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HLA-D identity in a family with multiple cases of multibacillary leprosy

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Summary HLA-D identity was determined by mixed lymphocyte reaction in a family with eight members affected by leprosy. The mother (BL) and all the children (1 LL, 4BL and 1BT) were HLA-D identical, whereas the father was HLA-D identical with four of the children except two BL cases. All the children formed HLA-D identical sib-pairs with the exception of one pair (LL and BL). Analysis of disease susceptibility frequency showed the mode of inheritance to be recessive, with the probability that the affected sibs would share both haplotypes.

Introduction

Family clustering of leprosy patients is known to occur, however it is unusual to see both parents and all six children affected with leprosy at the same time.[1] The occurrence of such a family offers a unique opportunity to study the development of a disease pattern in a sample of the population that may share both common environmental exposure and possibly shared genetic susceptibility.

Recent studies have shown that the HLA system contained genes that predispose to tuberculoid leprosy by analysing the segregation pattern of parental haplotypes as observed in affected children.[2] The observations were originally made in Surinam, but were later confirmed in India.[3] In addition to these, a significantly increased frequency of HLA-DR2 antigen was observed among tuberculoid leprosy patients in multiple case family studies in India. However, a consistent association between the lepromatous leprosy and the HLA system is lacking. In this paper we report the occurrence of HLA-D identity in a mother and her children affected with multibacillary leprosy.

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Patients and methods

PATIENTS

The family studied came from the Ambo region in the Shoa Administrative Region in central Ethiopia, a distance of about 260 km from Addis Ababa from an area referred to as 'the lepromatous corner' by leprosy field workers because of the high incidence of lepromatous leprosy in the area (unpublished observations).

The patients were classified by one of us (PAS) according to the Ridley–Jopling scale.[4] The clinical diagnosis was supported by skin smear bacilloscopy for acid-fast bacilli, and skin biopsies taken from representative lesions. Furthermore, skin testing with lepromin and lymphocyte transformation test (LTT) to *M. leprae* confirmed the diagnosis and classification.

MIXED LYMPHOCYTE REACTION (MLR) AND LYMPHOCYTE TRANSFORMATION TEST (LTT)

Lymphocytes were separated from defibrinated blood on Ficol–Isopaque, gradient.[5] All cultures of lymphocytes were made in triplicate in 96-well round-bottomed trays (Cooke laboratory products, Alexandria, Va.) in 200 μl of RPMI 1640 medium containing 20% pooled, heat inactivated, normal human serum, 2 mmol/l L-glutamine and antibiotics. All cultures were incubated at 37°C in 5% CO_2—humid air, and the cells were labelled on the appropriate day with 1 μ Ci ^{3}H-thymidine per well and harvested 20 hr later with a multiple cell culture harvester (Skatron, Lierbylen, Norway), ^{3}H-thymidine incorporation was determined by scintillation counting (LKB, Bromma, Sweden).

HLA-D identity was determined by mixed lymphocyte culture and was with standard methods.[6] Briefly 5×10^5 stimulator cells in 100 μl (treated with 0·25 mg 1 ml Mitomycin C) were added to 5×10^5 responder cells in 100 μl of medium in a microtitre well. All tests were carried out in triplicate and labelled with ^{3}H-thymidine after 5 days.

The lymphocyte transformation response to *M. leprae* was performed by adding 25 μl of a 10^6 bacilli/ml preparation to 2×10^5 lymphocytes in 200 μl of medium per well in triplicates. Cells were labelled with ^{3}H-thymidine on day 5 of culture.

The stimulation index (SI) and the increase in counts/min above control (cells cultured without antigen), i.e. the CPM were calculated from the mean of the triplicate cultures. An average SI >2 was considered to be positive for the mixed lymphocytes reaction and an SI >3 for the lymphocyte transformation response.

ESTIMATION OF DISEASE SUSCEPTIBILITY, GENE FREQUENCY AND INHERITANCE PATTERN

The technique for studying the inheritance pattern of HLA haplotypes in family members affected by a disorder has been described. The basic assumption in the technique is that there is a disease susceptibility locus effectively within the HLA region. Assuming that the parents carry four different HLA haplotypes between them and these are used as markers, one can then calculate the likelihood of getting a sib-pair with the disease for each possible disease and haplotype combination of the parents. Thus, for each parental arrangement, the probabilities of the affected sib-pairs having both, one or no HLA haplotypes in common can be calculated.

Results

Patient classification and LTT response to M. leprae *antigen*

Table 1 gives clinical data and the LTT response to *M. leprae*. Five of the children and the mother had multibacillary leprosy as shown by bacilloscopy, whereas one child was paucibacillary. The father had a BI of 2, however clinical and histopathological examination revealed no signs of leprosy. Histopathological classification revealed that four of the children and the mother had BL leprosy

Table 1. Clinical data and lymphocyte transformation test (LTT) result in patients under study

			Bacilloscopy					
Initials	Age	Sex	BI	MI	Clin* DX	Hist† DX	Lepromin	LTT¶
Cha‡	33	F	3	0	BL	BL	Neg	0 (<1)
Leg§	42	M	2	—	?L	Normal	10 mm	26·1 (5·1)
Tes	12	M	4	3	BL	BL	Neg	0 (<1)
Ahe	11	F	4·8	7·8	LL (H)	LL	Neg	0 (<1)
Biz	10	F	2·5	2	BL	BL	Neg	0 (<1)
Ale	7	M	0	0	BT	BT	8 mm	56·7 (13·3)
Dir	3	M	2	—	BL	BL	Neg	0 (<1)
Jil	1	M	3	2	?L	BL	Neg	0 (<1)

* Clinical diagnosis.
† Histological diagnosis.
‡ Mother.
§ Father.
¶ $\Delta CPM \times 10^{-3}$ (SI).

whereas one child had LL leprosy. All multibacillary patients were anergic in the lepromin test. The mother who was in reaction at the time of examination had a good LTT response like the BT child and the father, whereas all the multibacillary children had no *in vitro* response to *M. leprae* antigen.

MLR and HLA-D identity

The results of the MLR in the family studied are presented in Table 2. An extramaternal child who is free from leprosy is included in this study. As shown in this table all children are HLA-D identical with the mother. However, two of the multibacillary children (Dir and Jil) are HLA-D identical with the father whereas all the rest are non-identical. The extramaternal child of the father was found to be HLA-D non-identical to all family members except the father.

DISEASE SUSCEPTIBILITY FREQUENCY

The multibacillary children in this family form 10 sib-pairs. However, one sib-pair is HLA-D non-identical. Thus the observed value for sharing haplotypes bearing HLA-D linked disease susceptibility is 0·9 (expected value 0·907). This is

Table 2. Results of determination of HLA-D-identity by mixed lymphocyte reaction

	Leg	Tes	Ahe	Biz	Ala	Dir	Jil	Ger‡
Cha	NID*	ID†	ID	ID	ID	ID	ID	NID
	Leg	NID	NID	NID	NID	ID	ID	ID
		Tes	NID	ID	ID	ID	ID	NID
			Ahe	ID	ID	ID	ID	NID
				Biz	ID	ID	ID	NID
					Ale	ID	ID	NID
						Dir	ID	NID
							Jil	NID

* HLA-D non-identical.
† HLA-D identical.
‡ Extramaternal child.

quite consistent with a recessive mode of inheritance and 'a disease gene' with a frequency (pD) of 0·05, with the probability that all affected sibs share both haplotypes.

Discussion

The results of this study show that the mother and children affected with multibacillary leprosy, with the exception of one lepromatous sib-pair, are HLA-D identical. However, studies done in the past have shown significant association between the occurrence of HLA-DR2 antigen in tuberculoid case children of non-affected parents,[3] whereas there is only one report of weak association between lepromatous leprosy with HLA-D antigen.[8] Furthermore, it was shown that normal people, HLA-D identical with lepromatous siblings, do not share the siblings specific unresponsiveness to *M. leprae*.[9]

The appearance of leprosy in mother and all six children born to her is unusual. Apart from a common environment, families may share certain genetic susceptibilities and this study may shed some light on the possible involvement of genetic factors under the special circumstances of the family studied. Furthermore, this study suggests that the HLA-D related disease susceptibility in the family studied favours a recessive mode of inheritance. However, with the small sample size a dominant mode of inheritance cannot be completely ruled out although the results are much more compatible with a recessive mode of inheritance, due to a high frequency of sharing HLA-D haplotype in the affected sibs. It must also be pointed out that the data presented in this paper are not adequate to reach any firm conclusions about the mode of inheritance because of the small sample size and possible methodological issues.[10] It will be of interest to obtain further HLA-D data in similar families if the suspected recessive mode of inheritance for familial lepromatous leprosy can be confirmed.

If sufficient numbers of affected siblings are available this method provides a very powerful test for discriminating between dominant and recessive modes of inheritance of 'disease susceptibility genes' provided the frequencies of these genes are small.[11]

Appropriate data from other HLA-leprosy associated studies should provide very useful information for this test. If the method developed[2] for analysis of non-random segregation among sibships of different sizes is combined with the method applied in this study a much more meaningful synthesis could be made from such studies.

Previous studies indicate that lepromatous leprosy occurs in individuals in which the frequency of HLA-D or other genetic markers are not increased. This suggests that the disease may occur in individuals with a normal capacity for immune response to *M. leprae* but who, due to various reasons, have subsequently become unresponsive to *M. leprae* with ensuing development of

multibacillary disease.[9] However, the occurrence of lepromatous leprosy in almost all children born to a lepromatous mother and occurring uncharacteristically at a much younger age may be influenced by genes in the HLA-D region or in linkage disequilibrium with the region. If this prediction is verified by further studies, then a method for detecting a certain proportion of pre-lepromatous individuals will be available.

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References

[1] Sarojini PA. Occurrence of leprosy in an eight member family—A case report. *Lepr Rev*, 1984; **55:** 47–50.

[2] De Vries RRP, Lai A, Fat RFM, Nijenhus LE, Van Rood JJ. HLA-linked control of host response to *Mycobacterium leprae*. *Lancet*, 1976; **2:** 1328.

[3] Fine PEM, Wolf E, Pitchard J, Watson B, Bradley DJ, Festenstein H, Chako JG. HLA-linked genes and leprosy: A family study in Karigiri, South India. *J Inf Dis*, 1979; **140:** 152.

[4] Ridley DS, Jopling WH. Classification of leprosy according to immunity. A five-group system. *Int J Lepr*, 1966; **34:** 255.

[5] Ridley DS. Bacterial Indices. In *Leprosy in Theory and Practice* Cochrane RG, Davey TF (ed), John Wright & Sons, Bristol, 1964, p. 620.

[6] Böyum A. Separation of lymphocytes from peripheral blood. *Scand J Clin Lab Invest*, 1968; **21:** 97.

[7] O'Leary J, Reinsmoen N, Yunis EJ. Mixed lymphocyte reaction. In *Manual of Clinical Immunology* Rose NR, Friedman H (ed.), American Society for Microbiology, Washington D.C. 1976; 820.

[8] Day NE, Simons MJ. Disease susceptibility genes—their identification by multiple case family studies. *Tissue Antigens*, 1976; **8:** 109.

[9] Sogiyama K, Izumi S, Matsuneto Y, Ohkawa S, Matsumato H, Miyozekii T, Juji T, Maeda H. Analysis of the immunogenetic background of Japanese leprosy patients by HLA and serum protein allotype. *Int J Lepr*, 1980; **4:** 48.

[10] Stoner GL, Touw J, Belehu A. *In vitro* lymphoproliferative response of *Mycobacterium leprae* of HLA-D-identical siblings of lepromatous leprosy patients. *Lancet*, 1978; **II:** 543.

[11] Fine EM. Immunogenetics of susceptibility to leprosy, Tuberculosis and Leishmaniasis. In *Immunological Aspects of Leprosy, Tuberculosis and Leishmaniasis.* Humber DP, (ed.), Excerpta Medica, Amsterdam, 1981; p. 214.

[12] Thomas G, Bodmer G. The genetic analysis of HLA and disease associations. In *HLA and Disease* Dausset J, Suejgaard A. (ed.), Williams & Wilkins Co., Copenhagen, 1981; p. 84.