Idiopathic dilated cardiomyopathy: lack of association between circulating organ-specific cardiac antibodies and HLA-DR antigens

A. L. P. Caforio, M. Martinetti, G. Schwarz, E. Bonifacio, A. Gavazzi, G. Graziano, R. Lorini, M. Cuccia, W. J. McKenna, G. F. Bottazzo. Idiopathic dilated cardiomyopathy: lack of association between circulating organ-specific cardiac antibodies and HLA-DR antigens. Tissue Antigens 1992: 39: 236–240.

Abstract: Organ-specific cardiac antibodies are serological markers of autoimmunity in dilated cardiomyopathy (DCM). HLA-DR4 and possibly DR5 are immunogenetic markers of susceptibility in DCM, but it is not known whether they are associated with autoantibody production. We studied the frequency of HLA-DR antigens and the presence of organ-specific cardiac antibodies in 80 DCM Caucasian patients from Northern Italy. HLA-DR typing was performed by serology; 289 healthy blood donors from the same region were tested as controls. HLA-DR frequencies in DCM were also compared with VIII International Workshop control data for Italy. Cardiac antibodies were detected by indirect immunofluorescence on human heart. Skeletal muscle was used to identify cross-reacting antibodies. The prevalence of cardiac antibodies in DCM was: organ-specific 34% and skeletal muscle cross-reactive 30%. The previously reported positive association between DCM and HLA-DR4 was confirmed using either the controls from the same region (21.25% vs 10.73% p = 0.02, relative risk = 2.30) or from all of Italy (21.25% vs 12.3%, p = 0.03). HLA-DR5 frequency was slightly but not significantly higher in DCM than in controls from the same region (46.25% vs 31.49% p = 0.02, relative risk of 1.87, p corrected = NS) or from all of Italy (46.25% vs 35.8% p = NS). HLA-DR3 frequency was lower in DCM than in controls from the same region (12.50% vs 29.41% p = 0.003, relative risk of 0.36, p corrected = 0.03). This negative association was not confirmed using the control data from the whole of Italy (12.50% vs 16.5% p = NS). No significant association was found between HLA-DR4, DR5 and/or DR3 and cardiac antibody status, age, sex, symptom duration. The lack of association between HLA-DR and the presence of cardiac antibodies may be due to disease heterogeneity and/or reduction of antibody levels with disease progression, which are well-recognised features of organ-specific autoimmune conditions.

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Key words: autoantibody – autoimmunity – dilated cardiomyopathy – HLA antigens

Received 30 May, revised, accepted for publication 15 November 1991

Introduction

Organ-specific cardiac antibodies are novel serological markers of autoimmunity in dilated cardiomyopathy (DCM) and have been recently reported in a patient population from England (1). The association with particular HLA antigens is a common feature of diseases in which an autoimmune pathogenesis is involved (2). These antigens are immunogenetic markers of disease susceptibility, although the etiologic mechanisms through which the presence of a particular HLA specificity confers increased risk for the development of the disease are not known. The class II antigens (DR, DP, DQ) play a critical role in antigen presentation and thymic deletion of autoreactive T-cell clones (3). Thus, it is possible that autoantibody production may occur in conjunction with overrepresentation of one or a few HLA-DR specificities.

A positive association between DCM and HLA-DR4 has been described in patients from the USA (4–5), and from Russia (6). We have recently reported a positive association with HLA-DR4 and DR5 antigens and a negative association with HLA-DR3 in Caucasian patients with DCM from Northern Italy (7). It is not known whether these HLA markers of disease susceptibility are associated with autoantibody production. In the present

study we have performed HLA typing and cardiac autoantibody screening in 80 patients from Northern Italy, 50 of whom had already been reported for the HLA association only (7), in order 1) to determine the prevalence of cardiac autoantibodies in this partient population; 2) to relate clinical features and presence of organ-specific cardiac antibody to the HLA markers (DR4 and possibly DR5 and DR3).

Material and methods

Subjects

Eighty Caucasian patients (65 male and 15 female, mean age of 43 ± 10 years) from the Cardiology Clinics of the S. Matteo Hospital (Pavia, Italy) were identified as having DCM. The diagnosis of idiopathic dilated cardiomyopathy was based on the demonstration of a dilated and poorly contracting left or right ventricle, or both, in the absence of any known etiologic factor for myocardial injury according to the World Health Organization criteria (8). In particular, patients were excluded if there was evidence of 1) coronary heart disease at selective coronary arteriography, 2) systemic blood pressure ≥ 150/90, 3) concomitant systemic or endocrine diseases that are known to cause left ventricular impairment, 4) pregnancy, 5) inflammatory myocarditis at endomyocardial biopsy, 6) excessive alcohol consumption, defined as a daily intake of at least 8 oz (0.24 liter) of hard liquor or 2 qt (1.9 liters) of beer for the preceding 10 yr (9). In all patients a detailed personal history for cardiovascular disease was obtained; age at disease onset and mean duration of cardiac symptoms at the time of diagnosis were clinically ascertained. All patients underwent right and left heart catheterization, selective coronary arteriography, echocardiography, right and/or left ventricular endomyocardial biopsy. Five patients were in New York Heart Association functional class I, 25 in class II, 48 in III and 2 in IV. Symptom duration ranged from 1 to 120 months, with a mean (± standard deviation) of 33 ± 34 months and a median of 12 months. All patients were HLA-DR typed.

The control group used for calculating normal frequencies of HLA alleles consisted of 289 healthy blood donors residing in Pavia area. Although the majority of DCM patients were from this area, some were from other regions of Italy, thus the HLA frequencies were also compared with VIII International Workshop control data for Italy.

HLA typing

HLA typing was performed on peripheral blood lymphocytes (PBL) isolated from whole heparinized blood by an isopaque ficoll density gradient technique (10). The B lymphocytes were separated from the PBL suspension by adherence to a nylon wool column (11–12). DR typing was performed in the standard microlymphocytotoxicity assay using adherent B lymphocytes freed from the column by agitation (2). HLA typing of patients and controls was performed in the HLA laboratory, AVIS, Pavia, Italy.

Cardiac autoantibody screening

Cardiac antibody screening was performed in the Dept of Immunology, London Hospital Medical College, London, U.K. Sera were transported from Italy in dry ice, and were tested by indirect immunofluorescence on 4 μm-thick unfixed fresh frozen cryostat sections of blood group O normal human atrium, ventricle and skeletal muscle, as previously described (1). Briefly, sera were diluted 1/10 in pH 7.2 phosphate-buffered saline and fluorescein isothyocyanate-labelled (FITC) sheep anti-human immunoglobulin (Ig) (Wellcome Lab) at 1/60 dilution was used to assess Ig binding. Control for non-specific fluorescein staining, obtained through omission of the patient serum on one tissue section, was included in every assay. As internal control for assay sensitivity two sera were chosen as standard positive and negative controls and titrated in every assay. All test sera were read against these standards by 2 observers who were blind to clinical and HLA data. The intensity of IFL of the positive standard at 1/40 dilution was used as the cut-off point for positivity. Ig classes of cardiac antibodies were determined using FITC-sheep anti-human IgG, IgM and IgA class-specific antisera (Nordic Laboratories).

Statistical analysis

The significance of the deviation of HLA-DR3, DR4 and DR5 antigen frequencies was calculated by Chi square test and relative risk values were determined using Woolf's formula (2, 7). The association between DCM and HLA-DR4 has been reported by other groups (4–6), thus no correction for multiple comparisons was used (2). The association with HLA-DR3 and DR5 was reported by our group (7). Since 50 of the 80 patients in the current study were also included in our previous report, the statistical analyses for DR3 and DR5 were corrected for multiple comparisons.

The association of HLA-DR with age and with symptom duration in the DCM patients was evaluated by one factor analysis of variance; the association with sex, New York Heart Association class and cardiac autoantibody status was assessed by Chi square test or Fisher's exact test. Confidence

Table 1.
Clinical features of dilated cardiomyopathy patients with and without DR3, DR4 or DR5

	Male/female ratio	Mean age (years)	New York Heart Association Class (I–II/III–IV)	Mean symptom duration (months)
DR3 +ve (n=10)	10/0	45±10	4/6	28±37
DR3 −ve (n=70)	55/15	43±11	26/44	35±33
DR4 +ve (n=17)	12/5	44+10	6/11	40±38
DR4 −ve (n=63)	53/10	43+11	24/39	32±32
DR5 +ve (n=37)	30/7	43±10	14/23	29±29
DR5 −ve (n=43)	35/8	44±11	16/27	37±37
DR4/5 +ve (n=8)	4/4	44±10	3/5	39±43
Not DR4/5 +ve (n=72)*	61/11	43±11	27/45	33±33

+ve: positive; −ve: negative.
* Not DR4/5 includes: DR5 −ve/DR4 −ve (n=34); DR5 −ve/DR4 +ve (n=9); DR5 +ve/DR4 −ve (n=29).

intervals for difference in proportions of antibody-positive and -negative patients among each HLA-DR specificity were calculated where appropriate (13). The association of HLA-DR with cardiac autoantibody status was also separately assessed in patients with shorter (≤12 months) and longer (>12 months) symptom duration by Chi square test or Fisher's exact test.

Results

Prevalence of cardiac autoantibody in DCM patients

Cardiac antibodies gave three staining patterns which have been demonstrated previously (5) and were classified as follows: 1) "organ-specific" antibodies which gave diffuse cytoplasmic staining of both atrial and ventricular myocytes but were negative on skeletal muscle; 2) "cross-reactive 1" antibodies which gave fine striational fluorescence on cardiac tissue, but weakly stained skeletal muscle sections; 3) "cross-reactive 2" antibodies that showed a broad striational pattern on longitudinal sections of heart and skeletal muscle. The cardiac specificity of the cytoplasmic immunofluorescence has been previoulsy confirmed by absorption studies with relevant tissues (1).

"Organ-specific" cardiac antibodies of IgG class were detected in 27/80 DCM patients (33.75%), cross-reactive 1 in 17/80 (21.25%) and "cross-reactive" 2 in 7/80 (8.75%). The prevalence of "organ-specific" cardiac antibodies in these patients from Italy was similar to that reported in patients from England (17/65, 26%) (1). These antibodies are found in only 3.5% of a healthy control population and are virtually absent in patients with other cardiac disease or with ischemic heart failure (1).

HLA-DR association

The previously reported association between DCM and HLA-DR4 was confirmed using either the controls from the same region (21.25% vs 10.73% p=0.02, relative risk=2.30) or from the whole of Italy (21.25% vs 12.3%, p=0.03). HLA-DR5 frequency was slightly but not significantly higher in DCM than in controls from the same region (46.25% vs 31.49% p=0.02, relative risk of 1.87, pc=NS) or from all of Italy (46.25% vs 35.8% p=NS). HLA-DR3 frequency was lower in DCM than in controls from the same region (12.50% vs 29.41% p=0.003, relative risk of 0.36, pc=0.03). This negative association was not confirmed using the control data from all of Italy (12.50% vs 16.5% p=NS).

Relation between clinical and immunological features and the HLA markers of susceptibility

Age, sex ratio, proportions of patients in New York Heart Association class I/II vs class III/IV and

Table 2.
Cardiac antibody status in dilated cardiomyopathy patients with and without DR3, DR4 or DR5

	Organ-specific or cross-reactive cardiac antibody:		Organ-specific antibody:	
	+ve n (%)	−ve n (%)	+ve n (%)	−ve n (%)
DR3 +ve (n=10)	8 (80)	2 (20)	3 (30)	7 (70)
DR3 −ve (n=70)	43 (61)	27 (39)	24 (34)	46 (66)
DR4 +ve (n=17)	12 (71)	5 (29)	9 (53)	8 (47)
DR4 −ve (n=63)	39 (62)	24 (38)	18 (28)	45 (72)
DR5 +ve (n=37)	19 (51)	18 (48)	10 (27)	27 (73)
DR5 −ve (n=43)	32 (74)	11 (25)	17 (39)	26 (61)
DR4/5 +ve (n=8)	5 (62)	3 (38)	3 (37)	5 (63)
Not DR4/5 +ve (n=72)*	46 (64)	26 (36)	24 (33)	48 (67)

+ve: positive; −ve: negative.
* Not DR4/5 includes: DR5 −ve/DR4 −ve (n=34); DR5 −ve/DR4 +ve (n=9); DR5 +ve/DR4 −ve (n=29).

mean symptom duration were similar in patients with and without DR3, DR4,or DR5, as well as in patients with and without DR4 and DR5 (Table 1).

The prevalence of cardiac antibodies of all types and of the "organ-specific" type was similar in patients with and without DR3, DR4 or DR5, as well as in patients with and without DR4 and DR5 (Table 2). Since there was an apparent trend towards a positive association between HLA-DR4 and "organ-specific" antibody ($p=0.059$) the 95% confidence interval was calculated and ranged from −1.6% to 37.8%.

No significant association was found between the presence of DR3, DR4 or DR5 antigens and cardiac antibody status among patients with shorter (≤ 12 months) and in those with longer (> 12 months) symptom duration.

Discussion

This report shows that HLA-DR4 and possibly DR5 are markers of genetic susceptibility in DCM patients from Northern Italy. These resuts were similar using either our control data or VIII International Workshop data for the whole of Italy. The frequency of DR3 was significantly lower in DCM compared with our controls; the negative association was not seen using the DR3 control frequency from Italy. This discrepancy might reflect the fact that, although the majority of patients were from the same region of Italy as our controls, some were from other regions. It is well known that different regions of Italy may have different HLA antigen frequencies due to founder effects and to the relatively geographically stable populations of this country. Independent confirmation of the HLA-DR3 association is warranted.

HLA-DR4 and DR5 were not associated with age, symptom duration or with the cardiac antibodies detected by indirect immunofluorescence. There was an apparent trend towards a positive association between HLA-DR4 and "organ-specific" antibody, but it is unlikely that this represents a real association, because many tests were performed and none reached the 5% level of significance. Even taking the upper 95% boundary for the difference in prevalence the association of HLA-DR4 with "organ-specific" antibody is hardly striking. Furthermore, when the antibody data were analyzed in patients with shorter and longer symptom duration and related to the presence of the HLA markers of susceptibility, again no significant association was found.

Our negative findings are in agreement with those reported so far in the majority of other autoimmune diseases (2). A possible explanation for the lack of association would be that the HLA-DR antigens do not influence antibody production. However, circulating autoantibodies to different cardiac antigens have been reported in DCM, using immunofluorescence (e.g. cytoplasmic and sarcolemmal antigens) (1, 14). ELISA (e.g. M7 mitochondrial antigen, adenine nucleotide translocator) (15–16), solid-phase enzyme immunoassay (e.g. myelin major protein) (17), ligand binding inhibition and immunoblotting (e.g. beta-1 adrenoreceptor) (18–19) and it has been suggested that the antibodies to the beta-1 adrenoreceptor are associated with HLA-DR4 (20). The cytoplasmic autoantigen(s) recognised by the organ-specific cardiac antibodies detected by immunofluorescence remain to be identified (1). It may be that different antibody specificities are associated with distinct HLA antigens. It will be of interest to search for additional immunogenetic markers such as HLA-DQ antigens (21) or immunoglobulin (Gm) (22) allotypes and to look for potential associations between such markers and the organ-specific cardiac antibodies detected by immunofluorescence.

An alternative explanation for the lack of association between these antibodies and the HLA-DR markers would be that cardiac antibody levels become undetectable with disease progression, similarly to islet cell antibodies in Type 1 insulin-dependent diabetes mellitus (1, 21); in this case the cross-sectional design of our study may have led to these negative results. Clinical and serological follow-up studies in DCM patients with recent onset of heart dysfunction are currently in progress and should clarify this important issue.

DCM is probably a heterogeneous disease (23) and this might also contribute to the lack of association between HLA antigens and clinical as well as immunological features (21). Although studies of HLA association in patient populations of Northern European Caucasian origin – in whom, traditionally, the association of autoimmune diseases with HLA antigens is stronger (2) – have not been performed, the fact that the reported relative risk values for both DR4 (4–7), and DR5 associations (7) were rather low, ranging from 2.08 to 2.96 lends some support to this possibility.

Acknowledgments

ALPC is a British Heart Foundation Research Fellow and EB is supported by the Juvenile Diabetes Foundation International (JDF-I). We are grateful to Dr J. Poloniecki for help in the statistical analysis and to Mrs C. Pizzochero for technical assistance.

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