HLA Antigen and Susceptibility to Leprosy[1]

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In leprosy there are a wide variety of clinical forms ranging from tuberculoid to lepromatous leprosy. Many studies indicate that the clinical and histologic spectrum of leprosy is determined by the immunologic reaction of the host against *Mycobacterium leprae* ([9, 11]). In lepromatous leprosy there appears to be a specific deficiency in cell-mediated immune responses ([5, 19]). A similar but less pronounced deficiency may be seen in patients with tuberculoid leprosy. A specific depression of cell-mediated immunity in the lepromatous form is a host-dependent characteristic which probably is genetically determined ([10]).

Experimental studies have shown that genes determining the immune responsiveness to certain antigens are closely linked to major histocompatibility genes ([1, 7]). The human major histocompatibility system, HLA, has recently been suspected to be closely associated with a variety of diseases. The amount of literature on associations between certain HLA antigens and various diseases is increasing rapidly, and a number of views have been published ([13, 16, 17]).

In this study we looked for deviations in HLA antigens both in leprosy patients and in nonleprosy individuals as controls.

MATERIALS AND METHODS

Test subjects. Test subjects were divided into three groups: 1) tuberculoid patients, 2) lepromatous patients, and 3) a control group of nonleprosy subjects. Fifty-nine leprosy patients (31 tuberculoid, 28 lepromatous) at the Kuryu-Rakusen-en, National Hospital, in Kusatzu, Japan were selected for the present study.

The classification of the patients was determined by several criteria: clinical manifestation, bacteriologic examination of smears, histologic examination of biopsies and cutaneous response to lepromin. All of the lepromatous patients and some tuberculoid patients had been under chemotherapy for varying periods of time.

The control group consisted of 125 healthy individuals, members of the staff of Ida Hospital in Kawasaki-City, who had had no known contact with leprosy patients in the past.

Lymphocyte preparation and HLA typing. Lymphocytes for HLA typing were separated from 2 ml of heparinized peripheral blood using the Ficoll-Conray discontinuous density gradient. The lymphocyte rich fraction was washed and resuspended in tissue culture medium 199 containing 0.5% fetal calf serum (TC-199). After incubation for 15 minutes at 37°C in a flat-bottom glass tube, non-glass-adherent cells were recovered. The concentration of lymphocytes was adjusted to 1×10^6 cells per milliliter of TC-199. Over 95% of these cells were viable when tested by the dye exclusion method.

HLA antigens were determined by the microdroplet lymphocyte toxicity method developed by Terasaki and known as the standard NIH method ([21]). A total of 58 highly selected antisera were used to detect 14 antigens from the new locus A series and 17 antigens from the new locus B series. A Lot. No. T-7 research tray, which was kindly supplied by Prof. Terasaki of UCLA, was used. In order to determine the specificity, at least two or more different sera were tested, except in tests for A9, AW23, AW25, AW26, BW37, and BW39, where only one serum was used.

Statistical analysis. The differences in frequencies of antigens were analyzed for statistical significance by the ordinary χ^2 test, and by the Yate's correction in cases of small samples. The statistical analyses were conducted at the Department of Technology, Keio University, Yokohama, Japan.

RESULTS

Frequencies of different HLA antigens in the group of leprosy patients and the control group are shown in Table 1. AW24 antigen showed the most remarkable deviation. The

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frequency of this antigen in leprosy patients was 25.4%, and that in normal subjects of the control group was 63.2%. The difference in frequency between the two groups was statistically significant with X^2 being 22.89, and the p value being less than 0.001 without correction for the large number of comparisons made. When the p value was corrected for the number of antigens being tested, this was also statistically significant at a 5% level. Uncorrected p values of the BW39, A9 and B8 antigens were also statistically significant. Frequencies of all these antigens were statistically significant at a 1% level.

Frequencies of A9 and AW24 antigens in leprosy patients were found to be decreased as compared to the frequencies of these antigens in individuals of the control group. Frequencies of B8 and BW39 antigens were found to be increased as compared to the controls.

Table 2 shows the frequencies of the different HLA antigens in patients with tuberculoid leprosy and in those with lepromatous leprosy. Frequencies of the majority of HLA antigens in tuberculoid leprosy patients were similar to those of corresponding HLA antigens in lepromatous leprosy patients. AW24

TABLE 1. *Frequency of HLA antigen in 59 leprosy patients and 125 controls.*

HLA antigen	Controls		Leprosy patients		
	No.	%	No.	%	X^2
A1	0	0	0	0	
A2	38	30.4	18	30.5	
A3	2	1.6	0	0	
A9	85	68.0	27	45.8	8.32[a]
AW23	21	16.8	4	6.8	2.63
AW24	79	63.2	15	25.4	22.89[b]
A10	39	31.2	14	23.7	
AW25	5	4.0	1	1.7	
AW26	8	6.4	1	1.7	1.03
A11	28	22.4	5	8.5	5.38
A29	0	0	0	0	
AW30	0	0	0	0	
AW31	22	17.6	7	11.9	
AW32	2	1.6	0	0	
B5	37	29.6	10	16.9	3.37
B7	18	14.4	6	10.2	
B8	1	0.8	6	10.2	8.27[c]
B12	17	13.6	4	6.8	
B13	5	4.0	1	1.7	
B14	2	1.6	2	3.4	
B18	5	4.0	2	3.4	
B27	0	0	2	3.4	
BW15	23	18.4	7	11.9	
BW17	2	1.6	0	0	
BW21	11	8.8	3	5.1	
BW22	30	24.0	7	11.9	3.67
BW35	27	21.6	16	27.1	
BW37	0	0	0	0	
BW38	1	0.8	0	0	
BW39	0	0	8	13.6	14.60[d]
BW40	35	28.0	15	25.5	

Comparison with control (Uncorrected p value)
[a] $p < 0.005$
[b] $p < 0.001$
[c] $p < 0.005$
[d] $p < 0.002$

and AW31 antigens were found more frequently in the lepromatous patients as compared to the tuberculoid, but the differences were not statistically significant even at a 5% level.

DISCUSSION

Previous studies have revealed the definite associations between certain HLA antigens and diseases in man. Examples in nonmalignant diseases include ankylosing spondylitis with B27 ([14]), multiple sclerosis with A3 and B7 ([6]), and psoriasis vulgaris with B13 and B17 ([20]). In infectious diseases, hemophilus influenzae was found to be associated with B17 ([12]), and infectious mononucleosis with B5 ([8]). Certain HLA antigens were also reported to be associated with malignant diseases. McDevitt and Bodmer suggested that these associations were due to the influence of disease-predisposing genes closely linked with genes for HLA antigens, and not due to a function of HLA antigens ([7]). Results of their studies also showed that when this linkage disequilibrium was not present, many of these associations could not exist.

In our studies, frequencies of four antigens, AW24, BW39, A9, and B8 in leprosy patients were found to be different with statistical significance at a 1% level from frequencies of these antigens in normal subjects of the control group, without correction for

TABLE 2. *Frequency of HLA antigen in 31 tuberculoid leprosy patients and in 28 lepromatous leprosy patients.*

HLA antigen	Tuberculoid leprosy patients		Lepromatous leprosy patients		
	No.	%	No.	%	X^2
A1	0	0	0	0	
A2	10	32.3	8	28.6	
A3	0	0	0	0	
A9	15	48.4	12	42.9	
AW23	1	3.2	3	10.7	
AW24	6	19.4	9	32.1	1.27
A10	7	22.6	7	25.0	
AW25	1	3.2	0	0	
AW26	1	3.2	0	0	
A11	2	6.5	3	10.7	
A29	0	0	0	0	
AW30	0	0	0	0	
AW31	1	3.2	6	21.4	3.08
AW32	0	0	0	0	
B5	5	16.1	5	17.9	
B7	4	12.9	2	7.1	
B8	4	12.9	2	7.1	
B12	1	3.2	3	10.7	
B13	1	3.2	0	0	
B14	2	6.5	0	0	
B18	1	3.2	1	3.6	
B27	2	6.5	0	0	
BW15	3	9.7	4	14.3	
BW17	0	0	0	0	
BW21	1	3.2	2	7.1	
BW22	4	12.9	3	10.7	
BW35	8	25.8	8	28.6	
BW37	0	0	0	0	
BW38	0	0	0	0	
BW39	3	9.7	5	17.9	
BW40	8	25.8	7	25.0	

Differences between tuberculoid and lepromatous leprosy patients are not significant.

the large number of antigen comparisons made.

However, studies by Thorsby *et al* ([18]) of 39 patients showed an increased frequency of BW21 antigen in leprosy patients as compared to the frequency of individuals of a control group of Africans of Caucasian-Negroid extraction. Also results of studies by Smith *et al* ([15]) indicated a possible association of A10 antigen with leprosy in Filipinos. However, the association of BW21 and A10 with leprosy was not found in the present study.

Dasgupta *et al* ([3]) reported an increased frequency of B8 and a decreased frequency of A9 in leprosy patients tested in India for the presence of 11 antigens. These findings were confirmed in our study.

In the present study, no significant difference was found between the predisposition of HLA antigens in patients with tuberculoid leprosy and that in patients with lepromatous leprosy. However, the specificity of B8 and BW39 was found only in leprosy patients. The frequency of these antigens has been found to be very low among the Japanese.

Findings obtained in the present study on AW24 antigen are of great interest, since this antigen has not been tested in earlier studies by other investigators. The AW24 antigen is one of the subclasses of A9 antigen, therefore the increased frequency of A9 may be influenced by the elevated frequency of AW24.

A close connection between A1 and B8 among members of the Caucasian race is well known and a number of diseases have also been reported to be associated with these antigens ([2]). Since A1 and B8 are nonexistent among Japanese, a significantly higher frequency of B8 in leprosy patients than in controls observed in this study is of considerable interest. Moreover, different results of frequencies of HLA antigens observed in earlier studies of Africans, Filipinos, Indians and Mexicans ([4]) may be due to racial differences or to different antisera used. Study of many more cases as well as a follow-up study are required in order to determine whether there is an association between HLA antigens and leprosy.

SUMMARY

Fifty-nine leprosy patients (31 tuberculoid, 28 lepromatous) have been HLA typed and compared to 125 healthy individuals who have had no known contact with leprosy patients in the past. HLA antigens were determined by the microdroplet lymphocyte toxicity method developed by Prof. Terasaki of UCLA. In order to detect 31 HLA antigens, a total of 58 antisera were used in a No. T-7 research tray.

AW24 antigen showed the most remarkable deviation. The frequency of this antigen in leprosy patients was 25.4% and that in normal subjects of the control group was 63.2%. The difference in frequency between the two groups was statistically significant when the p value was corrected for the number of antigens being tested. Uncorrected p values of the BW39, A9, and B8 antigens were also statistically significant. Frequencies of the majority of HLA antigens in tuberculoid leprosy patients were similar to those of corresponding HLA antigens in lepromatous leprosy patients.

RESUMEN

Se tipificaron 59 pacientes con lepra (31 tuberculoides y 28 lepromatosos) en cuanto a sus antígenos HLA y los resultados se compararon con los obtenidos en 125 individuos sanos quienes nunca antes habían estado en contácto con enfermos de lepra. Los antígenos HLA se determinaron por el micrométodo de citotoxicidad desarrollado por el Profesor Terasaki de la UCLA. Se utilizaron 58 antisueros con los que se pudieron detectar 31 antígenos HLA en placas de microcitotoxicidad No. T-7.

El antígeno AW24 mostró la desviación más aparente. La frecuencia de este antígeno en los pacientes con lepra fue del 25.4% en tanto que en el grupo control fue del 63.2%. La diferencia en las frecuencias de los grupos comparados fue estadísticamente significativa cuando el valor de *p* se corrigió por el número de antígenos probados. Los valores sin corregir de *p* para los antígenos BW39, A9 y B8, también fueron estadísticamente significativos. La mayoría de los antígenos HLA tuvieron una frecuencia similar en los pacientes con lepra tuberculoide y en aquéllos con lepra lepromatosa.

RÉSUMÉ

Cinquante-neuf malades de la lèpre (31 tuberculoïdes, 28 lépromateux) ont été étudiés en ce qui concerne le type de HLA; ils ont été comparés à 125 témoins en bonne santé qui n'avaient aucun antécédent de contact connu avec des ma-

lades de la lèpre. Les antigènes HLA ont été déterminés par la méthode de toxicité d'une micro-gouttes pour les lymphocytes développés par le Professeur Terasaki de UCLA. Dans le but d'identifier 31 antigènes HLA, un total de 58 antisera ont été utilisés dans un plateau de recherche n° T-7.

L'antigène AW24 a présenté la déviation la plus remarquable. La fréquence de ces antigènes chez les malades de la lèpre était de 25,4%; la fréquence chez les sujets normaux du groupe témoin était de 63,2%. La différence de fréquence entre les deux groupes était statistiquement significative, lorsque les valeurs de p étaient corrigées pour le nombre d'antigènes étudiés. Les valeurs de p non corrigées, étaient également statistiquement significatives pour les antigènes BW39, A9 et B8. Chez des malades atteints de lèpre tuberculoïde, les fréquences observées pour la majorité des antigènes HLA étaient similaires à celles des antigènes HLA correspondants chez des malades souffrant de lèpre lépromateuse.

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