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HLA and Disease Susceptibility in Tamil Nadu, S. India

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Abstract

Results on HLA polymorphism in the populations of Tamil Nadu, South India are analysed for HLA association with various diseases like pulmonary tuberculosis, leprosy, psoriasis, rheumatoid arthritis, iridocyclitis and Eale's disearse. These studies revealed: i) association of psoriasis with HLA B17 and DR7 is highly significant in Vellala related group and this is attributed to founder effect - hitch hiking of a disease producing gene linked to these alleles , as the population migrated, ii) different castes differ from one another in their HLA allelic and haplotype polymorphism, iii) HLA DR2 predispose for more severe form of pulmonary tuberculosis which transcends ethnic barrier, the susceptibility presumably being due to a generalized MHC dependent immunogenetic and pathological mechanism, iv) HLA B27 and DR4 are associated with iridocyclitis, rheumatoid arthritis and low back pain; the susceptibility may be a HLA dependent molecular mimicry process, v) HLA alleles DR2 and DR4 predispose for severe forms of the respective diseases, though not for initial susceptibility.

Key words: HLA polymorphism, disease association, founder effect, molecular mimicry

Introduction

The population of India is comprised of many ethnically diverse groups essentially having migrated to this land over a long period of history. With her 3867 communities and 187 languages and dialects (Singh, 1993), with many histories of migrations and the resultant linguistic, socio-cultural and ethnic diversity, it is essential and urgent to study the epidemiology of various inherited disorders and diseases in this country. This is of paramount importance considering the load of infectious diseases such as tuberculosis, leprosy, filariasis and also overcrowding and poor environmental sanitations. The influence of genes contributing for a disease will be identified only in an endemic area. Further many autoimmune disorders are known to be a sequel of subtle infections, though indirectly. Thus studying the correlation of immune response genes (HLA) to a disease in an endemic area is the first approach to study the immunogenetic basis of disease susceptibility. Though many diseases have been studied for HLA association, many of them have been equivocal which can be attributed to study design and sampling

strategy: it is also possible that these diseases were studied in an area where the diseases were sporadic (Tiwari &Terasaki, 1985; Dausset and Colombani, 1972; Pitchappan, 1990). We have earlier studied different populations (castes) in Tamil Nadu (South India), for their HLA polymorphisms (Pitchappan et al. 1984; Rajasekar et al. 1987; Pitchappan et al. 1989: Brahmajothi et al 1991; Balakrishnan, 1993). The results on disease susceptibility from studies hither to carried out by us in the population of Tamil Nadu are presented here and its implications discussed.

Materials and Methods

Controls: Healthy volunteers, students and staff of our University and various colleges in Madurai were enrolled at random and HLA typed earlier. Appropriate matched controls were obtained from this data base for comparison with disease.

Patients: Patients with tuberculosis, rheumatoid arthritis, low back pain, iridocyclitis and psoriasis were sampled at Govt. Rajaji Hospital, Madurai (Number of samples presented in the tables). Patients with Eale's disease were obtained from Aravind Eye Hospital, Madurai. Respective attending physicians and the post graduate students working for their project work with us were responsible for the clinical diagnosis and sampling. Details on Nativity, caste group, health status etc., were obtained in a pre-coded questionnaire. Ten ml of peripheral blood was obtained by venipuncture through cubital vein using sterile disposable syringes and needles, defibrinated and tested the same day.

HLA typing: HLA typing was performed by serological methods employing two stage micro-lymphocytotoxicity assay (Terasaki and Mc Clelland, 1964) and either eosin or two color fluorescence method was employed to read the results (Van Rood et al; 1976). Lymphocytes were isoloated from the defibrinated blood samples on a Ficoll-Conray density solution (Boyum, 1968). HLA class II typing for HLA-DR and -DQ were performed on B lymphocytes isolated using a miniature nylon wool column and a long incubation period (Maniksundri et al 1984) . Standard HLA typing sera, two or three per each HLA-A,B and DR specificity, having an value >0.75 were used and the typing results were evaluated for quality as described earlier (Pitchappan & Arulraj, 1989).

Statistical Analysis: A computer data base for immunogeneic studies in Turbo-pascal developed in this laboratory (Pitchappan & Arulraj, 1989) was used to calculate allelic frequencies (Bernstein's formula), haplotype frequencies (Mathing et al 1970, Baur & Daniloves, 1980) relative risk by a modified method of Woolf and Haldane (Svejgaard et al, 1983) and etiological fraction and preventive fraction (Green, 1982).

Results and Discussion

Table 1 presents the results on tuberculosis susceptibility. While HLA A10 & B8 were associated with pulmonary tuberculosis irrespective of their severity and clinical

Table 1: HLA-DR2 predispose for more severe forms of pulmonary tuberculosis.

	Control	Patient Subgroups					
		Total	Sputum		AFB in smear		
			Negetive	Positive	+	++	+++
	N= 273	178	54	124	43	47	34
HLA-DR2 %PF	27.1	41.00	8.70	57.30	46.90	53.2	76.50
RR		1.90		3.60	2.30	3.00	6.60
X^2		9.40		32.00	6.70	12.30	31.40
EF		0.19		0.41	0.27	0.36	0.61

* = associations were valid in culture status as well as radiological lesions.(Brahmajothi 1990). RR: Relative risk, PF: Prerentive Fraction, EF: etiological fraction.

status (Brahmajothi et al. 1991), HLA-DR2 was associted with a far advanced (radiological lesion), sputum positive and culture positive (+++) pulmonary tuberculosis. While the percent phenotype frequency of HLA DR2 was around 30 percent in Indian population, it increased to 41% in patients with pulmonary tuberculosis, to 51% in sputum positive pulmonary tuberculosis and about 76% in far advanced disease: it is important to note that the DR2 was only 7% in sputum negative patients. This implies that HLA DR2 predispose for a more severe form of the disease. In other words, having been infected, if the patient has HLA DR2, the probability that the disease becomes more severe is very high. A similar correlation between HLA B8 and severe pulmonary tuberculosis has been described earlier (Al Arif et al. 1979). This finding on HLA-DR2 has been now convincingly confirmed in Russians (Khomenko et al, 1990) and Indonesians (Bolhemly et al. 1989). Our recent observation on chemotherapy of these tuberculosis patients have shown that HLA DR2 may have a role to play in recovery by chemotherapy and also in nurturing drug resistant organism (Brahmajothi et al 1991). Of all the HLA DR alleles, HLA DR2 infact has generic peptide binding capacity which may predispose it for many diseases and disorders (Falk et al. 1983) hitherto shown to have associations. Nonetheless the exact mechanism of these disease associations need to be elucidated.

Apart from the disease pathology, we also observed that many of the diseases studied viz, tuberculosis, rheumatoid arthritis and iridocyclitis revealed the same HLA association (Table 2) irrespective of subdivision of the sample in to Major groups (a collection of more akin caste groups, (Pitchappan et al. 1985). The disease association thus transcends ethnic barrier: this implies a generalized HLA dependent immunological phenomenon of molecular

Table 2: HLA-disease associations

			Total	MGII	MGIII
Associations transcending ethnic barriers					
1. *HLA-DR2 in Sputum positive pulmonary tuberculosis:*					
Control	%PF	(N)	27.1 (273)	29.8 (94)	19.8 (62)
Patients	%PF	(N)	57.3 (124)	60.0 (35)	63.4 (41)
RR			3.6	3.5	6.9
X^2	(1df)		32.0	9.5	19.1
2. *HLA-DR4 in Rheumatoid arthritis:*					
Control	%PF	(N)	14.2 (111)	27.3 (36)	63.4 (41)
Patients	%PF	(N)	51.2 (80)	69.2 (26)	33.3 (30)
RR			6.2	5.6	4.6
X^2	(1df)		27.3	9.8	5.6
EF			0.43	0.57	0.26
3. *HLA-B27 in Irridocyclitis:*					
Control	%PF	(N)	4.5 (111)	5.6 (36)	5.9 (34)
Patients	%PF	(N)	39.7 (78)	40.8 (20)	41.7 (24)
RR			12.8	9.4	9.4
X^2	(1df)		28.60	9.10	9.70
EF			0.37	0.36	0.37
Association unique to a major group: *HLA-B37 in Psoriasis:*					
Control	%PF	(N)	9.10 (77)	13.80 (29)	8.00 (25)
Patients	%PF	(N)	53.70	27.30 (11)	72.00 (25)
RR			10.90	2.30	23.20
X^2	(1df)		29.50	0.30	8.80
EF			0.48	0.16	0.69

MG II = Major group II; (Kallar, Maravar, Agamudayar, Vadar and related castes)
MG III = Major group III (Vellalar, chettiar, Vaicqer, Telugu speaking Naidu and related castes)
RR, PF, EF as in Table 1.

mimicry, or peptide consensus motif (Falk 1993; Hill 1991,1992) as the cause of the disease pathogenesis.

Our study on psoriasis has shown that the HLA DR7 and B17 association may be unique to Vellala related communities in Tamil Nadu: when our total sample was subdivided based on the major groups, the relative risk increased multifold in this group. This implies that the allele in the MHC region predisposing for psoriasis may be linked to the indicated HLA alleles and their identification in this subdivided population, with a high relative risk when compared to general population, may be due to founder effect. Nonetheless in the absence of any epidemiological data on the disease in Tamil Nadu, it is difficult to substantiate this.

In recent years it has been documented that the immune response genes play an important role in susceptibility and resistance to various diseases by their inherent peptide binding capacity (Benaceraf, 1981; Bjork man et al. 1985; Hill et al. 1992; Falk 1993) and the resultant immune response to various epitopes. By virtue of the fact Ir genes and extended haplotypes the HLA association need not be the same in various ethnic groups. It is known that there are ethnic differences in the prevalence of various diseases and immunological disorders as well.

The present study on the patients from Tamil Nadu, comparing the disease associations in various major groups revealed that while certain associations are unique and more significant in some diseases (psoriasis and HLA-B17), associations have the same relative risks and significance in many other diseases (tuberculosis, rheumatoid arthritis and iridocyclitis). It is possible that different mechanisms operate as revealed by these associations; in the first instance wherein the association was stronger in one group than the other, has suggested that a gene in the MHC region linked to the associated allele (B17) is responsible for the disease, where as the second case where in the relative risks are the same in many groups may either be due to an infective component in their etiology which the castes may be sharing through a common environment or due to generalized immunopathological or autoimmune disorders. Our observations on the anti-microbial antibodies in rheumatoid arthritis patients suggest a MHC dependent differences in the antibody profile Mahcn-dran (1992). The study reported here has thus indicated the importance of the sample stratification based on ethnic affinity and on clinical subtypes in an approach to identify the MHC association in disease susceptibility; After all many times a disease is christeined based on the clinical picture and not on the aetiopathology and the course of the disease.

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