

# A Study of Cell-Mediated Immunity and Histocompatibility Antigens in Leprosy Patients in Iran<sup>1</sup>

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The clinical manifestations of leprosy are heterogeneous and the disease is usually observed in the following forms: tuberculoid, lepromatous, borderline or intermediate and indeterminate (2). There is evidence showing that development of the disease is related to some immunologic mechanism, whether humoral or cellular (1, 10, 16).

Various methods may be employed to study the overall immunologic status and specific defects of immunity in leprosy patients. There are observations showing that a proportion of leprotic patients can present an impaired delayed hypersensitivity in the presence of different antigens (6, 11, 22).

Patients with the tuberculoid form of the disease do not show any specific perturbation of cell-mediated immunity (CMI) (22), whereas the characteristics of the lepromatous form are as follows:

1. Negative reaction to intradermal injection of lepromin (22).
2. Weak cellular hypersensitivity to the intradermal injection of various antigens (22).
3. Weak response to dinitrochlorobenzene (DNCB) (18).
4. Prolonged survival of allogenic skin grafts (18).

It has also been shown that all those who come into contact with the leprosy bacillus do not necessarily develop the disease. These observations along with other evidence favor a genetic and geographic predisposition

for the disease. Even in countries with a relatively homogeneous population, leprosy shows a predilection for certain particular areas (13).

Iran is situated in a region with a high incidence of leprosy, yet virtually no immunologic studies have been carried out on the disease in this region. We therefore felt that it would be worthwhile to investigate certain immunologic aspects of leprosy patients in Iran.

## MATERIALS AND METHODS

Seventy patients (56 male, 14 female) with ages ranging between 11 and 62 years, from the Society for Assistance to Leprosy Patients, were studied. All patients were classified according to the Ridley-Jopling delineation (18). Healthy controls were blood donors coming to the Iranian National Blood Transfusion Service with ages ranging from 22 to 55 years.

**T cell determinations.** Lymphocytes from peripheral blood were separated by Ficoll-Hypaque for the following tests:

**Sheep erythrocyte rosettes.** A lymphocyte suspension containing  $5 \times 10^6$  cells/ml in Hanks' balanced salt solution (HBSS) were mixed with washed sheep erythrocytes suspended in fetal calf serum (FCS) (Difco-Lab) at room temperature. The mixture was centrifuged at  $400 \times g$  for 5 minutes and then kept overnight at 20°C. The percentage of rosette forming cells (RFC) was determined. At least 300 lymphocytes were counted in triplicate tubes and only rosettes possessing three or more adherent erythrocytes were counted (4, 9).

**Complement dependent rosettes (EAC rosettes).** A 2.5% suspension, v/v, of human erythrocytes was incubated for 30 minutes at 37°C with an equal volume of a subagglutinating dilution of normal rabbit serum containing natural heterophil antibodies to human erythrocytes (HEAC). Undiluted

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mouse complement in the amount of 0.1 ml was added to 2 ml of the sensitized erythrocyte suspension and incubated for an additional 30 minutes at 37°C. This mixture (HEAC) was then washed with HBSS and adjusted to 5%. Then 0.1 ml of the lymphocyte suspension ( $2 \times 10^6$  cells/ml) was mixed with an equal volume of HEAC at room temperature in triplicate. This preparation was immediately centrifuged at  $200 \times g$  for 5 minutes at room temperature. RFC were then counted (4, 5, 20).

**Leukocyte migration test (LMT).** The LMT was performed by the technic of Soborg and Bendixen (21) as modified by Rosenberg and David (12, 19). The supernatant, containing leukocytes from 20 ml of heparinized venous blood, was centrifuged at  $200 \times g$  for 10 minutes and the cell pellet washed three times in HBSS. The washed leukocytes were resuspended in TC 199 medium (Difco-Lab), aspirated into capillary tubes, sealed with Seal-Ease (Hynz Company) and centrifuged for 5 minutes at  $300 \times g$ . The tubes were cut at the cell-fluid interface and that portion of the capillaries which contained the leukocytes was placed in migration chambers. The tests were set up in triplicate. The chambers were filled with TC 199 medium + 10% FCS containing antigens (phytohemagglutinin, PHA, Difco-Lab) at different concentrations. They were incubated for 18 hours at 37°C. The migration pattern was projected, traced and the area of migration was measured by planimetry. Inhibition of migration was considered significant when the migration index (MI) in the presence of PHA was less than

80% of the control figure without PHA. The MI was expressed as:

$$\text{MI} = \frac{\text{Area of migration in the presence of PHA}}{\text{Area of migration in control chambers}}$$

HLA antigens were defined by a modified N.I.H. lymphocytotoxicity test using known antisera from N.I.H. and local sources in order to type 48 patients and 100 controls. Seven antigens of the A and 12 antigens of the B locus were defined.

## RESULTS

The percentage of T and B lymphocytes detected by E and EAC rosette-formation in leprosy patients and normal controls is shown in Table 1. There is a significant difference ( $p < 0.01$ ) in T cells between tuberculoid and lepromatous forms of the disease as compared to normal controls. We did not observe any differences in EAC rosette cells.

The results of LMT index determinations in patients and controls are shown in Table 2. It will be noted that the MI is significantly higher in controls than in leprosy patients for concentrations of both one and two microliters of PHA and this suggests a good production of leukocyte inhibitory factor (LIF) by T cells of normal controls (Table 2).

The distribution of the A and B locus antigens of the HLA system in both leprosy patients and controls is shown in Tables 3 and 4. Although there is no significant difference in the distribution of the A locus antigens between leprosy patients and controls, a higher percentage of A-11 was obtained in leprosy patients.

TABLE 1. Relative distribution of total T cells and B lymphocytes in the peripheral blood of leprosy patients and normal controls.

Test	Cells	Normal controls	Leprosy patients	
			Tuberculoid	Lepromatous
Rosette formation	Total T cells	73.9% ± 11.35	36% ± 4.2	38% ± 6
	B lymphocytes	20.2% ± 4.45	20% ± 6.5	21% ± 3.2

TABLE 2. The mean migration index in the presence of PHA among normal controls and leprosy patients.

Concentration of antigen	Normal controls	Leprosy patients	
		Tuberculoid	Lepromatous
0.01 ml	0.34	0.73	1
0.02 ml	0.19	0.48	0.65

A slight elevation of B-5 antigen incidence was observed, but these results are preliminary and our information regarding the B locus is incomplete, so that it is difficult to establish any precise relationship between HLA antigens and leprosy at this stage.

### DISCUSSION

The different factors responsible for the immunologic defect in lepromatous patients are still unknown, but the significant decrease of T cells in these patients suggests an impairment of CMI. This impairment of CMI could be due to a reduction of the thymus-dependent lymphocyte population<sup>(14)</sup>, to humoral inhibitory factors<sup>(7)</sup>, or to deficient responsiveness of T cells to effector systems<sup>(4)</sup>. Finally, it is also possible that T suppressor cells have some part to play in this context<sup>(2)</sup>.

The observed decrease of MI in leprosy patients in the presence of PHA could be due to the low production of LIF by T lympho-

cytes<sup>(7,15,16)</sup> and this postulate correlates well with the observed decrease in peripheral T lymphocytes.

Differences in the geographic distribution of HLA antigens have been demonstrated by several groups<sup>(8,17)</sup>, and there are several reports showing an association between HLA and disease<sup>(13)</sup>. Dasgupta *et al*<sup>(3)</sup>, in a preliminary communication, have reported an increase of HLA-B8 in leprosy patients in India. We have not observed a similar association in Iran and an increase in HLA-B5 was noted amongst our patients. These observations from India and Iran purport to associate the distribution of HLA antigens with a proclivity for the disease. In reality, the hidden markers in this system and its extreme polymorphism make it very difficult to hypothesize emphatically until the results of more precise studies become available.

We have shown that there is a defect of CMI in these patients. On the other hand, the close linkage of the HLA system and the

TABLE 3. *Distribution of A locus antigens among leprosy patients and normal controls.*

HLA specificity	Leprosy patients (total no. = 48)		Normal controls (total no. = 100)	
	No.	%	No.	%
A1	6	12.4	22	22
A2	18	37.5	28	28
A3	11	22.9	18	18
A9	13	27	37	37
A10	7	14.5	16	16
A11	11	22.9	3	3
A28	0	0	6	6

TABLE 4. *Distribution of B locus antigens among leprosy patients and normal controls.*

HLA specificity	Leprosy patients (total no. = 48)		Normal controls (total no. = 100)	
	No.	%	No.	%
B5	27	56.2	39	39
B7	1	2	8	8
B8	2	4	7	7
B12	5	10.4	10	10
B13	4	8.3	9	9
B14	2	4	5	5
B17	2	4	12	12
B18	4	8.3	9	9
B27	2	4	5	5
BW35	9	18.7	15	15
BW40	3	6.2	4	4

immune response (Ir) genes is well-defined. Although there is no clear evidence for the existence of a gene which could be responsible for the immune response to a specific disease, a relationship between specific HLA antigens and certain diseases has been proven<sup>(13)</sup>.

This preliminary study in Iran points to the importance of further investigation along the same lines, most particularly including family studies.

### SUMMARY

Fifty-six male and 14 female leprosy patients, aged 11-62, were studied for cell-mediated immunity (CMI) and histocompatibility antigens. Healthy blood donors were used as normal controls. All patients were receiving antileprosy drugs. T and B cells were detected by E and EAC rosette formation techniques, and the leukocyte migration test (LMT) was done in the presence of PHA. HLA antigens were defined by a modified N.I.H. lymphocytotoxicity test in order to type 48 patients and 100 controls.

There was a significant difference ( $p < 0.01$ ) in the number of T cells between tuberculoïd and lepromatous forms of the disease as compared to normal controls. We did not observe any differences in EAC rosette cells. It should be noted that the migration index is significantly higher in controls than in leprosy patients for PHA.

There are no significant differences in the distribution of the A locus antigens between leprosy patients and controls, although a higher percentage of A-11 was obtained in leprosy patients. A slight elevation of B5 antigen was observed but these results are preliminary and our information regarding the B locus is incomplete. Thus, it is difficult to establish any precise relationship between HLA antigen and leprosy at this stage.

### RESUMEN

Se estudiaron la inmunidad celular y los antígenos de histocompatibilidad en 56 hombres y 14 mujeres afectados de lepra cuyas edades oscilaron entre los 11 y los 62 años. Como controles normales se usaron donadores de sangre sanos. Todos los pacientes estudiados estaban bajo tratamiento con drogas antileprosas. Las células T y B se determinaron por la técnica de las rosetas E y EAC, y la prueba de la migración de los

leucocitos se hizo en presencia de PHA. Los antígenos HLA se tipificaron por una prueba de linfocitotoxicidad modificada para poder estudiar 48 pacientes y 100 controles.

Hubo una diferencia significativa ( $p < 0.01$ ) en el número de células T entre las formas tuberculoïde y lepromatosa de la enfermedad, en comparación con los controles sanos. No se observó ninguna diferencia en el número de rosetas EAC.

El índice de migración fue significativamente más elevado en los controles que en los pacientes con lepra.

No hubieron diferencias importantes en la distribución de los antígenos del locus A entre los pacientes con lepra y los controles aunque se encontró un mayor porcentaje de A-11 en los pacientes con lepra. Se observó una ligera elevación del antígeno B-5 pero estos resultados son preliminares y nuestra información en relación al locus B es incompleta. Así, por el momento, es difícil establecer alguna relación precisa entre los antígenos HLA y la lepra.

### RÉSUMÉ

On a étudié l'immunité cellulaire (CMI) et les antigènes d'histocompatibilité chez 56 malades de la lèpre de sexe masculin et chez 14 malades de sexe féminin, âgés de 11 à 62 ans. Des donneurs de sang normaux ont été pris comme témoins. Tous les malades étaient soumis à une thérapeutique par médicaments antilépreux. On a détecté des cellules T et B au moyen de la technique de formation de rosette E et EAC; l'épreuve de migration des leucocytes (LMT) a été pratiquée en présence de phytohémagglutinine. Des antigènes HLA ont été identifiés par une épreuve modifiée de lymphocytotoxicité N.I.H., dans le but de classer 48 malades et 100 témoins.

On a observé une différence significative ( $p < 0.01$ ) dans le nombre de cellules T chez les malades atteints de la forme tuberculoïde de la maladie et chez les lépromateux, par rapport aux témoins normaux. Aucune différence n'a été observée dans la formation de cellules en rosette EAC. Il faut noter que l'index de migration pour le phytohémagglutinine (PHA) est significativement plus élevé chez les témoins que chez les malades de la lèpre.

Aucune différence significative n'a été mise en évidence pour les antigènes du locus A, entre les malades de la lèpre et les témoins, encore qu'un pourcentage plus élevé de A-11 ait été observé chez les malades de la lèpre. Une augmentation légère des taux d'antigène B5 a été observée, mais ces résultats ne sont que préliminaires et les informations actuelles concernant le locus B sont incomplètes. Il est dès lors malaisé d'établir de façon précise une quelconque relation entre l'antigène HLA et la lèpre, à ce stade.

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