

Association of Hypersensitivity to the Nematode *Anisakis simplex* with HLA Class II DRB1*1502-DQB1*0601 Haplotype

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ABSTRACT: Anisakiasis as well as allergic and anaphylactoid reactions to *Anisakis simplex* antigens are recently identified clinical entities. They are relatively frequent in countries with habitual raw food consumption, often in the form of large amounts of fish and sea food products. In this communication the relationship between HLA class II alleles and the IgE-specific immune response to *A. simplex* allergen was studied in a defined population in Northern Spain. Individuals with immediate-type Anisakis hypersensitivity and healthy controls were examined for HLA-DRB1, DQB1 and DQA1 alleles by sequence-specific oligonucleotide probe typing. Analysis of the HLA data among patients revealed increased phenotypic frequencies for DRB1*1502 and DRB1*0404 compared

to healthy controls ($p < 1 \times 10^{-7}$ and < 0.01 , respectively). Analysis of haplotypic frequencies showed that the DRB1*1502-DQB1*0601 haplotype is significantly higher in patients with Anisakis hypersensitivity in comparison with the control population from the same region ($p < 4 \times 10^{-8}$). The data suggest that this haplotype can be considered to be a susceptibility factor for hypersensitivity to *A. simplex* antigens. *Human Immunology* 61, 314–319 (2000). © American Society for Histocompatibility and Immunogenetics, 2000. Published by Elsevier Science Inc.

KEYWORDS: *Anisakis simplex* allergy; HLA-DRB1; HLA-DQB1

ABBREVIATIONS

HF haplotypic frequency
LD linkage disequilibrium

RR relative risk

INTRODUCTION

Anisakis simplex is a common nematode (Superfamily: *Ascaridoidea*; family: *Anisakidae*) [1, 2] that parasitizes sea mammals [3, 4], fish [5], and cephalopods [6] being frequent intermediate hosts. Human infestation can be caused by eating raw or undercooked fish or cephalopods through the gastrointestinal invasion of third-stage larvae [6–8]. Anisakiasis is a clinical entity relatively frequent in countries with high rates of fish and seafood consumption. Although most cases were reported in

Japan and other points of Asia, in recent years new cases have been reported in countries such as France [9], the United States [10] and Indonesia [11] associated with the increasing popularity of raw food. Consumption of undercooked or raw fish is also frequent along the Cantabrian coast and other areas of Spain. Hence, anisakiasis can be considered an emerging parasitosis in Spain. In addition, allergic and anaphylactoid reactions have also been observed in Japanese and Spanish patients with gastric anisakidiosis [12–15]. Hypersensitivity and infestation can occur either separately or together.

One of the most characteristic features of the immune response against helminths is a dramatic elevation and maintenance of serum IgE and IgG4 levels, usually associated with marked eosinophilia. In addition, it is well known that immune responses vary between individuals with similar helminth exposure, influenced by genetic,

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behavioural (including nutritional habits), and other infectious processes (reviewed in [16]).

Accumulated evidence indicates that: (i) in human IgE-mediated hypersensitivity the antigen (or antigens) implicated can be identified [17]; (ii) high serum IgE concentrations seem to be clearly associated with HLA-DR and microsatellite markers on chromosomes 5q31 and 11q13 [18]; and (iii) several studies using highly purified antigens have shown significant association between IgE-mediated hypersensitivity and specific HLA class II alleles, as well as HLA class II molecules restricting the T-cell recognition of antigenic determinants of defined allergens (for a review, see [19]). In spite of this, there are very few reports of HLA associations with helminthic disease and/or hypersensitivity response to helminth antigens.

In the present report, the relationship between sequence polymorphisms of HLA-DRB, DQA1, and DQB1 genes of people living in Northern Spain in which Anisakis allergy is well-documented was studied.

MATERIALS AND METHODS

A total of 46 unrelated caucasian patients recruited in the region of Cantabria with Anisakis hypersensitivity, were available for HLA typing. The definition of a case for the present study was: any individual with symptoms suggesting allergy (such as urticaria, angioedema, and/or anaphylaxis occurring <6 h after fish ingestion), elevated serum IgE, sensitization to *A. simplex* demonstrable by a positive skin prick test, and positive serum specific IgE to *A. simplex* antigen who was not receiving allergen immunotherapy. The total serum IgE and specific IgE to *A. simplex* were determined by nephelometry and the RAST-CAP system (Pharmacia Diagnostics, Uppsala, Sweden), respectively. In our study group, total serum IgE and specific IgE to *A. simplex* antigen were 633.32 ± 176.07 IU/ml and 24.17 ± 6.07 kU/l, respectively. Results of over 100 IU/ml and 0.35 kU/l, respectively, were considered positive.

As controls, 200 unrelated individuals who have lived in the same area for at least three consecutive generations were randomly selected from all the Cantabrian municipalities [20]. Demographic details (age, male/female ratio) were comparable between patients and controls. They had no *A. simplex* hypersensitivity or family history of the disorder, and all of their IgE levels were within normal range.

Genomic DNA was extracted from peripheral EDTA-blood lymphocytes as previously described [20]. HLA-DRB1, HLA-DQA1, and HLA-DQB1 alleles were detected by PCR-SSO-reverse dot blot, by PCR amplification with sequence-specific primers (PCR-SSP), and by PCR-RFLP, respectively, as described [20]. Gene

and phenotype frequencies were calculated by direct counting. Haplotype frequencies (HF) and linkage disequilibrium (LD) were analyzed by the maximum likelihood method assuming the Hardy-Weinberg law. Comparison between patients and controls were performed using the χ^2 test with Yates' continuity correction as described [20]. Fisher's exact test was used when the theoretical numbers were <3. The probability (p) values were corrected for the number of comparisons made, when appropriate, and were considered significant if they were < 0.05. All calculations were performed using the SYSTAT computer programs as described [20]. The relative risk (RR) was calculated by Woolf's formula: $RR = (a \times d)/(b \times c)$, where a and c are the phenotype frequencies of patients and controls with the marker, respectively, and b and d are the phenotype frequencies of patients and controls lacking this particular marker, respectively [21].

RESULTS AND DISCUSSION

The distribution of the DR and DQ frequencies are shown in Table 1. In the *A. simplex*-allergic patients, the DRB1*0404 and DRB1*1502 phenotypic frequencies were significantly higher than in the normal matched population ($p < 0.01$ and $p < 3.4 \times 10^{-7}$, respectively). For the risk of allergy to Anisakis allergen associated with the DRB1*0404 and DRB1*1502 alleles, the RR was found to be 4.9 and 18.3, respectively.

The frequencies of the DQB1*0202, DQB1*0601 and DQB1*0603 alleles were significantly increased in the allergic patients ($p < 4 \times 10^{-7}$, $p < 4 \times 10^{-6}$ and $p < 0.05$, respectively).

The analysis of the HF in both populations indicates a significant increase in DRB1*1502-DQB1*0601 haplotype ($p < 4 \times 10^{-8}$) in the Anisakis allergic group (the HF was 0.048 and 0 in the allergic and control populations, respectively). The linkage disequilibrium value for this haplotype in comparison with the rest of the haplotypes in the allergic population is extremely high (LD=0.041, $p < 1 \times 10^{-11}$). Other haplotypes were found to be similarly distributed in both populations and these differences were not significant (data not shown).

Atopic allergy is an excellent model for molecular studies of antigen presentation. Unlike autoimmune disorders, the antigens can be clearly defined and a direct relationship between IgG and IgE production against them and disease expression can be recognized. This association is particularly well studied for highly purified antigens and specific peptides, e.g., *Dermatophagoides* spp- and *Artemisia artemisiifolia*-derived antigens [19]. Although a putative chemically defined antigen from *A. simplex* is still lacking, some indirect information as to

TABLE 1 Gene and phenotype frequencies in *Anisakis* allergic individuals and in normal control group

Alleles	Normal group N = 200			Patient group N = 46			RR	χ^2	p Value			
	n = 400	Phen fr	Gene fr	n = 92	Phen fr	Gene fr						
<i>DRB1</i> *												
0101	39	0.195	0.0975	8	0.1739	0.086	18.3	26	$<3.4 \times 10^{-7}$			
0102	9	0.045	0.0225	5	0.1086	0.0543						
0104	4	0.02	0.01	3	0.065	0.032						
1501	48	0.024	0.12	5	0.1086	0.0543						
1502	3	0.015	0.075	10	0.2173	0.1086						
1601	10	0.05	0.025	1	0.021	0.0108						
0301	39	0.195	0.0975	5	0.1086	0.0543						
0401	26	0.13	0.065	2	0.043	0.0217						
0402	13	0.065	0.032	3	0.065	0.032						
0403	15	0.075	0.0375	4	0.086	0.0434						
0404	6	0.03	0.015	6	0.1304	0.0652	4.9	6	<0.01			
1101	3	0.015	0.075	0	0	0						
1103	2	0.01	0.005	0	0	0						
1104	21	0.105	0.0525	4	0.086	0.043						
1301	30	0.15	0.075	3	0.065	0.032						
1302	18	0.09	0.045	5	0.1086	0.0543						
1401	14	0.07	0.035	0	0	0						
0701	57	0.285	0.1425	16	0.3478	0.1739						
0801	16	0.08	0.04	3	0.065	0.032						
0804	2	0.01	0.005	1	0.021	0.0108						
0805	4	0.02	0.01	0	0	0	0.3	4.1	<0.05			
0806	5	0.025	0.0125	1	0.021	0.0108						
0901	5	0.025	0.0125	4	0.086	0.0434						
1001	11	0.055	0.0275	3	0.065	0.032						
<i>DOB1</i> *												
0501	65	0.325	0.1625	17	0.3695	0.1847				21.3	25.7	$<3.9 \times 10^{-6}$
0502	12	0.06	0.03	1	0.021	0.0108						
0503	16	0.08	0.04	0	0	0						
0601	0	0	0	6	0.1304	0.0652						
0602	59	0.295	0.1475	10	0.2173	0.1086						
0603	49	0.245	0.1225	4	0.086	0.0434						
0604	20	0.1	0.05	3	0.065	0.032						
0201	72	0.36	0.18	17	0.3695	0.1847						
0202	0	0	0	7	0.1521	0.076						
0301	50	0.25	0.125	7	0.1521	0.076						
0302	36	0.18	0.09	13	0.2826	0.141	0.3	4.1	<0.05			
0303	7	0.035	0.0175	2	0.043	0.0217						
0402	14	0.07	0.035	5	0.1086	0.0543						
<i>DOA1</i> *												
0101	67	0.335	0.1675	19	0.413	0.206				25.7	<4.01 $\times 10^{-7}$	$<3.9 \times 10^{-6}$
0102	83	0.415	0.2075	18	0.3913	0.1956						
0103	33	0.165	0.0825	6	0.1304	0.0652						
0104	0	0	0	1	0.021	0.0108						
0201	65	0.325	0.1625	16	0.3478	0.1739						
0301	56	0.28	0.14	17	0.3695	0.1847						
0401	20	0.1	0.05	3	0.065	0.032						
0501	76	0.38	0.19	12	0.2608	0.1304						
0601	0	0	0	0	0	0						

N and n are the number of individuals and alleles, respectively. No blank or null alleles were observed in these series. RR values were only calculated in significant positive associations (RR was not computed for the DQB1*0601 and DQB1*0202 alleles because of 0 values in the normal group).

the chemical characteristics of the responsible antigen is available, namely that the antigenic determinant is not a cross-reactive carbohydrate; the putative antigen is thermoresistant since hypersensitivity to *A. simplex* was de-

monstrable in patients even after eating boiled parasitized fish, and this is a glycoprotein synthesized by *A. simplex* larvae, of molecular weight 14 kD [22].

Reports dealing with HLA and large parasite infesta-

tions are rare. This is not surprising, because the specific immunity to large parasites is complex and probably involves several antigens that are not well defined [16]. Recently, however, a few studies involving DNA typing at an allelic level have been published. Examples show: (i) susceptibility to larval infection with *Echinococcus multilocularis*, which seems to be linked to HLA-DQB1*02, whereas a reduced risk for disease development is associated to HLA-DRB1*11 [23]; (ii) susceptible HLA class II alleles in Chinese patients with schistosomal hepatic fibrosis are DRB1*1202, DRB1*1404, and DRB1*1405 whereas DRB1*11011, DRB1*0409, and DRB1*0701 are resistant alleles [24]; and (iii) HLA-DQ and -DP molecules are associated with the intensity of the immune response to *Onchocerca volvulus* in Nigerians [25, 26].

The association of hypersensitivity to *A. simplex* with DRB1*1502-DQB1*0601 deserves further comment. First, the DRB1*1502 allele is relatively uncommon among caucasians, particularly in Northern Europe, which includes the Cantabrian and Basque provinces of Spain [20, 27]. However, this allele is frequent among Japanese, Korean, Thai, Vietnamese, and Canadians of Indian ancestry (the so called "Thai" allele [27, 28]). Second, the DQB1*0601 haplotype frequency is highest among Wailibri people from the Australian Northern Territory, Asians, and the Welsh (the "Welsh" allele [27, 28]). Finally, the DRB1*1502-DQB1*0601 haplotype is highest in Asian populations [29]. In Europe, this haplotype is relatively frequent among Scottish Highlanders, Spanish gypsies, Romanians, and Italians, but absent or very rare in several other European countries such as Germany, Denmark, Norway, Hungary, France, and Spain [30, 31].

A review of specific associations of these alleles with diseases, yielded the following information: (i) the frequencies of DRB1*1502 and DQB1*0601 alleles are significantly increased in Japanese patients with psoriasis vulgaris [32], Takayasu arteritis [33], and progressive systemic sclerosis [34]; (ii) DRB1*1502 has been shown to have a positive association with ulcerative colitis in Turkish [35] and Japanese [36] patients; (iii) haplotypes containing these alleles seem to confer susceptibility to progressive systemic sclerosis and Takayasu arteritis [32, 33]; (iv) while on the contrary, the frequencies of DRB1*1502 and DQB1*0601 alleles, among others, show a significant decrease in Japanese patients with Behcet's disease [37]. None of our patients showed clinical and laboratory finding compatible with these diseases.

Fish and several other marine products represent the staple food in many Asian countries as well as in areas of Northern Spain. According to the Food and Agriculture Organization (FAO) of the United Nations, Japan and Spain rank 4th and 16th, respectively, among 189 coun-

tries in world total fish catches, total marine fish processing, and fish and seafood consumption. These countries rank second and ninth, respectively, among 65 recorded in fish domestic supply (data from 1997, <http://apps.fao.org>). These considerations taken into account, together with the fact that anisakiasis [8] and Anisakis allergy [13–15] were first documented in Japan and Northern Spain, respectively, suggests that the pressure of this eating habit selected for an association with this haplotype in two areas as far apart as Japan and Spain.

The DRB1*1502-DQB1*0601 allele is absent among Norwegians [31], despite Norway being a country with high rates of fish production and consumption (eighth and tenth, respectively, according to the FAO records). However, it is also true that only sporadic cases of anisakiasis or Anisakis hypersensitivity have been reported in Scandinavia countries or Northern Europe.

In conclusion, this article appears to be the first study reporting a significant HLA class II association with hypersensitivity to *A. simplex* antigens. Allergy to *A. simplex* is significantly associated with DRB1*1502-DQB1*1601; consequently, determination of the MHC type could potentially predict susceptibility to *A. simplex* allergy. Nevertheless, additional studies, both in Japanese and other Asian communities to correlate Anisakis allergy and HLA class II allotypes, will further extend the value of such studies.

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