Tuberculoid leprosy in Mexicans is associated with HLA-DR3

CLARA GORODEZKY, J FLORES, N AREVALO,
LE CASTRO, A SILVA & O RODRIGUEZ

Departamento de Inmunogenética, ISET, SSA and Centro Dermatológico, 'Dr Ladislao Pascua', SSA. Mexico City

Accepted for publication 22 April 1987

Summary HLA-A, B, C, and DR antigens were investigated in a total group of 76 leprosy patients, classified according to the system of Ridley and Jopling in 30 TT and 46 LL individuals. A group of 100 healthy subjects were included for comparison. Patients and controls were all Mexican Mestizos born and inhabitants of Mexico. Typing of blood group systems was also performed. All TT patients showed a positive lepromin skin test, while all LL patients were negative to the specific antigen. The distribution of red-cell antigens was similar in both groups. The results of HLA typing demonstrated a significant association of DR3 with the tuberculoid type ($X^2 = 11.85$; $p = 0.0007$; $p_c = 0.04$). The RR value for DR3 carriers was 4.93 and the EF was 0.24. It is concluded that a Class II-linked susceptibility gene plays an important role in the expression of tuberculoid leprosy. This gene is closely linked to DR3 in the Mexican population.

Introduction

A considerable number of studies have been reported looking for an association between leprosy and gene products of the major histocompatibility complex (MHC) in humans. Firstly, concerning the HLA class I antigens,¹⁻¹⁰ and later to the molecules belonging to the D region.¹¹⁻¹⁶ An analysis of the published results up to 1983 has been reviewed by Serjeantson *et al.*¹⁷ Even though technical problems in HLA typing were sometimes evident, and the control group matching or the disease classification were not strictly considered, and a consistent association between A or B antigens could not be established from population studies, inheritance of HLA haplotypes in families provided evidence of the participation of HLA genes in the susceptibility to leprosy.¹⁷⁻²¹ Typing of class II products, which include HLA DR, DQ and DP antigens, clearly showed the relationship between DR antigens and tuberculoid leprosy¹¹⁻¹⁶ in random population as well as in families.

Leprosy in Mexico is an endemic disease with an incidence of 21/100,000 inhabitants and the patients are mainly located in the central plateau and the west area of the country.²² The most common type of leprosy according to the classification of Ridley and Jopling²³ is the lepromatous form; 62% of the cases are LL and BL, 23% are BB and only 15% are TT and BT.²⁴ For this reason, the first group we studied in 1973² looking for genetic factors was of LL patients. At that time, we found a decrease of A2 and A3, but only 8 antigens were explored. In a further work done by us in

1976²⁵ with a larger set of antisera and including locus C antigens, the previous observations were not confirmed, and thus we concluded that class I antigens are not related to LL type in Mexicans. Moreover the very well known MHC linkage disequilibrium relationships could readily account for the inconsistent class I associations found in LL in different populations. On the contrary, TT was clearly shown to be associated with DR2 in India,¹¹ Thailand¹⁶ and Japan^{12,14} and segregation of haplotypes carrying DR2 has also been confirmed.^{11,16,18,20,26} Among a group of a Caucasoid–Negroid patients originating from Surinam, a significant increase of DR3 was observed in TT, and was almost absent in LL.²⁷

Rea & Terasaki²⁸ reported an insignificant increase of DR2 in 19 Mexican TT patients. Because this is the only study done for TT in a Mexican group and many of the individuals were of Mexican–American ancestry, we decided to explore as many TT Mexican Mestizos as possible, with no other ethnic admixture. A better insight on the susceptibility genes that might be involved in the expression of the disease in this racial group can therefore be achieved.

Materials and methods

Leprosy patients and controls were all Mexican Mestizos (an admixture of Caucasian and Indian genes) born and inhabiting Mexico. One hundred unrelated healthy adults with no history of leprosy were included for comparison. Leprosy patients were classified according to the system of Ridley and Jopling, by experienced leprologists of the Centro Dermatológico Pascua and other specialized centres in Mexico. The total patients group consisted of 76 individuals; 30 were diagnosed as TT and 46 as LL, based on clinical and histopathological findings. Skin testing with lepromin-A resulted in a positive response for all TT patients (induration above 5 mm) and a negative one in every LL individual. Seventy per cent of them were natives of the central highland of the country, 10% of the southeast and 15% of the southwest. The average age for the total group of patients was 47.3 ± 5 , and did not differ between patients with TT (48.4 ± 5) and LL (46.4 ± 5). The average age of the controls was 30.2 and 48% were females. Among LL patients, 78.3 were males, while in the TT group 53.3% belonged to this sex.

T and B cells from peripheral blood samples were separated using nylon wool columns.²⁹ A set of 303 sera were used to define HLA-A, B, C and DR antigens. Sera identified 20 locus A, 28 HLA-B, 7 HLA-C and 9 HLA-DR antigens, and the reagents were obtained locally, from NIH and by international exchange. The standard microcytotoxicity assays described in the 8th International Histocompatibility Workshop³⁰ were used for typing. Detection of blood group antigens of the ABO and Rh systems was done by the established agglutination methods.

Statistics. Gene frequencies were calculated as $p = 1 - \sqrt{1 - A}$, where A = antigen frequency. The significance of the observed associations was determined comparing proportions using the chi-square test corrected by Yates. p values were corrected (p_c), multiplying by the total number of antigens tested. Relative risk (RR) as described by Woolf,³¹ was evaluated as a measure of the strength of association with a given antigen: $RR = a \times d / b \times c$; a, patients positive for the antigen; b, patients negative for the antigen; c, controls positive for the antigen; d, controls negative for the antigen. The aetiological fraction (EF) was obtained according to Bengtsson and Thomson³² as $(RR - 1) / RR$ (a) / a + b. This value indicates how much of the disease is 'due' to the associated factor.

Results

The distribution of blood group antigens in the patients showed no significant deviation in comparison with the control group, either as a whole or considering the LL and TT groups separately (Table 1).

Frequencies of class I antigens are equivalent among patients and controls (data not shown).

Table 1. Distribution (%) of blood group antigens in patients and controls

Antigen	Controls (N = 100)	LL patients (N = 46)	TT patients (N = 30)	Total patients (N = 76)
A	23	19.6*	30	23.7
B	7	8.7	10	9.2
AB	1	2.1	0	1.3
O	69	69.6	60	65.8
Rh +	98	100	100	100

Table 2. Gene and antigen frequencies (%) of HLA-DR antigens in patients and controls

Antigen HLA	Controls (N = 100)		Leprosy patients (N = 76)		LL Patients (N = 46)		TT patients (N = 30)	
	AG	GF	AG	GF	AG	GF	AG	GF
DR1	12	6.2	22.4	11.9	28.3	15.3	13.3	6.9
DR2	31	16.9	31.6	17.3	23.9	12.8	43.3	24.7*
DR3	8	4.1	22.4	11.9†	17.4	9.1	30.0	16.3†
DR4	27	14.6	36.8	20.5	45.7	26.3	23.3	12.4
DR5	19	10.0	22.4	11.9	23.9	12.8	20.0	10.6
DRw6	10	5.1	7.9	4.0	8.7	4.4	6.7	3.4
DR7	33	18.2	23.7	12.7	19.6	10.3	30.0	16.3
DR8	24	12.8	11.8	6.1	13.0	6.7	10.0	5.1
DRw9	5	2.5	2.6	1.3	0	0	6.7	3.4
DRX	31	9.6	18.4	2.4	19.5	2.3	16.7	0.9

* DR2 showed a non-significant increase ($\chi^2 = 2.17$; $p = \text{NS}$).

† DR3 was significantly increased in the total group: $\chi^2 = 8.541$; $p = 0.003$; $p_c = \text{NS}$. Particularly, in tuberculoïd patients: $\chi^2 = 11.85$; $p_c = 0.04$. RR = 4.93 and EF = 0.24

Table 2 shows the antigen and gene frequencies of HLA-DR antigens in all individuals submitted to the study. The proportion of all class II antigens was similar in controls and the total group of patients, except for DR3 that was significantly increased (GF = 5.5% in controls vs. 11.9% in the patients; $\chi^2 = 8.541$ $p = 0.003$; $p_c = 9.19$). Moreover, when the group was separated in LL and TT, DR2 was found increased in TT patients (24.7% vs. 16.7%) and DR3 was significantly elevated (16.3% vs 4.1% $\chi^2 = 11.85$; $p = 0.0007$; $p_c = 0.04$). The RR for DR3 carriers was 4.93 and the EF = 0.24.

Discussion

The accumulating evidences presented by different groups have shown that HLA markers encoded by the D region are important in the expression of the 2 polar types of leprosy. It is clear, from this report and from others, that LL is not related to any particular class I or DR antigen, although DQw1 has recently been reported associated to LL in the Venezuelan population.¹⁵ The cosegregation

analyses done in 50 families integrated in the 9th International Workshop of Histocompatibility²⁶ and the 6 families that were included by us in the same study³³ showed that siblings affected with BL or LL shared 1 haplotype more often than expected ($p = 0.025$).

The strong association between DR2 and tuberculoid leprosy is supported by observations in several populations, as already mentioned, but DR2 does not seem to participate in the development of the TT form in Mexicans, as shown in the present report and the one from Rea and Terasaki²⁸ even if in both studies DR2 was more common in the patients. Since Mexican Mestizos do have a Mongoloid gene pool ancestry, a DR2 association might be expected. However, the influence of Caucasian genes is indubitable as demonstrated by the HLA frequencies in normal Mexicans.^{34,35} Surprisingly, the association of DR3 in Mexicans is in accordance with the results found in a mixed Caucasoid—Negroid population from Surinam.²⁷

It is interesting to point out that in Rea and Terasaki's report, the antigen frequency of DR3 was higher in their controls than in ours (20% *vs* 8%). This difference may be due to the fact that Mexican Americans are a more heterogeneous group, with a greater white component than Mexicans native from this country. An association with this antigen may be missed, especially if a small group of patients is analysed. Moreover, the authors claimed their patients were BT, and the affected in the present report were TT, as confirmed by histological criteria.

Lepromin testing was positive in all TT individuals, as expected, but still DR3 antigen seems to play an important role in the susceptibility to TT, although not necessarily due to an immune defect, because genes in chromosome 6 participate in non-immunological functions as well.^{36,37}

The RR value of 4.93, suggests that DR3 carriers are almost 5 times more likely to develop TT, but the EF of 0.24 indicates that other factors are also implicated, and DR3 contributes only in a partial way. Thus, the involvement of a DR-linked susceptibility gene is more likely, and depending on the ethnic background, the manifestation can be through an association with different DR alleles in each population. The data shown here, previous population studies and those in multicase families show that class II determinants play an important role in determining the clinical manifestations of *M. leprae*, despite the origin of the patient.

Finally, thinking about the possible mechanism of DR3 association, Van Eden *et al.*³⁸ demonstrated a DR restriction phenomenon, indicating a role of HLA-DR products in *M. leprae* specific proliferative responses. They concluded that T-cell recognition in conjunction with DR3 occurs poorly or differently. However, in view of the immunological features of tuberculoid leprosy, the nature of the phenomenon must be clarified.

In 2 manuscripts that are currently being prepared, we will present our data on multicase families and of a DQ-linked immune suppression gene related to the non-responsiveness in leprosy.³⁹

Acknowledgments

We are sincerely grateful to the following doctors for submitting tuberculoid patients to the study: Luis Ramírez, Yolanda Ortiz and Teresa Loyo. Our gratitude to Patricia Escobar, Héctor Debaz, Gilberto Eroza, Carmen Mora and Laya Hun for their technical help and Raquel Zavala for typing the manuscript.

References

- Thorsby, E, Godal T, Maryvang B. HLA antigens and susceptibility to diseases. II: Leprosy. *Tissue Antigens*, 1973; 3: 373–7.
- Escobar-Gutiérrez A, Gorodezky C, Salazar-Mallén M. Distribution of the HLA system lymphocyte antigens in Mexicans. II: Studies in atopics and in lepers. *Vox Sang*, 1973; 25: 151–5.
- Reis AP, Maia F, Reis VF, Andrade IM, Campos AAS. HLA antigens in leprosy. *Lancet*, 1974; 2: 1384.

- ⁴ Kreisler M, Arnaiz A, Perez B, Fernández-Cruz E, Botello A. HLA antigens in leprosy. *Tissue Antigens*, 1974; **4**: 197–201.
- ⁵ Smith GS, Walford RL, Shepard CC, Payne R, Prochazka GJ. Histocompatibility antigens in leprosy. *Vox Sang*, 1975; **28**: 42–9.
- ⁶ Dasgupta A, Mehra NK, Ghei SK, Vaydia MC. Histocompatibility antigens (HL-A) in leprosy. *Tissue Antigens*, 1975; **5**: 85–7.
- ⁷ Nakajima S, Kobayashi S, Nohara M, Sato S. HLA antigens and susceptibility to leprosy. *Int J Lep*, 1977; **45**: 273–7.
- ⁸ Takata H, Sada M, Ozawa S, Sekiguchi S. HLA and mycobacterial infection: increased frequency of B8 in Japanese leprosy. 1978; *Tissue Antigens*, **11**: 61–4.
- ⁹ Greiner J, Schleiermacher E, Smith T, Lenhard V, Vogel F. The HLA system and leprosy in Thailand. *Hum Genet*, 1978; **42**: 201–3.
- ¹⁰ Nocera A, Barrocci S, Chiarla W. HLA antigens and leprosy. *Int J Lep*, 1980; **48**: 69–70.
- ¹¹ de Vries RRP, Mehra NK, Vaydia MC, Gupta MD, Khan PM, Van Rood JJ. HLA linked control of susceptibility to tuberculoid leprosy and association with HLA-DR types. *Tissue Antigens*, 1980; **16**: 294–304.
- ¹² Miyana K, Juji T, Maeda H, Nakajima S, Kobayashi S. Tuberculoid leprosy and HLA in Japanese. *Tissue Antigens*, 1981; **18**: 331–4.
- ¹³ Serjeantson SW, Vaidya MC, Chan SH, Juji GT, Mahra NK, Nark S, Sasasuki T. Leprosy. In: *Proceedings of the Second Asia-Oceania Workshop and Conference*. Simmons M, Tait B. (eds). Victoria: Australia Immunopublishing, 1981, pp. 379–401.
- ¹⁴ Izumi S, Sugiyama K, Matsumoto Y, Ohkawa S. Analysis of immunogenetic background of Japanese leprosy patients by the HLA system. *Vox Sang*, 1982; **42**: 243–7.
- ¹⁵ Ottenhoff THM, González NM, de Vries RRP, Convit J, Van Rood JJ. Association of HLA specificity LB-E12 (MB1, DC1, MT1) with lepromatous leprosy in a Venezuelan population. *Tissue Antigens*, 1984; **24**: 25–9.
- ¹⁶ Schauf V, Ryan S, Scollard D, Jonasson O, Brown A, Nelson K, Smith T, Vithayasai V. Leprosy associated with HLA-DR2 and DQw1 in the population of Northern Thailand. *Tissue Antigens*, 1985; **26**: 243–7.
- ¹⁷ Serjeantson S. HLA and susceptibility to leprosy. *Immunol Rev*, 1983; **70**: 89–112.
- ¹⁸ De Vries RRP, Fat RFM, Nijenhuis LE, van Rood JJ. HLA linked genetic control of host response to *Mycobacterium leprae*. *Lancet*, 1976; **iii**: 1328–30.
- ¹⁹ Fine PEM, Wolf E, Pritchard J, Watson B, Bradley DJ, Festenstein H, et al. HLA-linked genes leprosy: A family study in Karigiri, South India. *J Infect Dis*, 1979; **140**: 152–61.
- ²⁰ Van Eden W, de Vries RRP, Mehra NK, Vaidya MC, Dámaro J, Van Rood JJ. HLA segregation of tuberculoid leprosy: Confirmation of the DR2 marker. *J Infect Dis*, 1980; **141**: 693–701.
- ²¹ Van Eden W, González NM, de Vries RRP, Convit J, van Rood JJ. HLA-linked control of predisposition to lepromatous leprosy: 1985; *J Infect Dis*, 1985; **151**: 9–14.
- ²² Boletín Mensual de Epidemiología. Dirección General de Epidemiología Éne. 1 1986; Vol. 1, México, D.F.
- ²³ Ridley DS, Jopling WH. Classification of leprosy according to immunity: A five group system. *Int J Lep*, 1966; **34**: 255–73.
- ²⁴ Boletín Mensual de Epidemiología. Dirección General de Epidemiología, Oct. 1981; Vol. 1, México, D.F.
- ²⁵ Gorodezky C, Escobar-Gutiérrez A, Latapí F, Castro E, Rodríguez O, Castaneda L, Franco R, Estrada-Parra S. Antígenos HLA en pacientes con lepra. I *Congreso Nacional de Inmunología*, Oaxtepec, México, 1976 (abstract).
- ²⁶ de Vries RRP, Serjeantson SW, Layrisse Z. Leprosy. Disease Report. In: *Histocompatibility Testing 1984*. Albert ED, Baur MP and Mayr WR (eds) Berlin: Springer-Verlag, 1984, pp. 362–7.
- ²⁷ van Eden W, de Vries RRP, Dámaro J, Schreuder I, Leiker DL, van Rood JJ. HLA-associated genetic control of the type of leprosy in a population from Surinam. *Human Immunol*, 1982; **4**: 343–50.
- ²⁸ Rea TH, Terasaki PI. HLA-DR antigens in tuberculoid and lepromatous leprosy. *Lep Rev*, 1980; **51**: 117–23.
- ²⁹ Danilovs J, Terasaki PI, Park MS, Ayoub G. B-lymphocyte isolation by thrombin-nylon wool. In: *Histocompatibility Testing 1980*. Terasaki PI (ed). Los Angeles: UCLA Tissue Typing Laboratory, 1980, pp. 287–8.
- ³⁰ *Histocompatibility Testing 1980*. Terasaki PI (ed). Los Angeles: UCLA Tissue Typing Laboratory, 1980.
- ³¹ Woolf B. On estimating the relation between blood groups and disease. *Ann Hum Genet*, 1955; **19**: 251–3.
- ³² Bengtsson BO, Thomson G. Measuring the strength of associations between HLA antigens and diseases. *Tissue Antigens*, 1981; **17**: 356–63.
- ³³ Gorodezky C, Alvarenga MV, Flores J, Castro LE, Erosa G, Debaz H, Castro-Bernal E, Hernández O, Rodríguez O. Immunogenetic Analysis in Mexican Families with Leprosy. *IX International Histocompatibility Workshop and Conference*. München-Wien, 1984 (abstract) p. 65.
- ³⁴ Castro Le, Flores J, Rangel B, Debaz H, Erosa G, Granados G, Hernández O, Gorodezky C. Frecuencias de Antígenos Clase I y Clase II en la población Mestiza Mexicana. *VI Congreso Nacional de Inmunología*, México D.F. 1985 (abstract), p. 149.

- ³⁵ Park MS, Gorodezky C, Terasaki P. HLA in a population of Mexicans. In: *Proceedings of the Third Asia and Oceania Histocompatibility Workshop—Conference* (in press).
- ³⁶ Class FHJ, van Rood JJ. The interaction of drugs and endogeneous substances with HLA Class I antigens. *Prog Allergy*, 1985; **36**: 135–50.
- ³⁷ Simonsen M, Skjodt K, Crone M, Sanderson A, et al. Compound receptors in the cell membrane: Ruminations from the borderland of Immunology and Physiology. *Prog Allergy*, 1985; **36**: 151–76.
- ³⁸ Van Eden W, Elferink BG, de Vries RRP, Leiker DL, Van Rood JJ. Low T-lymphocyte responsiveness to *Mycobacterium leprae* antigens in association with HLA-DR3. *Clin exp. Immunol*, 1984; **55**: 140–8.
- ³⁹ Gorodezky C, Granados G, Flores J. Role of Class II MHC products in the ability of monocytes to eliminate *M. leprae*. *VI International Congress of Immunology*. Toronto: 1986 (abstract), pp. 579.

LEPROSY CONTROL AND FIELD WORK

WHO Guidelines for protection of personnel; HIV infection

'Guidelines for personnel involved in collection of skin smears in leprosy control programmes for the prevention and control of possible infection with HIV' is a WHO document—WHO/CDS/LEP/87.1, issued recently, giving details of protection of personnel in areas where HIV infection is known or suspected to exist. The full text was printed in *Leprosy Review*, (1987) Number 3, **58**, 207 and also appears in the *International Journal of Leprosy*, (1987) Number 2, **55**, 421. Copies will be included in all future issues of the *Technical Guide for Smear Examination for Leprosy* (Leiker and McDougall, published by the Leprosy Documentation Service (INFOLEP), Amsterdam).

Training Disabled People in the Community; WHO and others

This is an A4 manual, of 652 pages, with hundreds of pictures and drawings. The full title is 'Training disabled people in the community; a manual on community-based rehabilitation for developing countries'. It has been written as part of a cooperative programme for the United Nations Decade of Disabled Persons, carried out by: United Nations Development Programme; United Nations High Commissioner for Refugees; United Nations Centre for Social Development and Humanitarian Affairs; United Nations Children's Fund; International Labour Organization, United Nations Educational, Scientific and Cultural Organization the World Health Organization. Date of publication May 1983. Principal authors and artists are: Einar Helander, Padmani Mendia and Gunnel Nelson. This is a superb publication; the drawings are amongst the best we have seen for this type of manual and the main concept of community-based training is one which should surely be studied by those working in leprosy. The end section 'Stories', includes one about an Indian villager, Ramasamy, with leprosy. Reference RHB/83.1. Office of Publications, WHO, Geneva. Cost not known.