

RAST and skin test results of 188 subjects exposed to *Chi t I* were negative in 93 (49%) individuals, and 95 (51%) subjects were sensitized to at least one of the allergens tested (including *Chi t I*). Screening of the sera for antibodies to *Chi t I* by RAST showed that of the 70 (37%) subjects who responded to the crude extract of *C. thummi*, 61 (87%) were sensitized to the major allergen *Chi t I*. Thirty-seven of 188 (20%) had IgE antibodies to grass pollens, 31 (16%) to tree pollens, 23 (12%) to animal danders, 19 (10%) to weed pollens, 16 (9%) to *Der p*, and 6 (3%) to mixed molds. In the 69 subjects who were skin tested with *Chi t I* and other allergens, the skin test results showed a good correlation with the RAST results; all subjects with positive skin test responses to *Chi t I* also had positive RAST results. In two cases (3%) subjects with negative skin test responses to *Chi t I* showed a positive RAST result. Thirty-six of the 188 subjects (19%) were responders to other allergens; 15 of the responses resulted from additional allergen extracts used in the RAST, and the others resulted from skin tests. Two individuals sensitive to *Chi t I* and one other person with negative results to the allergen spectrum tested in the RAST showed a positive skin test response to one of the allergens tested.

After distribution into four groups according to differential sensitization (see Methods section), the levels of total IgE differed in the four subgroups, as shown by the cumulative distribution curves in Fig. 1. Group 1 (nonsensitive) and group 2 (*Chi t I*-monosensitized individuals) both showed lower total IgE distributions than the subjects of the atopic groups 3 and 4. Student's *t* test (two-tailed) showed that the geometric mean (GM) levels of total IgE were significantly different between the control group 1 (GM = 17 U/ml) and the sensitized groups 3 (GM = 98 U/ml) and 4 (GM = 230 U/ml) with a *p* value of less than 0.05. Also between group 2 (GM = 37 U/ml) and group 3, as well as between groups 2 and 4 and between groups 3 and 4, the GM levels of total IgE differed significantly (*p* < 0.05). No significant difference was found between groups 1 and 2.

FIG. 1. The GM levels of total IgE were different among the four study groups as shown by cumulative distribution frequencies: group 1, 17 U/ml; group 2, 36 U/ml; group 3, 98 U/ml; group 4, 230 U/ml. As determined by Student's *t* test (two-tailed), the statistics showed that these differences were significant between all groups with *p* < 0.05, except for the comparison of group 1 and group 2 for which no significant difference was found.



HLA-D typing and DNA sequencing

The primer sets used worked well for amplification of the *DRB1*, *DRB4*, and *DRB5* genes but less well for *DRB3*. Amplification was successful for all *DQB1* alleles, although the *DQB1*0400* group was only weakly amplified, probably because of a polymorphic difference in the third base from the 3' end of primer DGM 7b.²⁸ The pseudogene *DQB2* was also amplified by the primer set used, but *DQB2*-specific sequences were excluded by the selection of SSOs used for hybridization. In the case of *DQA1*, the specific primer pair DQAAMP-A/DQAAMP-B amplified all *DQA1* alleles well. Allele *DQA1*03012* was not found in our study subjects. HLA-D typing results in the control group (group 1) and in the total panel coincided well with previously published phenotype frequencies of broad specificities in Caucasian panels.²⁹

With the SSO set used in this study we were not able to distinguish between *DRB1*0101* and *DRB1*0102* (which differ only at codons 85 and 86). We wished to investigate further the precise sequence of the associated specificities among the monosensitized responders to *Chi t I*. Sequencing of the *DRB1* second exons of the 13 *Chi t I*-monosensitized individuals who had a *DRB1*0101/02* allele including one patient from group 4, who most likely has a *DRB5* gene in addition to *DRB1*0101/02*,²⁰ revealed the "normal" *DRB1*0101* sequence, which is the predominant DR1 encoding allele in Caucasians. Thus there was no micropolymorphism in the second exon DNA, which might account for susceptibility to the allergen *Chi t I*.

Analysis of HLA associations by nonparametric statistics

With the use of the Chi square test, the proportions of the different *DRB1*, *DRB3*, *B4* and *B5*, *DQA1*, and *DQB1* alleles were compared by nonparametric analyses in the three groups of allergic individuals and the exposed control group. ■Table I shows that *DRB1*0101/02*, *DQA1*0101*, and *DQB1*0501* are positively

associated with IgE antibody to *Chi t I*. The strongest association was found for *DRB1*0101/02* between *Chi t I*-monosensitized individuals and healthy control subjects ($p < 0.01$) or subjects in group 3 who are only sensitized to other allergens ($p < 0.025$). Also, we found a significant difference for *DRB1*0101/02* between groups 2 and 4 (additionally sensitized to other allergens), both of which consist of individuals sensitized to *Chi t I* ($p < 0.025$). In the case of *DQB1* and *DQA1*, the differences are still significant for *DQA1*0101* and *DQB1*0501* between *Chi t I*-monosensitized individuals (group 2) and those who are not sensitized to any allergen (group 1, $p < 0.05$), as well as between group 2 and those who are exclusively sensitized to other allergens (group 3, $p < 0.025$ for *DQA1*0101*; $p < 0.05$ for *DQB1*0501*). For *DQB1*0501* and *DQA1*0101* no significantly different distributions were noted between groups 2 and 4.

TABLE I. Associations between HLA class II alleles and IgE antibody positivity to ***Chi t I*** and other common allergens

	Specific IgE			
	Negative group 1 (n= 93)	<i>Chi t I</i> alone group 2 (n= 24)	<i>Chi t I</i> -negative but positive to other allergens group 3 (n= 34)	<i>Chi t I</i> + with other allergens group 4 (n= 37)
<i>DRB1*0101/2</i>	20%	50%	18%	18%
	$p < 0.01$	$p < 0.025$	NS	
	RR = 3.89	RR = 4.67		
<i>DQB1*0501</i>	23%	48%	18%	24%
	$p < 0.05$	$p < 0.05$	NS	
	RR = 3.14	RR = 4.43		
<i>DQA1*0101</i>	25%	50%	18%	27%
	$p < 0.05$	$p < 0.025$	NS	
	RR = 3.04	RR = 4.67		

The HLA class II alleles, for which significant associations with the IgE immune response to *Chi t I* were found by nonparametric analysis in three groups of allergic subjects and the control group are shown. For *DRB1*0101/2*, a significant difference was also found between groups 2 and 4 ($p < 0.025$, relative risk = 4.28). No significant differences were found for all other *DRB*, *DQB*, or *DQA* alleles. The relative risk calculated for the different groups for each allele is given below the p value. RR, Relative risk; NS, not significant.

Stepwise regression analyses

With parametric analysis, the influence of variables on the magnitude of specific IgE antibody level could be evaluated. In three sets of stepwise regression analyses involving *HLA-DRB1*, *DQB1*, or *DQA1*, we investigated the influence of log[total IgE], age, sex, and HLA-D type on log[*Chi t I* IgE antibody].²⁶ The results of these stepwise regression analyses are shown in ■Table II. In the analysis of *DRB1* specificities in groups 1 + 2, in which subjects were either IgE antibody negative to any tested allergen or IgE antibody positive to *Chi t I* alone, log[total IgE] and *DRB1*0101/02*, respectively, were the best predictors of log[IgE antibody] level to *Chi t I*. In the analysis of groups 1 + 2 + 3 + 4, log[total serum IgE] and *DRB1*0101/02* were still predictors of log[IgE antibody] level to *Chi t I*, although the significance of the *DRB1*0101/02* association was much diminished. The *DRB1*0101/02* association was not observed in the group 3 + 4 analysis. Other positive associations observed in these last two stepwise regression analyses (i.e., *DRB1*0803*, *DRB1*1001*, *DRB1*0700*) can be attributed to the *Chi t I* positive subjects in group 4 in which,

as determined by *t* test, log[IgE antibody] levels to *Chi t I* were significantly greater than those in group 2 (GM = 20 and 7, respectively, *p* = 0.02).

TABLE II. Independent variables included in regression equation resulting from stepwise regression analyses with log[IgE antibody] to ***Chi t I*** as the dependent variable

Independent variables		<i>p</i> Values
<i>DRB1</i>		
Groups 1 + 2 (<i>n</i> = 117)		
log[total IgE]		<0.001 (+)
<i>DRB1*0101/02</i>		0.002 (+)
Groups 3 + 4 (<i>n</i> = 71)		
log[total IgE]		0.001 (+)
Age (yr)		0.080 (+)
<i>DRB1*0400</i>		0.053 (–)
<i>DRB1*0803</i>		0.077 (+)
Groups 1 + 2 + 3 + 4 (<i>n</i> = 188) log[total IgE]		0.001 (+)
<i>DRB1*0803</i>		0.009 (+)
<i>DRB1*1001</i>		0.016 (+)
<i>DRB1*0700</i>		0.013 (+)
<i>DRB1*0101/0102</i>		0.056 (+)
<i>DQB1</i>		
Groups 1 + 2 (<i>n</i> = 116)		
log[total IgE]		<0.001 (+)
<i>DQB1*0301/04</i>		0.009 (–)
<i>DQB1*0501</i>		0.038 (+)
<i>DQB1*0601</i>		0.054 (+)
Groups 3 + 4 (<i>n</i> = 71)		
log[total IgE]		0.001 (+)
Age (yr)		0.052 (+)
Group 1 + 2 + 3 + 4 (<i>n</i> = 187) log[total IgE]		<0.001 (+)
<i>DQB1*0301/04</i>		0.030 (+)
<i>DQA1</i>		
Groups 1 + 2 (<i>n</i> = 117)		
log[total IgE]		<0.001 (+)
<i>DQA1*0101</i>		0.058 (+)
<i>DQA1*0201</i>		0.105 (+)
Groups 3 + 4 (<i>n</i> = 71)		
log[total IgE]		0.002 (+)
Age (yr)		0.089 (+)

<i>DQA1*0300</i>	0.052 (–)
<i>DQA1*0601</i>	0.079 (+)
Groups 1 + 2 + 3 + 4 (<i>n</i> = 188) log[total IgE]	<0.001 (+)
<i>DQA1*0601</i>	0.012 (+)
<i>DQA1*0201</i>	0.038 (+)
<i>DQA1*0300</i>	0.051 (–)

Positive and negative signs indicate positive and negative associations, respectively.

In the analysis of *DQB1* specificities of groups 1 + 2, log[total IgE] and *DQB1*0501*, respectively, were the best positive predictors of log[IgE antibody] level to *Chi t I*. However, the association of *DQB1*0501*, was not observed in either groups 3 + 4 or groups 1 + 2 + 3 + 4.

In the analysis of *DQA1* specificities in groups 1 + 2, log[total IgE] and *DQA1*0101*, respectively, were the best predictors of log[IgE antibody] level to *Chi t I*. In the analysis of groups 1 + 2 + 3 + 4, log[total IgE serum IgE] and *DQA1*0101* were still predictors of log[IgE antibody] level to *Chi t I*, although the significance of *DQA1*0101* association was much diminished. The *DQA1*0101* association was not observed in the group 3 + 4 analysis.

Discussion

Using nonparametric methods, we have shown associations between IgE antibody responsiveness to the chironomid major allergen, *Chi t I*, and the possession of the HLA class II specificities, *DRB1*0101/02*, *DQA1*0101*, and *DBQ1*0501*, which are in strong linkage disequilibrium³⁰ (Table I). The associations were strongest in *Chi t I*-monosensitized subjects (group 2) when compared with nonsensitized subjects (group 1). The associations were, however, not very striking in view of the large numbers of analyses performed.

To examine these associations further, we performed a series of stepwise multiple regression analyses, which consider log[*Chi t I* IgE antibody] as a metric trait and do not have the problem of multiple analyses of different HLA types. These regression analyses confirmed the positive associations found for *DRB1*0101/02*, *DQA1*0101*, and *DQB1*0501* for groups 1 + 2 (nonsensitized subjects and *Chi t I*-monosensitized subjects). We point particularly to the DR1 (*DRB1*0101/02*) data, for which the following differences were significant as determined by the nonparametric analyses in Table I: differences between the nonatopic subjects of group 1 and the *Chi t I*-monosensitized subjects of group 2 ($p < 0.01$), as well as for those between group 2 and the multiply sensitized group 3 ($p < 0.025$) and group 4 ($p < 0.025$). These findings were confirmed for *DRB1*0101/02* by parametric statistical analyses of the groups 1 + 2 and of groups 1 + 2 + 3 + 4 (Table II). Also, Fisher et al.,³¹ who studied HLA-D associations for the IgE response to *Bet v I*, found a specific DR association only in *Bet v I*-monosensitized subjects.

The weak associations found in our study as compared with association studies of other purified allergens (e.g., *Amb a V*, *Amb a VI*, *Lol p II*, and *Lol p III*; relative molecular mass = 5000 to 11,000)^{1-5, 27} may be partially explained by the observation that more than one T-cell epitope on *Chi t I* (relative molecular mass = 16,000) is recognized by different class II molecules.^{8, 32} We also recognize that the different individuals in our study probably had quite different levels of exposure to *Chi t I*; this is a problem common to HLA association studies and is particularly acute in our study. This factor would militate against our finding any HLA association.

Experiments in which peptides of *Chi t I* are used for stimulation of peripheral blood lymphocytes have shown that the whole molecule is immunologically active.^{12, 33} Our association data suggest that a major T-cell epitope on *Chi t I* may be restricted by DR1 or DQ5, which are encoded by alleles of genes that are in strong linkage disequilibrium,³⁰ with stronger evidence for an association with DR1 than with DQ5. For *Amb a V*, a DR restriction was found with specific T-cell clones.³⁴ Our recent experiments with monoclonal antibodies against HLA-DR, DQ, and DP have shown that HLA-DR-specific antibodies can significantly inhibit proliferation of T cells incubated with *Chi t I* and the relevant antigen presenting cells, whereas

antibodies specific for HLA-DQ and DP did not inhibit T-cell stimulation in all cases (unpublished results). Taken together, these findings implicate HLA-DR1 rather than DQ5 as the class II major histocompatibility complex molecule involved in antigen presentation of *Chi t I*. Further experiments with *Chi t I*-specific T-cell clones should clarify this issue.

Sequencing of DR1-specific second-exon DNA did not reveal a unique *Chi t I* responder sequence. This is in agreement with the results observed for the pollen allergen *Amb a V* in which "normal" DR2-encoding sequences were detected in responders.¹⁸

The mean values of log[total IgE] levels in both the *Chi t I*-monosensitized subjects and the nonsensitized subjects were significantly lower than the mean value of log[total IgE] levels in subjects with IgE antibodies to multiple allergens other than *Chi t I* (group 3 or 4). Furthermore, the mean value of log[*Chi t I* antibody] levels in *Chi t I*-monosensitized subjects were significantly lower than the mean value of log[*Chi t I* antibody] levels in subjects with IgE antibodies to *Chi t I* and multiple other allergens (group 4). These observations might be an indication for a different predisposition for the IgE-specific immune response in subjects who respond only to one specific allergen when compared with subjects who are responders to multiple allergens. These results are similar to those of Ansari et al.⁴ who observed, in a group of subjects with positive skin test results, a significant association between HLA-DR3 and the IgE antibody response to *Lol p III*. They found that among subjects with IgE antibody to *Lol p III*, those who had low total IgE levels had a high DR3 frequency, which decreased as total IgE level increased.

Thus we have observed that subjects who produce high levels of total IgE tend to produce high levels of IgE antibody to *Chi t I* with relatively little dependence on HLA-D type; whereas low IgE producers appear to require a particular HLA specificity, DR1 or DQ5, in order to respond to *Chi t I*. Previously, Willcox and Marsh³⁵ postulated that genetically predisposed *low* IgE antibody producers may require a more optimal class II molecule for antigen presentation than *high* IgE producers, perhaps reflecting different regulatory controls on T-cell responsiveness in the two categories of individuals. Recently, Bousquet et al.³⁶ claimed that there might be a degenerative binding of epitopes to different class II major histocompatibility complex molecules in responders with high total serum IgE levels. In other related studies, Pène et al.³⁷ noted that the release of interleukin-4 is different in monosensitized versus polysensitized subjects, who have "low" and "high" total IgE levels, respectively. All of these data seem to point to a difference in the regulation of IgE antibody responsiveness in the two groups of atopic subjects. The *Chi t I* system would seem to offer a useful model for investigation of the molecular basis for this difference.

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