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HLA class II and response to interferon-beta in multiple sclerosis

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Objective –To study the relationship between human leucocyte antigen (HLA) genotype and clinical response to interferon-beta (IFN-β). *Methods* –We analysed the HLA class II genotypes of 96 multiple sclerosis (MS) patients treated with IFN-β. The patients were classified as responders or non-responders according to clinical criteria: one or more relapses or a sustained increase after 1 year treatment compared with the year prior to IFN-β therapy of ≥0.5 points on the Expanded Disability Status Scale (EDSS). *Results* –There were 66 (69%) responders and 30 (31%) non-responders. Baseline clinical characteristics were similar. We found no association between HLA class II alleles and clinical response to IFN-β. *Conclusions* –HLA genotype does not appear to influence the clinical response to IFN-β in MS patients.

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Epidemiological studies and genome screenings suggest that genetic factors influence susceptibility to multiple sclerosis (MS). The genomic region that codes for the major histocompatibility complex (MHC) has been most consistently associated with MS (1–3). The human leucocyte antigen (HLA) class II DR2 haplotype (DRB1*1501, DQA1*0102, DQB1*0602) has been associated with MS in Caucasians (1–5), including patients from our geographical area (6). The association of DR2 with some clinical characteristics possibly related to disease severity (female sex, younger age at onset) has been reported. However, other studies employing larger numbers of patients have generally failed to show any significant effect of HLA on disease course or outcome among Europeans (7). Thus, the pathogenic role of HLA in MS remains to be elucidated, particularly its relationship to the response to immunomodulatory therapies.

Interferon-beta (IFN-β) has proved to be effective for the treatment of MS in the relapsing–remitting (RR) and secondary progressive (SP) phases in patients still experiencing relapses. IFN-β reduces the attack rate and probably slows the sustained progression of disability (8). However, nearly 30% of patients either fail to respond or respond suboptimally to this treatment as currently administered (9). Patients with increased or unchanged disease activity, either due to relapses or to gradual disability progression, should be considered as non-responders or suboptimal responders (10).

Prognostic markers, either clinical or genetic, are needed for the early discrimination of IFN-β responders from non-responders to help improve treatment options for individual patients. To investigate prognostic markers of the response to IFN-β therapy we therefore assessed the possible relationship between treatment response and HLA class II allele distribution.

Patients and methods

Patients

From the cohort of MS patients followed prospectively at our hospital since 1990, we included in this

open study all HLA-genotyped patients with clinically definite MS according to Poser's criteria (11) and who had been treated exclusively with IFN-β [8 MUI subcutaneous (s.c.) IFN-β-1b every other day; 6 MUI-30 mcg intramuscular (i.m.) IFN-β-1a once a week or 6 MUI-22 mcg s.c. IFN-β-1a three times a week] over a minimum of 1 year. All patients gave their informed consent prior to inclusion in this study. Patients were evaluated prospectively every 3 months and in the event of any exacerbations during the study period. Exacerbations and Expanded Disability Status Scale (EDSS) were recorded by a single evaluating neurologist, who was blinded to the assigned treatment. Visual acuity and walking distance were measured by a study nurse at each visit. Relapses were defined according to Poser's criteria, i.e. the occurrence of a symptom or symptoms of neurological dysfunction lasting more than 24 h, confirmed objectively by the patient's neurologist.

Patients were classified, from the database, as suboptimal responders or non-responders if they had increased or unchanged clinical activity as compared with the year before starting IFN-β treatment, defined as: one or more relapses or sustained progression, confirmed at a 6-month interval, of 0.5 or more points on the EDSS after 1 year of IFN-β treatment. Two pairs of independent neurologists performed this classification and any disagreement was resolved by consensus after conjoint review of the clinical files.

The analysis of demographic and clinical characteristics included sex, age, age at onset, symptoms at onset, duration of MS, type of MS according to Lublin's classification at the moment of initiation of treatment (12), the relapse rate and EDSS score (13) confirmed every 6 months in each year of the study, time of progression for the SP patients and type of IFN-β preparation. All SP patients included in this study presented exacerbations, due to the fact that in Spain, treatment with IFN-beta is only registered for RRMM and SPMS while still experiencing exacerbations.

We analysed the HLA class II subregions DRB1, DQA1 and DQB1 by polymerase chain reaction and with sequence-specific oligonucleotide probe hybridization (PCR/SSO, Inno-Lipa®; Innogenetics, Ghent, Belgium) for DRB1 and DQB1 and with sequence-specific primers (PCR/SSP, Dynal®, Oslo, Norway) for DRB1 subtypes and the DQA locus.

Statistical analysis

The demographic, clinical and HLA data were entered in a database and analysed with SPSS, version 11.5 for Windows (SPSS Inc., Chicago, IL, USA).

The frequencies of the DRB1, DQA1 and DQB1 alleles of the responders and non-responders were compared using the chi-square test with the Bonferroni correction for multiple comparisons. *P*-values < 0.05 were considered to be statistically significant after multiple comparisons correction by the Bonferroni method.

Results

The 96 MS patients included in the study were on IFN-β treatment for a mean duration of 5 ± 1.5 years, with a minimum of 1 year and a maximum of 8 years treatment. Sixty-six (68.8%) of these patients were responders and 30 (31.3%) were non-responders to IFN-β (Table 1). Thirty-three of the 96 patients (34%) were treated with s.c. IFN-β-1b, 26 (27.1%) with i.m. IFN-β-1a and 37 (38.6%) with s.c. IFN-β-1a.

The demographic characteristics and the clinical onset features of the two groups of responders to IFN-β were similar. However, there were differences in the clinical characteristics between responders and non-responders relating to differences at the start of IFN-β treatment: there was a higher proportion of patients with SPMS in the non-responders (56.7%) compared with the responders (27%; $P = 0.006$), and the mean EDSS score at the beginning of treatment was significantly ($P = 0.01$) higher in the non-responders (3.7 ± 1.8) than the responders (2.7 ± 1.7).

Table 1 Demographic and clinical characteristics of responders and non-responders to INF-β therapy

	Responders ($n = 66$)	Non-responders ($n = 30$)	*P*
Women	43 (65.2%)	22 (73%)	0.42
Age (years)	38 ± 7 (21–60)	39 ± 9 (20–55)	0.80
Age at onset (years)	25 ± 7.3 (13–52)	25.9 ± 7.8 (12–43)	0.97
Symptoms at onset			
Pyramidal	28 (42%)	6 (20%)	0.02
Cerebellar	7 (10%)	3 (10%)	0.91
Brainstem	12 (18%)	6 (20%)	0.85
Sensory	29 (43%)	10 (33%)	0.29
Bowel-bladder	1 (1.5%)	1 (3%)	0.57
Visual	9 (13%)	4(13%)	0.95
Mental	0	0	–
Clinical type			
RR	48 (72.7%)	13 (43%)	0.006
SP	18 (27.3%)	17 (56.7%)	
Progression time in SP patients (years)	6.2 ± 5.3 ($n = 18$)	6.4 ± 5.6 ($n = 17$)	0.94
Disease duration (years)	10.5 ± 7.3 (1–36)	14.2 ± 6.8 (6–30)	0.02

RR, relapsing–remitting; SP, secondary progressive. *P*-values < 0.05 were considered to be statistically significant after multiple comparisons correction by the Bonferroni method.

Table 2 Phenotypic frequencies of HLA class II DRB1 alleles of responders and non-responders to INF-β therapy

Alleles	Responders ($n = 66$)	Non-responders ($n = 30$)	P
DRB1			
*01	8 (12.1)	5 (16.7)	0.57
*03	18 (27.3)	3 (10)	0.05
*04	16 (24.2)	4 (13.3)	0.22
*07	15 (22.7)	11 (36.7)	0.15
*08	4 (6.1)	3 (10)	0.49
*09	1 (1.5)	0	0.49
*010	1 (1.5)	0	0.49
*11	13 (19.7)	6 (20)	0.97
*13	4 (6.1)	1 (3.3)	0.57
*14	5 (7.6)	0	0.12
*1501	30 (45.5)	17 (56.7)	0.30
*16	3 (4.5)	3 (10)	0.30

Table 3 Phenotypic frequencies of HLA class II DQA1 alleles of responders and non-responders to INF-β therapy

Alleles	Responders ($n = 66$)	Non-responders ($n = 30$)	P
DQA1			
*0101	11 (16.7)	6 (20)	0.69
*0102	29 (43.9)	18 (60)	0.14
*0103	7 (10.6)	3 (10)	0.92
*0104	1 (1.5)	0	0.49
*0201	16 (24.2)	10 (33.3)	0.35
*0301	17 (25.8)	5 (16.7)	0.32
*0303	2 (3)	1 (3.3)	0.93
*0401	2 (3)	1 (3.3)	0.93
*0501	28 (42.4)	8 (26.7)	0.13

Thirty-five of the 96 patients were in the SP phase of the disease (36%). All of them had relapses while in treatment, with an annual relapse rate of 0.4 ± 0.7 in the responder group and 0.8 ± 1.2 in the suboptimal responder group.

After correction for the number of comparisons, none of the HLA class II alleles or haplotypes was significantly associated with any of the demographic or clinical characteristics studied.

None of the HLA class II alleles or haplotypes was significantly associated with the response to IFN-β. The phenotypic frequencies of each allele tested (DRB1, DQA1 and DQB1) are presented in Tables 2–4.

Discussion

The present study was designed to examine the possible association between HLA and the clinical efficacy of IFN-β. The lack of response to IFN-β did not appear to be determined by any demographic or HLA aspects, but more by the clinical type of MS and mainly by the EDSS status at the start of the treatment.

We found that 32% of our MS patients were suboptimal responders to IFN-β therapy. There is

Table 4 Phenotypic frequencies of HLA class II DQB1 alleles of responders and non-responders to INF-β therapy

Alleles	Responders ($n = 66$)	Non-responders ($n = 30$)	P
DQB1			
*0201	16 (24.2)	4 (13.3)	0.22
*0202	16 (24.2)	9 (30)	0.55
*0301	15 (22.7)	8 (26.7)	0.67
*0302	12 (18.2)	3 (10)	0.30
*0303	2 (3)	1 (3.3)	0.93
*0402	2 (3)	1 (3.3)	0.93
*0501	9 (13.6)	5 (16.7)	0.69
*0502	3 (4.5)	4 (13.3)	0.12
*0503	5 (7.6)	0	0.12
*0601	0	1 (3.3)	0.13
*0602	26 (39.4)	17 (56.7)	0.11
*0603	9 (13.6)	2 (6.7)	0.32
*0604	1 (3)	0	0.33

no generally accepted definition of response to IFN-β treatment as there is no acknowledged clinical marker of response. The conclusions of a recent consensus meeting of 16 neurologists held in Florida about the suboptimal responders' identification question were to consider suboptimal responders as those patients who document one or more relapses, or an increased relapse rate or a sustained progression during a period of 6 months to 1 year after initiation of a disease modifying agent. There was less consensus among the panel members on the use of MRI markers to monitor therapeutic response (14).

Then, clinical response in MS can be evaluated by relapse rate or by measuring variations in disability progression. We chose to take into account both aspects of the disease, to have a more comprehensive and clinically relevant measure of the impact of a given therapy.

Importantly, to make our criteria for response to treatment clinically relevant we based them on a deviation from the reported natural history of the MS. The reported natural history of MS, in international series of patients (15) and in our own series (16), sets a mean annual relapse rate of one relapse per year and a mean progression index of 0.5 points per year. Taking this into account, the cut-off point for a suboptimal response in the non-responders was fixed at an increase after 1 year of IFN-β treatment, compared with the year before treatment, of one or more relapses or progression of 0.5 or more points on the EDSS score. Furthermore, as we are aware of the inter-observer variability for the EDSS scale, particularly for changes of 0.5 points, we attempted to minimize this effect by having the same neurologist undertake the clinical evaluations of each patient.

Although some clinical characteristics of MS, such as female sex and younger age at onset, are

possibly related to disease severity and may also be involved in the response to IFN-β therapy, we found no association between the clinical characteristics of the MS patients and their HLA distribution. In another study of the clinical response of MS patients to IFN-β therapy (17), a different clinical profile was proposed as a marker of IFN-β response, based exclusively on the relapse rate. Responders were defined as having a lower relapse rate on IFN-β compared with the year or 2 years prior to IFN-β therapy. The authors concluded that a higher relapse rate in the year prior to IFN-β treatment in the RR patients, and a higher Disability Status Scale (DSS) score at initiation of therapy in the SP group were associated with a better response to IFN-β therapy. However, this study included no data on HLA genetic background.

Few studies have addressed the question of whether the HLA could influence the clinical response to immunological therapies in MS. As part of a study of the relationship between HLA-DRB1*1501 and the response of RRMS patients to copolymer-1 and IFN-β, 22 of 39 (56.4%) of the IFN-β-treated RR-MS patients were classified as responders (18). The evaluation criteria in that study were clinical: patients with one or more moderate or severe relapses and/or an increase in the EDSS of at least one point were classified as non-responders. No difference was observed in the DRB1*1501 distribution between responders and non-responders to IFN-β. At present, no data have been reported on HLA alleles other than DRB1*1501 individually or the haplotype DR2 (DRB1*1501, DQA1*0102, DQB1*0602). Our results confirm the lack of an association between DRB1*1501 and the response to IFN-β treatment, and extend this finding to the other HLA class II alleles that we studied (DRB1*, DQA1*, DQB1*).

In conclusion, HLA class II did not determine the response to IFN-β, whether in direct or indirect association with the clinical characteristics.

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