HLA antigens in idiopathic dilated cardiomyopathy

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SUMMARY Disturbances of humoral and cellular immunity are common in patients with idiopathic dilated cardiomyopathy and they may contribute to the initiation and maintenance of myocardial damage. HLA antigens were studied in 102 patients with dilated cardiomyopathy and a control hospital population. HLA-DR4 was significantly more common in patients with idiopathic cardiomyopathy (41 patients, 40%) than in the control group (123 patients, 24%). The distribution of other antigens was not significantly different in the two groups. The distribution of blood group antigens, immunoglobulin concentrations, and disease severity was similar in patients with the HLA-DR4 antigen and those without it.

These results suggest that HLA-DR4 antigen may be a genetic marker for susceptibility to dilated cardiomyopathy.

Idiopathic dilated cardiomyopathy is a clinical entity of unknown and probably heterogeneous aetiology. In the search for pathogenetic mechanisms, considerable attention has been focused on the possibility that disturbed immunity may be important.¹⁻⁹ Several abnormalities in cellular¹⁻⁵ and humoral⁶⁻⁹ immunity have already been described in patients with idiopathic dilated cardiomyopathy and are thought to be triggered by an initial episode of myocardial damage (for example after viral infection) that sets the stage for an autoimmune response. Development of autoimmunity is the summation of diverse genetic traits that give rise to a genetic predisposition expressed both as an increased susceptibility to infectious (for example viral) agents and to organ specific autoimmune reactions.¹⁰

In most reported cases a predisposition to autoimmune disease is under the control of immune response genes^{10,11} and as a result autoimmune disorders show preferential associations with the products of immunoglobulin, complement, or HLA genes. For example, there is a significant increase in the frequency of HLA-B27 antigens in ankylosing spondylitis, HLA-DR3/DR4 in juvenile diabetes mellitus, and HLA-DR3 in myasthenia gravis (for a review, see Tiwari and Terasaki¹²). Furthermore, expression of class II HLA genes is thought to be of overriding importance in the presentation of self-

antigens that leads to the pathological manifestations of organ-specific autoimmunity.¹³ Such expression may be induced in response to viral infection or as a result of the initial tissue damage induced by the putative initiating agent. To examine the possible role of immune response factors in the pathogenesis of idiopathic dilated cardiomyopathy, we compared the frequency of histocompatibility antigens A, B, C, and DR with that expected in a control population.

Patients and methods

We studied 102 white patients (aged 16-65, mean (SD) 43.8 (10)) with idiopathic dilated cardiomyopathy; none was alcoholic. There were 18 (17.6%) women. About 90% of these patients were of Northern European (predominantly Scandinavian) origin. Cardiac symptoms had been present for 3.51 (1.0) years. There were no diseases known to be associated with specific HLA antigens (such as diabetes mellitus, ankylosing spondylitis, Graves' disease, myasthenia gravis, or rheumatoid arthritis). The severity of myocardial dysfunction in these patients was indicated by a low ejection fraction (20.5 (4%)), raised pulmonary capillary wedge pressure (26.8 (5) mm Hg), low cardiac index (2.1 (0.2) l/min/m²), and high concentrations of plasma noradrenaline 3.4 (0.4) nmol/l. We selected controls for this patient group from a local hospital population. Six hundred and seventeen individuals were typed for HLA A, B, and C loci and 511 for HLA-DR loci (mean age 48 (9) years and 316 (28%) were women).

Lymphocytes for HLA antigen typing were

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isolated from 20 ml heparinised blood by Ficoll-Hypaque (Pharmacia) density gradient centrifugation.¹⁴ Purified B lymphocytes for HLA-DR typing were prepared by the method of Danilovs *et al.*¹⁵ Histocompatibility antigens A, B, and C were assayed on lymphocyte suspensions by the microcytotoxicity method¹⁶ while HLA-DR typing was performed in microlymphocytotoxicity assays with prolonged incubation times on isolated B lymphocytes.¹⁷ In two patients, only Class I HLA antigens were tested. The statistical evaluation of the results followed the methods of Svejgaard *et al.*¹⁸ Chi squared 2×2 contingency tables were used for comparisons between patient and control groups. Associations based on small numbers were tested by Fisher's exact test. *p* values were corrected for the number of antigens tested and associations were regarded as significant if $p < 0.05$. Relative risk and aetiological factor were calculated as previously described.¹⁸

Results

Tables 1–4 give the HLA antigen frequencies for patients with cardiomyopathy and controls. The

Table 1 HLA-A antigen frequencies in patients with idiopathic dilated cardiomyopathy (IDC) and in controls

HLA type	IDC (n = 102)		Controls (n = 617)	
	n	%	n	%
A1	37	36.3	169	27.4
A2	57	55.9	304	49.3
A3	28	27.4	154	25.0
A11	9	8.8	71	11.5
Aw24	18	17.6	120	19.4
A26	6	5.9	50	8.1
A28	10	9.8	51	8.3
A29	4	3.9	27	4.4
Aw30	8	7.8	28	4.5
Aw32	5	4.9	48	7.8

None of the *p*_c values was significant.

Table 2 HLA-B antigen frequencies in patients with idiopathic dilated cardiomyopathy (IDC) and controls

HLA type	IDC (n = 102)		Controls (n = 617)	
	n	%	n	%
B7	24	23.5	153	24.8
B8	20	19.6	121	19.6
B13	7	6.9	24	3.9
B14	6	5.9	49	7.9
B27	11	10.8	47	7.6
Bw35	7	6.9	101	16.4
Bw39	4	3.9	26	4.2
Bw44	36	35.3	143	23.2
Bw51	8	7.8	61	9.9
Bw62	17	16.7	67	10.9

None of the *p*_c values was significant.

Table 3 HLA-C antigen frequencies in patients with idiopathic dilated cardiomyopathy (IDC) and controls

HLA type	IDC (n = 102)		Controls (n = 617)	
	n	%	n	%
Cw1	3	2.9	46	7.5
Cw2	7	6.9	60	9.7
Cw3	19	18.6	124	20.1
Cw4	13	13.0	136	22.1
Cw5	7	7.0	72	11.7

None of the *p*_c values was significant.

Table 4 HLA-DR antigen frequencies in patients with idiopathic dilated cardiomyopathy (IDC) and controls

HLA type	IDC (n = 102)		Controls (n = 511)	
	n	%	n	%
DR1	17	17.0	102	20.0
DR2	23	24.0	161	31.5
DR3	31	30.0	118	23.1
DR4	41	40.0*	123	24.0*
DR5	20	20.0	94	18.4
DRw6	22	22.0	102	20.0
DR7	18	18.0	115	22.5
DRw8	4	4.0	17	3.4
DRw9	2	2.0	10	2.0

**p*_c = 0.001.

frequencies of HLA-A and HLA-C were similar in the two populations and indistinguishable from the expected frequencies in North American populations. Among the HLA-B group, only HLA-Bw44 was slightly overrepresented in the cardiomyopathy group (35.3% compared with 23.2% in the controls, *p* = 0.05). The difference, however, was not statistically significant after correction for the number of antigens tested (*p*_c = 0.5). On the other hand, HLA-DR4 was found in 41 (40%) of the cardiomyopathy patients compared with 123 (24%) of the controls (*p* = 0.001). The latter association represented a risk association of 2.20 and an aetiological factor of 0.25. There was no clinical or haemodynamic characteristic that distinguished the HLA-DR4 positive patients from the HLA-DR4 negative patients (table 5). Similarly, the distribution of ABO blood groups was not significantly different in the patients (52% were blood group A, 35% were blood group O, and 13% were blood group B). The distribution of ABO blood groups among patients who were HLA-DR4 positive and those who were negative patients was similar. Concentrations of serum immunoglobulins were normally distributed (IgG 9.63 (1.40) g/l, IgA 2.36 (0.43) g/l, IgM 1.06 (0.19) g/l, IgE 193.92 (36.0) g/l).

Table 5 Clinical characteristics (mean (SD)) of patients with idiopathic dilated cardiomyopathy

	HLA-DR4 (+) (n = 40)	HLA-DR4 (-) (n = 62)
Age (yr)	46 (7)	47 (8)
Sex (F/M)	8/40	10/62
Duration of symptoms (yr)	3.3 (0.4)	3.4 (0.7)
Ejection fraction (%)	21.0 (1.5)	18.2 (1.6)
Pulmonary capillary wedge pressure (mm Hg)	28.1 (2.1)	27.3 (2.9)
Cardiac index (l/min/m ²)	2.06 (0.20)	1.98 (0.16)
Plasma noradrenaline (nmol/l)	3.53 (0.48)	2.96 (0.49)

Discussion

There is considerable evidence for immunological dysfunction in patients with dilated cardiomyopathy, and abnormalities of both cellular and humoral immunity have been reported. Das *et al*, for example, showed that a large proportion of cardiomyopathy patients had impaired lymphocyte responses to mitogens.⁸ In Chagas's disease, cytotoxic T lymphocytes are directed against both the parasite, *Trypanosoma cruzi*, and heart muscle cells.² Jacobs *et al* found cell mediated cytotoxicity against cultured myocardial cells in 30% of patients with cardiomyopathy, 24% of those with other forms of heart disease, and 4% of healthy individuals.³ An abnormality in suppressor T cell function in patients with dilated cardiomyopathy has been suggested⁴ but does not seem to be a uniform finding.⁵ Although subgroups of cardiomyopathy patients seem to have abnormalities in lymphocyte function, the relation to the pathogenesis of the disease and clinical severity has not been easy to establish.¹⁹

Abnormalities in humoral immunity have also been implicated in the pathogenesis of dilated cardiomyopathy. Anti-heart antibodies were detected by immunofluorescence^{6,8} or the antiglobulin consumption test⁷ and the frequency was correlated with the severity of heart failure and the duration of the disease. Circulating antibodies against specific antigenic determinants, such as sarcolemmal proteins,⁶ the adenine nucleotide translocator,⁹ and the β adrenoceptor²⁰ were also reported. Although these findings strongly suggest that immunological responses are important in dilated cardiomyopathy, they do not elucidate the relation of these abnormalities to the initiation and maintenance of myocardial dysfunction.

The possible contribution of genetic factors to the immunologically-associated damage in cardiomyopathy has not received adequate attention. It is known, however, that genetic factors have a role in the pathogenesis of dilated cardiomyopathy, as shown by the cases of familial cardiomyopathy,²¹ for

which both recessive and dominant modes of inheritance have been described.²² It is not known, however, how the genetic traits are expressed as myocardial dysfunction in cardiomyopathy. Although, in some cases, metabolic defects of the cardiac myocyte have been implicated, in others, an enhanced susceptibility to environmental agents or to immunological responses to them may be at fault. An immunological component was shown for the progression to chronic cardiac disease in experimental and human viral myocarditis.^{23,24} It is widely assumed that many cases of dilated cardiomyopathy are the end result of a preceding viral infection the serological evidence for which has long since disappeared. It is suggested that the initial virus induced myocardial damage sets up an immunological reaction against intracellular constituents which further sustains and aggravates myocardial dysfunction. Immunological mechanisms would thus have a secondary role and contribute to a variable extent to the progression of the myopathic process. It is also possible, however, that genetically controlled immunological mechanisms participate more directly in the initiation of myocardial damage either through control of the susceptibility to the aetiological viral infection, or through modulation of the immunological response to the putative viral agents. There is precedent for both mechanisms: for example, it is known that histocompatibility antigens determine the cardiotoxicity of Coxsackie B viruses²⁴ or their interaction with specific tissues. Also, experimentally induced myocarditis induced in mice through induction of antimyosin antibodies is largely determined by the H-2 locus.²⁵

This suggested that it would be useful to look for immunologically associated genetic markers of dilated cardiomyopathy in humans. The HLA antigens were selected because of their known participation in the regulation of immune responses and the previously reported association between specific haplotypes and autoimmune diseases. The results of our study suggest that the frequency of HLA-DR4 was significantly higher in patients with dilated cardiomyopathy than in controls. This accords with the results of a smaller series reported by Anderson *et al*.²⁶ On the other hand, the increase in HLA-B27 and the underrepresentation of HLA-DRw6 reported by Anderson *et al* were not confirmed in our series. In a series of patients with ischaemic cardiomyopathy, a preponderance of HLA-DRw6 antigen was found,²⁷ whereas in patients with alcoholic cardiomyopathy there was no association with any particular HLA haplotype. Also there is some evidence that HLA-DR3 is the antigen linked to hypertrophic cardiomyopathy.²⁸ This would indicate a certain degree of specificity in the association of different types of

cardiomyopathy with immune genetic markers.

The calculated aetiological factor of 0.25 in our series probably underestimates the significance of the association with HLA-DR4 because of the heterogeneity of the pathological processes involved in dilated cardiomyopathy. For example, in a continuing study we found that the prevalence of auto-antibodies against cardiac β adrenoreceptors is almost 70% in HLA-DR4 positive patients with cardiomyopathy compared with only 25% in HLA-DR4 negative patients.²⁹ It is likely, therefore, that the increase in certain HLA antigens is linked to specific immunological disturbances, such as anti-receptor (or other autoantibody) production. Subsets of cardiomyopathy may then join the list of auto-immune diseases associated with anti-receptor antibodies. This has important implications for the classification and the pathophysiology of dilated cardiomyopathy as well as for new treatments. Support for such a pathogenetic role of class II HLA antigens may come from studies of their expression in the diseased myocardium. These antigens are not normally expressed in the heart³⁰ but may be abnormally expressed in pathological conditions such as rheumatic carditis³¹ and cardiac transplant rejection.³⁰ It is not known whether myocardiopathic tissue expresses these antigens and it is important to find out if it does.

We found no correlation in this study between the presence of HLA-DR4 and the severity or duration of the disease. This may not be unexpected if the association is a marker for susceptibility to the disease but does not influence its progression or severity—a situation that is common to autoimmune diseases. On the other hand, if HLA-DR4 is a marker for a specific pathogenetic pathway (that is anti-receptor autoantibodies), there may be a correlation with disease severity within this subset of cardiomyopathy patients. The design of our study, which includes patients with severe haemodynamic impairment does not allow us unequivocally to exclude the possibility that HLA-DR4 positive patients have more severe disease or more rapid course. A prospective study of a population with a wider range of haemodynamic abnormalities is currently underway and should help resolve this issue.

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