HLA Alleles in Anti-Endothelial Cell Antibody Positive Indian SLE Patients

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ABSTRACT Anti-endothelial cell antibodies (AECA) are heterogeneous group of antibodies against a variety of antigenic determinants of endothelial cells (EC). AECA plays an immunopathogenic role in triggering EC activation leading to vascular damage. The presence and the strength of AECA have been found to correlate with disease activity in various systemic vasculitic diseases like systemic lupus erythematosus. To assess the involvement of HLA alleles in AECA production, 45 clinically and histopathologically proven cases of class IV lupus nephritis were studied for their HLA A and HLA B alleles by standard NIH microlymphocytotoxicity assay. All patients fulfilled ARA classification criteria for SLE. AECA were detected by indirect immunofluorescence using cultured human umbilical vein endothelial cells (HUVEC). Forty percent of the SLE patients possessed the AECA antibodies. The HLA alleles A9 (24) (OR=2.90, EF=0.29,p value 0.08) and B21 (OR=74, EF= 0.11, p value 0.038) were significantly increased while HLA A1 (OR=0.27, PF= 0.35, p value 0.039) and B40 (OR= 0.29, PF= 0.25, p value 0.076) were significantly reduced among AECA positive SLE patients when compared with AECA Negative patients. Further two-locus haplotype analysis revealed that A19-B35, A3-B21, and A28-B21 were observed with significant T value among AECA positive patients. The common clinical symptoms among the AECA positive patients observed were lupus nephritis (84%), involvement of skin (22%), involvement of joints (17%) and CNS as well as hematological involvement (11%). Our findings suggest that immunogenetic mechanism may be involved in AECA antibody production leading to the immunopathogeneis in a subset of SLE patients.

INTRODUCTION

Circulating autoantibodies that bind to human endothelial cells cultured in vitro have been detected in a variety of autoimmune diseases (Moroni et al. 1996). Anti-endothelial cell antibodies (AECA) are a heterogeneous group of antibodies directed against a variety of antigenic determinants of endothelial cells (ECs) in these autoimmune disorders, which share a varying degree of vessel wall damage. Some of the target autoantigens of AECA are yet to be ascertained. The mechanism through which AECA potentiates EC activates apoptosis and vessel wall damage is to be explored (Moroni et al. 1996). Further reports (Meroni et al. 2005; Constans et al. 2003), show increasing evidence that AECA might have pathogenic role in

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inducing autoimmune vascular disease. AECA has been found to be associated with various autoimmune disorders mainly in the active stage of diseases like systemic lupus erythematosus (SLE), scleroderma, Wegener's granulomatosus (WG), mixed connective tissue disorders (MCTD), Sjogren's syndrome, rheumatoid arthritis (RA), systemic vasculitis, lupus nephritis (LN), Kawasaki disease, progressive systemic sclerosis (PSS) etc (Meroni et al. 2005). Therefore the measurement of AECA may improve understanding the etiopathogenetsis of various autoimmune disorders.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of a broad spectrum of autoantibodies and it has been suggested that several autoantibody specificites play an important role in the pathogenesis of this disease. The proportion of AECA positivity ranges from 16% to 83 % in SLE (D' Cruz et al. 1991) In patients with acute lupus nephritis AECA was found to be associated with histological evidence of active renal injury leading to endothelial cell dysfunction supportive of pathogenic involvement in Lupus Nephritis (LN). Further 365 of patients with

LN had IgG-AECA correlating with high titer of anti-ds DNA antibodies (D' Cruz et al. 1999).

Earlier studies have shown that genetic factors are involved in the pathogenesis of SLE. Multiple loci within the major histocompatibility complex (MHC) have been implicated in SLE susceptibility. HLA Class II alleles complement components, tumor necrosis factor (TNF) loci, have been shown to be involved. HLA association studies from Indian SLE patients are considerably limited (Mehra et al. 1993). A significant increase in HLA A1, A2, B27, DRB1*03, DQB1*0302 and DQB1*0601 were observed among SLE patients from western India (Shankarkumar et al. 2003).

MATERIAL AND METHODS

A total of forty-five clinically and histo-pathologically proven SLE patients fulfilling the revised ARA classification were studied. The details of clinical, histopathological and laboratory findings were also recorded. The study was carried out after obtaining the requisite Ethics Committee permission. SLE disease activity index was assessed by the SLE disease activity index (SLEDAI) (Zhang et al. 1999; Westphal et al. 1994). All the patients were in active stage of disease and were untreated at the time of presentation. Patients had no history of taking any drugs like hydralazine, propylthiouracil etc. The HLA A and HLA B locus antigens were identified by a National Institute of Health two-stage microlymphocy-totoxicity assay using indigenous and commercial HLA antisera. The allele and haplotype frequencies, odds ratio, probability value and chi-square were estimated using our database and computer program. The p value was corrected by the Bonferoni inequality method.

Isolation and culture of endothelial cells (HUVEC) was done as follows. ECs were isolated from sterile human umbilical cords of blood group) positive foetus by collagenase (0.2% v/v) digestion of interior walls of umbilical veins by passing an umbilical catheter through it (Bombardier et al. 1992). The cells were grown in tissue culture medium IMDM (Sigma, USA), supplemented with 10% FCS (Gibco) supplemented with 2 ml glutamine, 100 U penicillin/ml and 100 mg of streptomycin/ml. The culture plates were incubated at 37^0C under 5% CO_2 atmosphere. The cells were supplemented with culture medium at two-day intervals and morphology was confirmed by phase contrast light microscopy showing the typical cobblestone monolayer appearance of endothelial cells. The confluent cells after second passage were used after giving two washes with PBS (0.01 M, pH 7.2) and adjusting the cell count to $1X10^6$ /ml. Homogenous population of actively growing ECs (>90% von Willibrand factor antigen positive) on second passage was obtained, which was detected by using anti-vWF monoclonal antibody (Dako, Denmark) by indirect immunofluorescence test (IIF).

Detection of AECA was performed by IIF test using HUVEC (Perry et al. 1993). HUVEC cells $(1X10^5)$ were diluted in PBS (0.01M, pH 7.2). The ECs were used to prepare a cytospun substrate using Hitachi Universal 16A cytocentrifuge and the test serum diluted 1: 20 was allowed to react for 45 minutes at 4^0C. Slides were further probed with FITC tagged polyvalent (IgG, IgM, IgA) anti-human globulin serum (Sigma, USA) using a fluorescent microscope (Nikon, Optiphot II) and microphotography was done using an automated photography system. (Nikon, AFX IIA, Japan). The positive results were interpreted in terms of titers i.e., test sera giving positivity at its highest dilution. Immunoglobulin subclass of AECA was identified in all AECA positive sera using FITC tagged IgG, IgM and IgA monovalent anti-human globulin serum. (Sigma, USA).

RESULTS

An increase in frequencies HLA A9 (24), A19 (31), B7, B15 (62), B21, B22, B27 were observed among the AECA positive patients (Table 1). Further a decrease in the frequencies of HLA A1, A3, A28, B5, B17, B40 were observed among the AECA positive patients. The HLA alleles A9 (24) (OR=2.90, EF=0.29,p value 0.08) and B21 (OR=74, EF= 0.11, p value 0.038) were significantly increased while HLA A1 (OR=0.27, PF= 0.35, p value 0.039) and B40 (OR= 0.29, PF= 0.25, p value 0.076) were significantly reduced among AECA positive SLE patients when compared with AECA Negative patients after Bonferoni's correction. Further two-locus haplotype analysis revealed that A19-B35, A3-B21, and A28-B21 were observed with significant T value among AECA positive patients. (Table 2) The common clinical symptoms among the AECA positive patients observed were lupus nephritis (84%), involve-ment of skin (22%), involvement of joints (17%)

Table:1 HLA association in AECA positive SLE patients from Mumbai, India

HLA	AECA +ve N = 18	AECA -ve N = 37	OR	Ki2	EF	PF	P value
	n (AF%)	n (AF%)					
A1	4 (11.11)	19 (25.67)	0.27	4.05		0.35	0.039**
A2	9 (25.00)	17 (22.97)	1.18	0.08			
A3	4 (11.11)	9 (12.16)	0.89	0.01		0.02	
A9	8 (22.22)	8 (10.81)	2.90	3.08	0.29		0.08**
A11	2 (5.55)	4 (5.40)	1.03	0.02			
A19	7 (19.44)	8 (10.81)	2.31	1.89	0.21		
A28	2 (5.55)	5 (6.75)	0.80	0.02		0.01	
A31	2 (5.55)	1 (1.35)	4.50	1.85	0.08		
B5	4 (11.11)	14 (18.91)	0.47	0.50		0.18	
B7	5 (13.88	4 (5.40)	3.17	3.03	0.18		
B12	4 (11.11)	4 (5.40)	2.54	1.26	0.13		
B15	1 (2.77)	0 (0.00)	37.00		0.05		
B17	2 (5.55)	7 (9.45)	0.54	0.62		0.06	
B18	1 (2.77)	1 (1.35)	2.12	2.09	0.03		
B21	2 (5.55)	0 (0.00)	74.00		0.11		0.038**
B22	3 (8.33)	3 (4.05)	2.27	2.23	0.09		
B27	3 (8.33)	3 (4.05)	2.27	2.23	0.08		
B35	6 (16.66)	9 (12.16)	1.56	1.56			
B40	3 (8.33)	15 (20.27)	0.29	0.33		0.25	0.076**
B53	1 (2.77)	2 (2.70)	1.03	1.22			
B55	1 (2.77)	1 (1.35)	2.12	2.09	0.03		
B56	1 (2.77)	1 (1.35)	2.12	2.09	0.03		
B62	1 (2.77)	0 (0.00)	37.00		0.05		

n=Number positive, Ki2=Chisquare with Yates correction, AF=(%) Allele frequency percentage, OR=Odds ratio
EF=Etiological fraction, PF=Preventive fraction, **Significant P value

Table 2: Significant Two-Locus Haplotypes identified among SLE patients.

Haplotypes	HF (%)	D (%)	Ki2	T val
AECA +ve				
A3-B21	5.72	5.04	11.09	2.19
A19-B27	8.71	6.81	8.44	2.80
A28-B21	5.72	5.39	26.59	2.19
A19-B35	10.89	6.88	4.22	2.36
AECA -ve				
A2-B17	5.92	3.73	4.46	2.43
A3-B21	2.78	2.43	9.77	2.08
A9-B40	6.76	3.94	3.92	2.11
A19-B27	4.71	3.67	8.64	2.49
A19-B35	6.96	4.40	5.52	2.39
A11-B53	2.78	2.62	25.41	2.09
A28-B21	2.78	2.60	22.12	2.09

HF (%) = Haplotype frequency in Percentage
LD (%) = Linkage disequlibrium in Percentage
Ki2 = Chi-square with Yates correction
T val = >2 is significant

and CNS as well as hematological involvement (11%).

DISCUSSION

Anti-endothelial cell antibodies (AECA) are a heterogeneous group of autoantibodies detected in number of systemic autoimmune diseases. Their occurrence has been well correlated with both disease activity and vessel wall inflammation. AECA directed to micro-vascular and macrovascular Endothelial cells (ECs) found to have a potential role in the development of vasculopathy or thrombosis in vascular injuries. AECA are also found to activate microvascular and macrovascular ECs via elevated expression of thrombomodulin, IL6 and adhesion molecules (P-selectin, E-selectin, VCAM-1), followed by enhanced monocytes activation to EC or induction of apoptosis (Ali et al. 1989; Hatfield et al. 1987).

The proportion of AECA positivity ranges from 16% to 85% in SLE[3]. Perry et al (1993), had reported 73% patients with LN in acute stage of disease with AECA positivity as compared with 44% incidence in SLE patients without evidence

of nephritis (Youinou et al. 1995). D' Cruz et al (1999), had reported 81 % AECA among LN patients and had found higher levels of AECA than those SLE cases without renal involvement[2]. Zhang et al (1999), had reported an incidence of 36.2% AECA among LN patients[5]. The over all incidence of AECA positivity was 38.4% with a slightly higher incidence of 39.6% among LN patients (Pradhan et al. 2002). Literature review shows that HLA allele involvements in AECA antibody production have been not very well understood and our findings could be the first attempt to explore the role of HLA in AECA antibody production. This is the first report to our knowledge in the role of AECA production in SLE associated with SLE.

Understanding how the HLA allele restriction involved in endothelial response to inflammation will give a better understanding of immuno-pathogenesis in vasculitis related disorders. In conclusion this study has demonstrated the immunogenetics has a role in the presence of AECA in SLE patients.

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