Lack of association between HLA genotype and chronic fatigue syndrome

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Summary

Although the aetiology of chronic fatigue syndrome is controversial, evidence that infective agents including viruses may have a role in the development of the condition has led to studies seeking an association with the immunomodulatory HLA genes. In the present study, we sought to extend previous work using a well-characterized patient group and modern HLA genotyping techniques. Fifty-eight patients were phenotyped for HLA A and B by microcytotoxicity and genotyped for HLA DRB, DQB and DPB by PCR oligoprobing, and the frequencies of antigens so assigned were compared with those from a control group of 134. No significant differences in HLA frequencies were found between patient and control groups. Thus, this study does not confirm previous findings of an HLA association with chronic fatigue syndrome, suggesting that neither presentation of viral antigen by HLA class I nor antigen processing genes in the HLA region is a major contributory factor in the development of the disease.

Introduction

Patients with severe exhaustion and associated symptoms for which no simple biomedical explanation is forthcoming are currently the focus of attention in a number of disciplines, as well as a source of controversy. The term 'chronic fatigue syndrome' (CFS) has recently been introduced to describe these symptoms, although the condition itself is probably not new. A number of aetiological theories have been proposed, but particular prominence has been given to the possibility that CFS represents a response to viral infection, or alternatively is associated with viral persistence (Kleinman & Straus, 1994). Clinically most patients seen in specialist care trace their illness to a viral episode which failed to resolve (Salit, 1985; Behan & Behan, 1988; Wessely & Powell, 1989; Komaroff & Buchwald, 1991; Hinds & McCluskey, 1993). Several studies have found retrospective evidence of increased exposure to viral agents, Epstein Barr virus being the favoured candidate in the USA and the enterovirus family in the UK. Other possible candidates have included human herpes virus-6 or a retrovirus (Kleinman *et al.*, 1994). There is also evidence of persistence of enteroviral genome in the muscles of sufferers (Gow *et al.*, 1991); in addition an abnormal response to a range of common infective agents has been proposed.

Although it is true that many of the above findings have proved irreproducible, or are based on studies whose methodologies may be questioned, the possibility that CFS is linked with an infective process remains a subject of great interest. This is reflected in the term 'post viral fatigue syndrome' which is a synonym for CFS in the UK, and the introduction of the term 'post infectious fatigue syndrome' in the case definition proposed by UK researchers (Sharpe *et al.*, 1991).

That a viral or other infective agent may be involved in the initiation of the disease has aroused some interest in the possibility that there may be specific associations between HLA antigens and CFS. Preliminary HLA studies from the USA and Australia have suggested a substantial overrepresentation of DR4 in patients with CFS (Keller *et al.*, 1994; A. Lloyd *et al.*, unpublished results). These studies were based on serological HLA typing, which may be inaccurate (Opelz *et al.*, 1991), and used broad definitions, especially in the assignment of DQ types. A larger study from Northern Ireland using restriction fragment length polymorphism (RFLP), a more accurate DNA-based method, showed no association between CFS and HLA class II genotypes, including DR4 (Middleton *et al.*, 1991), although a non-significant increase in the frequency of DR14 in CFS patients was noted. The most recent contribution from Ireland, using a limited PCR method, has also suggested a negative association with DR4 (Fitzgibbon *et al.*, 1996). If such an association with immune regulatory genes were to be confirmed it would significantly enhance our understanding of this enigmatic condition.

In the present study we have sought to reassess the associations with HLA in a new, well-characterized patient group, using methods based on PCR amplification and oligonucleotide probing, which allows more accurate and higher resolution genotyping.

Methods

Fifty-seven subjects were selected from those referred by hospital consultants or general practitioners to a clinic

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specializing in CFS. All fulfilled the UK consensus criteria (Sharpe *et al.*, 1991). In addition, patients with somatization disorder were excluded, bringing the sample into line with the recent case definition proposed by the Center for Disease Control (Fukuda *et al.*, 1994), which was not available when the study commenced. There was an excess of female patients (70%) and also of patients with high socio-economic status, as is characteristic of all specialist samples of CFS recruited to date. Duration of illness was relatively long (mean 4.4 years), well in excess of the minimum period of 6 months stated by all the case definitions, and again characteristic of CFS (Euba *et al.*, 1996). Thirty-three (67%) reported a viral illness at onset. Most patients had taken part in a multicentre diagnostic study of CFS, the results of which confirmed that they are typical of their counterparts attending similar clinics in the UK, the USA and Australia (Deale *et al.*, 1997).

Control subjects comprised 134 Northern European Caucasian volunteers. Patients and controls were typed by microcytotoxicity for HLA A, B and DR/DQ, using sera provided by the United Kingdom Transplant Support Services Authority (Bristol, UK) and genotyped for HLA DRB, DQB and DPB as below. Antigen assignments were made for broad antigens only. Class II positive cells for DR typing were separated using immunomagnetic beads (Dynal UK Ltd, New Ferry, Wirral, UK) (Doherty *et al.*, 1991).

Genomic DNA was prepared from lymphocyte suspensions using proteinase K digestion and salt precipitation. Genomic DNA was amplified by PCR with primers specific for the variable second exon regions of the HLA DRB, DQB and DPB genes. PCR products were denatured by heating to 95 °C, and 2 μl was dotted by hand onto replicate positively charged nylon membranes (Boehringer UK, Lewes, UK). Genotyping was performed by sequence-specific oligonucleotide hybridization, using 22 DRB probes, 16 DQB probes and 18 DPB probes labelled with digoxygenin. Primers and probes were supplied in the sequence-specific oligonucleotide (SSO) class II typing kit (British Society for Histocompatibility and Immunogenetics). Stringency washes were performed in TMAC at 58 °C and hybridization patterns revealed by enhanced chemiluminescence (Boehringer UK) on X-ray film. Membranes were stripped in 0.4 M NaOH and re-probed up to 3 times. The patterns of reaction of the probes were used to assign 29 DRB alleles, 16 DQB alleles and 19 DPB alleles, although some are grouped together because of the very low frequency of rarer alleles (see Table 2 below). Frequencies of each allele were compared between patient and control groups using the chi-squared test. Probable haplotypes were assigned on the basis of known linkage disequilibrium (Doherty *et al.*, 1992).

Results

None of the antigens or alleles tested was present at significantly higher or lower frequency in patients compared with controls (Tables 1 and 2). Similarly, there were no significant differences in haplotype frequencies between patient and control groups. The frequency of DR4 in the patient group was 33% compared with 40% in the control group (P = NS). In every case, in both patients and controls, the alleles DRB1*0401–11 were found with DQB1*0301 or DQB1*0302, as would be expected from the strong linkage disequilibrium between DRB and DQB. However, since, in the absence of family data, we cannot assign definitive haplotypes, we must note that alleles of DR4 (i.e. DRB1*0401–11) occur together in the same individual with alleles of DQ1 (i.e. DQB1*0501–4 and DQB1*0601–5) in 10 (18%) of the patient group and 31 (18%) of the control group. DR4 was found without DQ1 in nine (16%) of the patients and 44 (25%) of the controls. Similarly, the alleles of DQ1 were found in haplotypes with DRB alleles, consistent with previously reported haplotypes not including DR4.

Alleles of DR14 were present in 7% of patients and 5% of controls, in contrast to the findings from Northern Ireland, in which DR14 was present in 11.9% of patients and 1.9% of controls (Middleton *et al.*, 1991).

Table 1. HLA A and B antigen frequencies by serotyping

	Patients (n = 58)		Controls (n = 134)	
Broad antigen	*n*	%	*n*	%
A1	15	28	51	38
A2	29	53	68	51
A3	13	23	36	27
A9	14	23	23	17
A10	7	12	9	7
A11	4	7	15	11
A19	15	28	34	25
A28	5	9	6	5
AX	14	24	26	19
B5	6	10	8	6
B7	18	31	29	22
B8	10	17	32	24
B12	16	28	45	36
B13	2	3	6	5
B14	4	7	10	8
B15	7	12	22	16
B16	6	11	3	2
B17	5	9	14	11
B18	3	5	8	6
B21	3	5	6	4
B22	2	3	5	4
B27	7	12	15	11
B35/53	6	11	17	13
B37	1	2	8	6
B40	5	9	16	12
B41	0	0	1	1
B46	1	2	0	0
B47	0	0	1	1
B70	0	0	1	1
BX	15	26	19	14

Table 2. HLA DRB, DQB and DPB alleles by PCR SSO hybridization

	Patients (n = 58)		Controls (n = 134)	
Allele	n	%	n	%
DRB1*0101	15	26	20	15
DRB1*0102	1	2	2	2
DRB1*0103	0	0	6	5
DRB5*0101	18	31	27	20
DRB5*0102	0	0	1	1
DRB5*0201/2	3	5	1	1
DRB1*0301/2	10	17	35	26
DRB1*04	19	33	54	40
DRB1*1101–4	6	10	16	12
DRB1*1201/2	1	2	3	2
DRB1*1301/4	7	12	19	14
DRB1*1302	0	0	5	4
DRB1*1303/5	2	3	3	2
DRB1*1401–5	4	7	6	5
DRB1*0701	16	28	37	28
DRB1*0801–4	3	5	7	5
DRB1*0901	2	3	5	4
DRB1*1001	1	2	1	1
DRBX	8	14	19	14
DQB1*0501	17	29	27	20
DQB1*0502	2	3	1	1
DQB1*0503	4	7	6	5
DQB1*0601	1	2	0	0
DQB1*0602	19	33	28	21
DQB1*0603	4	7	8	6
DQB1*0604	2	3	12	9
DQB1*0605	1	2	2	1
DQB1*0201	20	35	61	46
DQB1*0301	18	31	45	34
DQB1*0302	11	19	39	29
DQB1*0303	7	12	14	10
DQB1*0402	2	3	8	6
DQB1*X	8	14	17	13
DPB1*0101	7	13	10	10
DPB1*0201	5	10	23	22
DPB1*0301	12	23	18	18
DPB1*0401	35	67	74	72
DPB1*0402	17	33	28	27
DPB1*0501	5	10	2	2
DPB1*0601	1	2	1	4
DPB1*0901	0	0	2	2
DPB1*1001	0	0	1	1
DPB1*1101	1	2	3	3
DPB1*1301	3	6	5	5
DPB1*1401	0	0	3	3
DPB1*1501	4	8	2	2
DPB1*1601	1	2	0	0
DPB1*1701	0	0	3	3
DPB1*1901	0	0	2	2
DPB1*X	13	25	29	28

Discussion

In contrast to some previous reports, this study clearly demonstrated no HLA association with CFS in the population studied. Although the increase in frequency of DR14 in the study from Northern Ireland was substantial (Middleton *et al.*, 1991), it was not significant after correction for multiple testing, and is not confirmed by the data presented here. The haplotype or pairing of DR4 and DQ1 reported from Australia is very unusual and was not found in either the patient or the control group in our study. These data do not confirm the previously reported HLA associations with CFS from Australia or the USA (Keller *et al.*, 1994; A. Lloyd *et al.*, unpublished results) but instead support the negative results of the study from Northern Ireland (Middleton *et al.*, 1991). The most likely reasons for this contrast are differences in the diagnosis of CFS or techniques employed for HLA typing. The differences in reproducibility of HLA typing techniques based on serology compared with DNA methods have been dramatically demonstrated (Opelz *et al.*, 1991). The PCR SSO technique used here is accurate and provides a greater degree of resolution of HLA DR and DQ alleles than either serology or RFLP (Bidwell, 1994). Since white populations from North America, Australia and Northern Europe have similar HLA distributions, variability in HLA associations as reported may be a result of differing environmental effects in the development of CFS.

The lack of an HLA association in CFS does not preclude the involvement of a virus in the aetiology of the disease. Despite the role of HLA class I in the presentation of viral antigenic peptides and the ever-increasing number of genes in the HLA region known to be involved in antigen processing and immune response, relatively few HLA associations with viral diseases have been discovered. These associations are typically not very strong and often related to severity or disease progression and not to infection *per se* (Thursz *et al.*, 1995). There is strong linkage disequilibrium across the HLA region, giving rise to a range of conserved haplotypes, although this linkage does not extend as far as DPB. Thus, given the spread of polymorphic loci tested here, it is unlikely that a strong association haplotype has been overlooked, though it is possible that weaker associations with linked genes of immune function (e.g. complement and tumour necrosis factor genes) could have been missed. Only a thorough study of these alleles will resolve the question, but since such weak associations would add little to our understanding of CFS, future investigations may be better directed into other areas of the human genome or the search for an infectious agent.

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