INTERNATIONAL JOURNAL OF LEPROSY Volume 53, Number 1
Printed in the U.S.A.

HLA-linked Control of Predisposition to Lepromatous Leprosy[1]

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The discovery of immune response genes linked to the major histocompatibility complex (MHC) ([7]), combined with the insight that the outcome of an infection with *Mycobacterium leprae* paralleled the cellular immune reactivity of the host to the bacillus ([5]), led to a considerable number of studies investigating the relationship between leprosy or leprosy type and HLA, the MHC of man ([3, 8]). Whereas population studies yielded some interesting but equivocal results, family studies provided evidence for a recessive HLA-linked gene predisposing to tuberculoid leprosy ([1, 2, 4, 8, 11]). No data or only a small amount of data ([6, 8]) were collected for the other leprosy types until very recently, when a family study conducted in Venezuela provided convincing evidence for HLA-linked control of predisposition to lepromatous leprosy as well (van Eden, *et al.*, unpublished). This study also showed clearly that these HLA-linked genes did not confer susceptibility to leprosy per se but, rather, determined the type of the disease to develop, as already suggested earlier ([3, 8, 10]).

The present family study has been undertaken to investigate the relationship between HLA and the susceptibility to leprosy or the predisposition to particular leprosy types in an endemic area of China. Considering the recent population study conducted in this area which indicated an association between lepromatous leprosy and HLA-DR2 ([13]), we were particularly interested in whether the HLA-linked control of predisposition to lepromatous leprosy indicated by the Venezuelan study could be confirmed. Furthermore, we have typed complete two-generation families in order to discover if susceptibility to or resistance to leprosy is controlled by HLA-linked genes. Finally, we have performed lepromin tests on eight whole families, containing at least two patients affected with lepromatous leprosy, in order to investigate whether the lepromin test results in healthy siblings are controlled by HLA-linked genes.

MATERIALS AND METHODS

Families. With the help of many local doctors, 28 two-generation families were selected on the basis of the following criteria: a) at least two siblings had to be affected with leprosy, and b) at least one healthy sibling older than the youngest affected sibling had to be present. Four more families (families 2, 15, 16, and 19) which did not fulfill the second criterion but contained at least two healthy siblings or siblings affected with different types of leprosy were included in the study. The mean age of the parents was 68 years (range 49–81) and of the children, 38 years (range 14–62). In the majority (21) of the families one parent had died, and in five families one of the children had died. These 32 families contained 71 leprosy patients of whom only 4 were parents. The mean age at onset of the disease among the children was 17.3 years (range 5–31); the mean elapsed time between the onset of the disease and the present study was very long, 22.4 years (range 2–39). All of the families live in a leprosy endemic area of the Jiangsu Province of China, and belong to the Han nationality.

Leprosy classification. All family members were checked for signs of leprosy by an experienced leprologist, and no new cases

[1] Received for publication on 3 April 1984; accepted for publication in revised form on 23 August 1984.

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of leprosy were discovered. The leprosy classification was based on the records which were present in all cases. The majority of the patients had originally been classified according to the Madrid classification, but for this study the patients were re-classified according to the Ridley-Jopling classification before HLA typing started. Although the adequate descriptions of pretreatment biopsies were only available for 32% of the patients and lepromin tests had only been performed in three cases before the present study, the clinical description was sufficient in most cases and bacteriological data were nearly always present. We felt confident with the Ridley-Jopling classification except for 6 patients who could only be classified as either tuberculoid (4 patients) or lepromatous leprosy (2 patients).

Lepromin testing. In eight families containing at least two siblings with lepromatous (BL or LL) leprosy (families 9, 11–15, 17, and 26), we performed a lepromin test in (nearly) all family members. The lepromin used was prepared at the National Hansen's Disease Center, Carville, Louisiana, U.S.A., from *M. leprae*-infected armadillos, contained 40×10^6 bacilli/ml, and was made available through the World Health Organization (WHO). After four weeks the mean diameter (in mm) was recorded. In LL patients the diameter was 0–1 mm, and this varied from 0–3 mm among BL patients.

HLA typing. Heparinized blood was drawn by venipuncture from all living parents and 151 of the 163 living children. Mononuclear cells were isolated on Ficoll-Isopaque gradients, and the HLA-A and HLA-B typing was performed at the Institute of Dermatology, Taizhou, according to the standard U.S. National Institutes of Health (NIH) micro-lymphocytotoxicity technique ([12]). A set of 60 well-defined sera was used, which allowed the assignment of 11 HLA-A and 20 HLA-B specificities. HLA-DR typing was performed also in Taizhou on the cells of about half the individuals. The results of the DR typing, which were completed in Leiden, including those of a sample of unrelated sporadic cases of lepromatous leprosy, will be reported separately.

Complete data of HLA-A and HLA-B phenotypes were available for 29 families. In three families we were not able to definitely assign the parental haplotypes from the HLA phenotypes, and these three families were excluded from the analysis. In another three of the remaining 26 families, the haplotypes of one parent (families 6 and 12) or both parents (family 14) could be assigned with the use of the results of DR-typing. In those families where one of the parents had died, we were always able to deduce the haplotypes of the deceased parent from the phenotypes of the children.

Statistical analysis. Deviations from randomness of the observed HLA-haplotype segregations in relation to leprosy status, indicating the involvement of HLA-linked genes in the susceptibility to leprosy and/or the development of particular leprosy types, were analyzed with the method developed by Nijenhuis ([1]). With this method, the observed difference between the number of siblings who inherited one haplotype from a parent and those who inherited the other haplotype (D) is compared with the expected difference (d), assuming random segregation. The method allows pooling of data from sibships of different sizes. The statistical test used is

$$\chi^2_{\nu=1} = [|\Sigma D - \Sigma d| - 0.5]^2 \div \Sigma\sigma^2 d$$

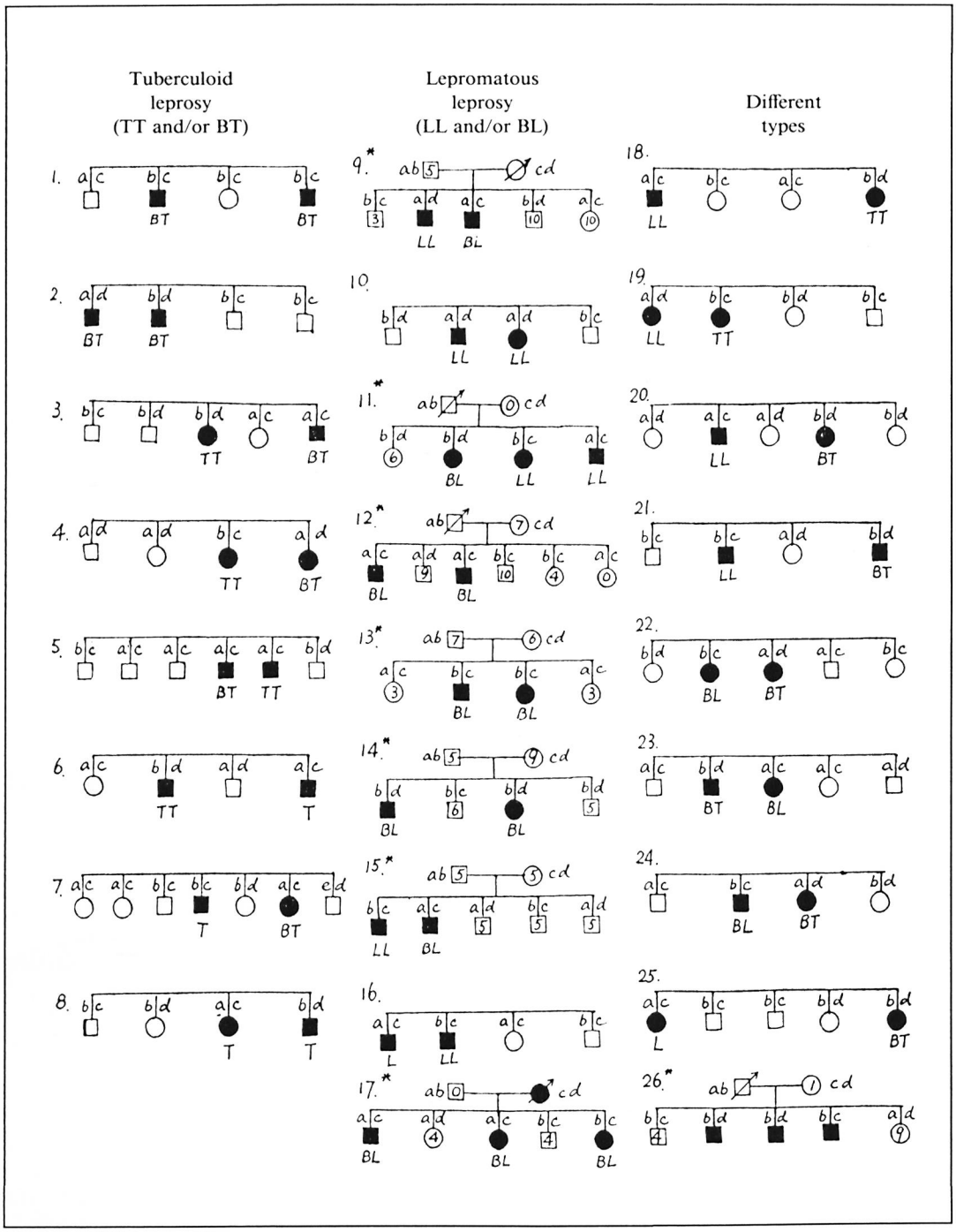
A variant of this method was used to analyze the occurrence of those haplotypes that are shared among all leprosy patients of a given sibship (S) among the healthy siblings of the same sibship. The expected number of healthy siblings carrying S was derived from the binomial distribution for $p = 0.50$ from which also d and $\sigma^2 d$ were derived ([1]). In this case the expected number of siblings carrying S (s) is simply the number of healthy siblings divided by 2 and the variance of $s = \frac{1}{2}s$

$$\chi^2_{\nu=1} = [|\Sigma S - \Sigma s| - 0.5]^2 \div \Sigma\sigma^2 s$$

The same methods were used to analyze the segregation of the lepromin test results in families 9, 11–15, 17, and 26.

RESULTS

The Figure shows the parental HLA-haplotype segregation and the leprosy status of the children from the 26 families which could be used for the analysis of the segre-



THE FIGURE. Leprosy status and HLA haplotypes of multicase leprosy families.

□ = healthy male ○ = healthy female

■ = leprosy male ● = leprosy female

⊠ = deceased male ⊘ = deceased female

a, b, c, d = HLA haplotypes (father = ab, mother = cd).

* = whole family tested with lepromin A.

[5], ③, etc. = lepromin test result, expressed as mean diameter in mm.

TABLE 1. *Analysis of parental HLA haplotype segregation observed among siblings in relation to leprosy status.*

Sibships analyzed	Family no. (see Fig.)	Σ D	Σ d	σ^2d	χ^2	p
Total sibships	1–26	84	87	90.6	0.07	N.S.[a]
Leprosy, irrespective of type	1–26	46	55	50.5	1.43	N.S.
Tuberculoid leprosy	1–8	12	16	16	0.77	N.S.
Lepromatous leprosy	9–17	28	20	17	3.31	0.05[b]
LL/LL or BL/BL	9–14, 17	24	15	13.5	5.35	0.01[b]
(B)L/LL	11, 15, 16	6	7	5.5	0.05	N.S.
Different leprosy types (L/T)	18–26	2	16	16	11.39	0.0005[b]
Healthy siblings	1–26	54	60	51	0.59	N.S.

[a] Not statistically significant.
[b] One-sided test, p value divided by two.

gation of parental HLA haplotypes in relation to leprosy status observed among the children. The results of the lepromin test (mean diameter in mm) among healthy family members are also indicated (families 9, 11–15, 17, and 26). (A list of the HLA-A and HLA-B genotypes of the parents of these 29 families may be obtained from any of the authors on request.) In one family (family 7) we observed an extrapaternal child who was excluded from the analysis. In one family (family 26) the results of the lepromin tests were incompatible with the clinical classifications; therefore, we only analyzed the healthy siblings of that family.

Only four parents were or had been affected with leprosy. Moreover, of at least three different types, namely, the father of family 3 with BL, the father of family 4 with BT, the father of family 22 with TT, and the mother of family 17 with T leprosy. Therefore, we did not take into account the leprosy status of the parents for the segregation analysis presented here.

The most important results of the analysis of parental HLA-haplotype segregation as observed among children in relation to their leprosy status are shown in Table 1. We first checked whether or not segregation of parental HLA haplotypes as observed among all children tested (both healthy and those affected with leprosy) occurred randomly, and it did indeed appear to be the case. This meant that a necessary assumption (i.e., random segregation) for the method of analysis used had been shown to be present in the material. We also observed a random segregation of parental HLA haplotypes among all siblings with leprosy, irrespective of type.

The families may be divided into three categories. The haplotype segregation observed in the first category of families, namely, those containing siblings affected with TT and/or BT leprosy (families 1–8), did not deviate from randomness. There were no families in which all affected children had polar tuberculoid (TT) leprosy. In two families (families 1 and 2) both children were affected with BT leprosy and they tended to share more often than expected a parental haplotype. In contrast to these two families, the remaining six families, which lacked siblings with a definitely identical Ridley-Jopling classification, showed the reverse pattern. However, neither of the latter two observations was significant.

The second category of families consisted of those with at least two siblings affected with lepromatous (BL and/or LL) leprosy. Irrespective of their Ridley-Jopling classification, the siblings affected with lepromatous leprosy (BL and/or LL) shared parental haplotypes more often than expected ($p < 0.05$). When the Ridley-Jopling classification was taken into account, however, the data showed heterogeneity. As to the combined results of those families in which the affected siblings had both LL or both BL leprosy, the haplotype sharing was more obvious ($p < 0.01$); whereas in those families in which one sibling was affected with LL and the other(s) with BL (families 11, 15, 16?), no significant deviation from random segregation was observed.

The third category of families consisted

TABLE 2. *Analysis of presence among healthy siblings*[a] *of those HLA haplotypes that are shared among all leprosy patients of that sibship.*

	Σ S[b]	Σ s[c]	p
Present study	16	13	N.S
Fine, *et al.* (6)	40	40.5	N.S
van Eden, *et al.* (Venezuela)	22+	24+	N.S
Total	78	77.5	N.S

[a] Healthy siblings older than youngest affected.
[b] S = observed number of healthy siblings carrying a haplotype shared by all siblings affected with leprosy.
[c] s = expected number of healthy siblings carrying that haplotype.

of sibships in which one sibling was affected with tuberculoid (TT or BT) and the other(s) with lepromatous (BL or LL) leprosy (families 18–25). These siblings shared a parental haplotype very significantly less than expected ($p < 0.0005$). In the majority of these families the tuberculoid sibling was classified as BT (families 20–25). Also in those families the same result which was again significant ($p < 0.005$) was observed.

Table 1 also shows the results of the analysis of the haplotype segregation observed among healthy siblings of all the families, which did not differ significantly from randomness. This was also true when analyzed separately for the three above-mentioned categories, and for only those healthy siblings older than the youngest affected siblings.

In order to answer more specifically the question of whether or not genes conferring susceptibility to leprosy are linked to HLA, we performed an additional analysis, the results of which are shown in Table 2. If genes conferring susceptibility to leprosy were linked to HLA, we would expect that those HLA haplotypes shared among all leprosy patients in a given sibship occurred less often than expected among the healthy siblings of that sibship. However, this is not the case, neither in all healthy siblings nor when only those siblings older than the youngest affected sibling were analyzed. The same was true when this analysis was performed on the data of the study by Fine, *et al.* (4) and the Venezuelan study by van Eden, *et al.* (unpublished).

Finally, we analyzed the co-segregation of parental HLA haplotypes and the lepromin test results in eight of the families in which at least two siblings were affected with lepromatous (BL and/or LL) leprosy (families 9, 11–15, 17, and 26). The data are few, particularly for lepromin-negative healthy siblings (The Figure). However, when we compared the results among healthy siblings and when the lepromin test results among healthy siblings were compared with the lepromin-negative lepromatous sibs in relation to HLA type, no evidence for co-segregation with HLA haplotypes was obtained.

DISCUSSION

This study has provided three sets of data: the results of the analysis of HLA haplotype segregation observed in patients, the results of the segregation observed in healthy siblings, and the lepromin data.

The excess of shared HLA haplotypes observed among sibships containing siblings affected with either BL or LL leprosy confirms a very recent observation, the HLA-linked control of the development of lepromatous leprosy shown in Venezuelan families (van Eden, *et al.*, unpublished). Whereas in the Venezuelan study only five children affected with BL leprosy were included, the data on BL leprosy in the present study indicate that the HLA-linked control of predisposition to lepromatous leprosy is probably not confined to LL leprosy. The random segregation, however, observed in those sibships in which one sibling is affected with BL and the other(s) with LL leprosy may suggest genetic heterogeneity between BL and LL leprosy. The significant deficit of shared HLA haplotypes observed among siblings discordant for leprosy type (tuberculoid including BT versus lepromatous including BL) combined with the apparently random segregation among sib-pairs affected with tuberculoid leprosy may be explained in two ways.

The first explanation is simple, namely, that only one HLA-linked gene predisposing to lepromatous leprosy is segregating in these families. The lack of this gene would then lead to tuberculoid leprosy and in the families with only tuberculoid leprosy, the gene might be absent. The second explanation is more complicated but should certainly also be considered because of previous studies showing excess sharing of HLA

haplotypes among affected siblings in families with only polar tuberculoid leprosy ([1, 2, 4, 8]) and genetic heterogeneity between TT and BT leprosy ([11]). For this second explanation we have to postulate that at least three different HLA-linked genes control leprosy type: one for TT, one for BT, and at least one for L. A fourth gene might even be necessary to explain the genetic heterogeneity between BL and LL leprosy suggested in the present study.

The second set of data consists of the parental HLA haplotype segregation observed among the healthy children. The present study is especially suited to analyze this point because of the nearly complete ascertainment of the sibships. The purpose of this analysis is to see whether only predisposition to certan leprosy types or also susceptibility and/or resistance to leprosy per se is controlled by HLA-linked genes. The random segregation observed among the healthy siblings of all three categories of families together with the random segregation among all children affected with leprosy, irrespective of leprosy type, already suggests that the latter does not seem to be the case. This random segregation is also observed when only those siblings older than the youngest affected sibling—who may be the most informative ones—are analyzed. Supposing one susceptibility and/or resistance gene for leprosy, in all of the cases mentioned one would expect to observe less often among the healthy siblings of a given sibship those haplotypes shared among all patients of the same sibship. This was clearly not the case, neither in the present study nor in a re-analysis of two previous studies ([4] and van Eden, *et al.*, unpublished). Therefore, one could envision that several different HLA-linked genes control both susceptibility to and the type of leprosy, as originally suggested by de Vries, *et al.* ([1]).

Finally, we did not find evidence for co-segregation of the lepromin test results and HLA haplotypes. Although we have to be careful with this conclusion because there are only few data, this could be compatible with the lack of evidence for co-segregation of susceptibility to leprosy and HLA discussed previously and with the lack of evidence for co-segregation of the *in vitro* low responsiveness to *M. leprae* and HLA as reported by Stoner, *et al.* ([9]). These observations indicate that cellular nonresponsiveness to *M. leprae* observed among healthy individuals may be controlled by factors different from those in the nonresponsiveness observed among lepromatous leprosy patients. Before arriving at a more logical conclusion with lepromin test results, more studies are needed.

We conclude from this study that: a) predisposition to lepromatous leprosy is controlled by HLA-linked genes, and b) HLA-linked genes do not confer susceptibility to or resistance to leprosy per se and an apparently different genetic background exists in tuberculoid and lepromatous leprosy.

SUMMARY

In order to investigate if genes conferring susceptibility to leprosy and/or predisposition to a particular leprosy type are linked to HLA, we performed HLA-A and HLA-B typing on members of 29 families containing at least two siblings affected with leprosy from a leprosy endemic area in Jiangsu Province, People's Republic of China. Moreover, we performed a lepromin test in nearly all of the members of eight families containing at least two siblings affected with lepromatous leprosy. For 26 families the data permitted analyses of the segregation of parental HLA-haplotypes observed among children in relation to leprosy status. Siblings affected with lepromatous (LL or BL) leprosy shared parental HLA-haplotypes significantly more often than expected ($p < 0.05$), and a highly significant deficit ($p < 0.0005$) of shared HLA-haplotypes was observed among affected siblings discordant for leprosy type. These data confirm the HLA-linked control of leprosy type and, in particular, recently obtained evidence for linkage of predisposition to lepromatous leprosy with HLA. Healthy siblings did not share a haplotype more often than expected, and those haplotypes which were shared among all leprosy patients of a sibship did not occur less frequently than expected among the healthy siblings of the same sibship. The latter data confirm the absence of linkage of genes conferring susceptibility or resistance to leprosy with HLA. An analysis of the lepromin test results observed among healthy siblings showed no evidence for co-segregation of the results with HLA.

RESUMEN

Para investigar si los genes que confieren susceptibilidad a la lepra y/o la predisposición a un tipo particular de la misma están ligadas al HLA, hicimos la tipificación de HLA-A y HLA-B, en los miembros de 29 familias con al menos dos casos (hermanos) afectados por la lepra en un área endémica de la Provincia de Jiangsu de la República Popular China. Además, efectuamos la prueba de la lepromina en casi todos los miembros de ocho familias con al menos dos hijos afectados por la lepra lepromatosa. En 26 familias, los datos permitieron el análisis de la segregación de los haplotipos HLA de los progenitores entre los hijos en relación a la condición leprosa. Los hijos afectados con lepra lepromatosa (LL o BL) compartieron los haplotipos de los padres con mayor frecuencia de los esperado ($p < 0.05$). Los hijos con tipos discordantes de lepra tuvieron un déficit altamente significativo ($p < 0.0005$) de haplotipos compartidos. Estos datos confirman que el control del tipo de lepra y la predisposición al estado lepromatoso están ligados al HLA. Los hermanos sanos compartieron un determinado haplotipo con la frecuencia esperada y aquellos haplotipos que fueron compartidos entre todos los pacientes con lepra de una familia, tuvieron una frecuencia similar a la encontrada entre los hermanos sanos de la misma familia. Los últimos datos confirman la ausencia de enlazamiento entre HLA y genes que confieren susceptibilidad o resistencia a la lepra. El análisis de los resultados de la prueba de la lepromina en los hermanos sanos no mostró evidencias de co-segregación de los resultados con el HLA.

RÉSUMÉ

Dans le but de mettre en évidence une liaison ("linkage") des gènes déterminant la susceptibilité à la lèpre avec le système HLA, de même qu'une prédisposition éventuelle à un type particulier de la maladie, on a procédé à des déterminations de HLA-A et de HLA-B chez les membres de 29 familles comptant au moins deux germains atteints de lèpre. Cette étude a été menée dans une région endémique pour la lèpre de la Province de Jiangsu, en République populaire de Chine. On a également procédé à une épreuve à la lépromine chez la plupart des membres de huit familles comprenant au moins deux germains atteints de lèpre lépromateuse. Chez 26 familles, les données obtenues ont permis de procéder à une analyse de la ségrégation chez les enfants des haplotypes HLA des parents, selon que ceux-ci et ceux-là étaient ou non atteints de lèpre. Les germains atteints de lèpre lépromateuse (LL ou BL) partageaient plus souvent les haplotypes HLA des parents. Cette différence était statistiquement significative ($p < 0.05$). Par contre, on a observé un pourcentage beaucoup plus faible d'haplotypes HLA communs chez les germains atteints de la maladie mais présentant des types différents de celle-ci. Cette différence était également significative ($p < 0.0005$). Ces données confirment que le type de lèpre est déterminé par le système HLA. Ces résultats sont en accord avec des observations récentes indiquant que le système HLA est impliqué dans la prédisposition à la lèpre lépromateuse. Les fréquences observées dans le partage d'un même haplotype chez des germains en bonne santé ne dépassaient pas les valeurs statistiquement attendues. Quant aux haplotypes communs à tous les frères et soeurs atteints de lèpre dans une même famille, ils ne présentaient pas une fréquence significativement plus basse chez les germains en bonne santé de cette famille. Ces données confirment l'absence de groupes de liaison avec le système HLA des gènes conférant la susceptibilité ou la résistance à la lèpre. Une analyse des résultats des épreuves à la lépromine pratiquée chez les germains en bonne santé n'a révélé aucune indication permettant de conclure à la co-ségrégation des valeurs observées en fonction du système HLA.

Acknowledgments. The basis for this Sino-Dutch collaborative project was laid during a visit by Professor van Rood to China in 1979. Since then many Chinese researchers and leprologists have done a lot of work to allow the present study to be completed successfully. Drs. Tao Mingbo, Wang Chengming, Wang Chuanji, and Yang Zhongmin did a major job in the organization, and excellent technical assistance was provided by Messrs. Ma Ling, Xue Zhengyong, and Zhou Wuqing, and Miss Zhao Yuping, under the supervision of Dr. Fan Lian. Professors Yang Lihe and Meng Meibai checked the records of the patients.

This project received financial and material (lepromin A) support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, the Chinese Academy of Medical Sciences, the Dutch Foundation of Medical Research (FUNGO), and the Dutch Ministry of Onderwijs en Wetenschappen.

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