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HLA Linked with Leprosy in Southern China; HLA-Linked Resistance Alleles to Leprosy[1]

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Leprosy is a chronic infectious disease caused by the acid-fast bacillus *Mycobacterium leprae*. It is still a serious endemic disease in the great majority of countries in Asia, Africa, and Latin America. The endemic areas of this disease in China are nearby the Yangzi River, the Shannan area, the Yungui highlands, the Sichuan mountains, and Tibet.

Many investigators have tried to find an association between leprosy and genetic markers, with the purpose of identifying susceptibility genes. Much attention has been paid to the major histocompatibility complex (MHC) also called human leukocyte antigen (HLA). Previous reports of serologic HLA analysis showed the associations with the HLA-DR2, DQ1 and DR53 in Japanese, Korean, Indian, Turkish, Egyptian, Greek and Southern Brazilian populations ([2, 6, 8, 12, 14]). Recent progress in HLA analysis has provided methods for analyzing the polymorphisms in HLA genes, i.e., the HLA alleles, at the DNA level. There are several reports showing the positive associations with specific HLA alleles: DRB1*1501, *150, *0405, *0803 and *0901, DRB5*0101 and *0102, DQA1*03, and DQB1*0401 in Japanese ([7]), DRB1*1501, *1502, *0404, *0701 and *1401, DRB5*0101 and *0102, DQA1*0102 and *0103, and DQB1*0601 and*0503 in North Indians ([11]).

However, many studies identified HLA class I associations with resistance to leprosy in addition to the well-known association with the HLA class II genes ([1, 2, 13]). These reports revealed associations with different markers in the different populations. Previously we analyzed 72 southern Chinese leprosy patients compared with 267 southern Chinese healthy volunteers as controls by serologic HLA typing, and found that the frequency of the HLA-B46 antigen was significantly decreased in the patients ([15]), suggesting that there might be an HLA-B-linked disease resistance. The present paper might be the first report on the association between leprosy and HLA-B antigens in a southern Chinese population.

On the other hand, a distinct family of the MHC class I gene recently has been identified within the human MHC class I region. The MICA (MHC class I chain-related A) gene in this family is a highly divergent member of the MHC class I family and has a unique pattern of tissue expression. The MICA molecule is polymorphic, and the high degree of linkage disequilibrium between the alleles of the MICA and HLA-B genes compels reevaluation of data linking several diseases to HLA class I genes, diseases such as Behcet's disease, acute anterior uveitis, ankylosing spondylitis, and several other inflammatory diseases. It is very important to investigate a possible correlation between MICA polymorphism and the development of these diseases ([4, 5, 10]). In addition, recent findings that the MICA molecule is recognized by gamma delta T cells and that the expression of the MICA molecule is induced by cell stress, as are the heat-shock proteins, have suggested that the MICA gene might be involved in the pathogenesis of chronic infectious disease ([3]).

Until now, association studies between leprosy and the HLA genes have not been

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TABLE 1. *Gene frequencies of HLA alleles in multibacillary (MB) or paucibacillary (PB) leprosy and controls.*

HLA[a]	Leprosy (2N = 138)	MB (2N = 100)	PB (2N = 38)	Controls (2N = 224)
B13	14 (0.101)	10 (0.100)	4 (0.105)	20 (0.089)
B27	4 (0.029)	3 (0.030)	1 (0.026)	6 (0.027)
B35	10 (0.072)	7 (0.070)	3 (0.079)	13 (0.058)
B37	1 (0.007)	1 (0.007)		2 (0.009)
B39	5 (0.036)	5 (0.036)		3 (0.013)
B46	10 (0.072)	4 (0.040)[b]	6 (0.185)[c]	29 (0.129)
B48	6 (0.043)	4 (0.040)	2 (0.058)	9 (0.039)
B51	5 (0.036)	5 (0.036)		13 (0.058)
B52	2 (0.015)	1 (0.010)	1 (0.026)	4 (0.018)
B54	3 (0.022)	2 (0.020)	1 (0.026)	9 (0.039)
B55	4 (0.029)	4 (0.029)		7 (0.031)
B56	4 (0.029)	4 (0.029)		3 (0.013)
B58	25 (0.181)	17 (0.170)	8 (0.211)	29 (0.130)
B60	23 (0.167)	18 (0.180)	5 (0.132)	40 (0.179)
B61	2 (0.015)	2 (0.015)		1 (0.004)
B62	13 (0.094)	10 (0.100)	3 (0.079)	28 (0.125)
B67	1 (0.007)	1 (0.007)		3 (0.013)
B75	1 (0.007)	1 (0.007)		3 (0.013)
blank	1 (0.007)	1 (0.007)		3 (0.013)
DRB1*1501	20 (0.145)	13 (0.130)	7 (0.184)	22 (0.098)
DRB1*1502	3 (0.022)	3 (0.030)		1 (0.005)
DRB1*1602	5 (0.036)	5 (0.050)		6 (0.027)

[a] HLA = Human leukocyte antigen.
[b] RR = 0.28, p <0.01, MB patients vs controls.
[c] RR = 0.22, p <0.02, PB patients vs MB patients.

performed in a southern Chinese population. To determine the association of HLA with leprosy and its subgroups, we examined 69 southern Chinese patients with leprosy and 112 healthy controls for the HLA-B and MICA genes by DNA typing using the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) method.

MATERIALS AND METHODS

Patients. Sixty-nine southern Chinese leprosy patients (59 males and 10 females) were selected for this study from Shantou, Chaoyang and Jieyang leprosy hospitals. Forty of them were lepromatous (LL), 10 were borderline lepromatous (BL), 15 were tuberculoid (TT), and 4 were borderline tuberculoid (BT). They were divided into two groups: MB (multibacillary, LL and BL) and PB (paucibacillary, TT and BT). All patients included in this study were free from other infectious diseases and received multidrug therapy (MDT) according to the recommendations of the World Health Organization (WHO). Their ages ranged from 22 to 84 years old (mean 63 years) with a mean age of 36 years at the time of diagnosis. The 112 healthy control subjects (54 males, 58 females; 18–70 years old) were normal unrelated individuals selected according to nation, occupation and geographical origin of the patients.

DNA typing of HLA genes by PCR-SSCP. DNA was extracted from peripheral blood granulocytes of each subject following the protocol of the 11th International Histocompatibility Workshop (9). The second exon of HLA genes of the genomic DNA sample was amplified by PCR with primer pairs as follows: a) for DR2-DRB1, DRB-Amp A (5′-CCGCTGCACTGTGAAGCTCT-3′) and DRB-Amp B (5′-TTCCTGTGGCAGCCTAAGAGAGG-3′); b) for Bw4, CG5 (5′-GACGACACGCTGTTCGTGA-3′) and CG2 (5′-GCTCTGGTTGTAGTAGCGGA-3′); c) for Bw6, CG5 and CG3 (5′CTCTGGTTGTAGTAGCCGC-3′); d) for exon 5 of MICA, MIC5F (5′-GCCCAGTGTATAACAAGT- CC-3′) and MIC5R (5′-GCCTTACCATCTCCAGAAAC-3′).

After PCR, the samples were heat denatured in the presence of 80% formamide at 95°C for 5 min, immediately chilled in ice

TABLE 2. *Gene frequencies of the microsatellite polymorphism in the exon 5 of the MICA[a] alleles in MB or PB leprosy groups in comparison with controls.*

Microsatellite repeats	Leprosy (2N = 138)	MB (2N = 100)	PB (2N = 38)	Controls (2N = 224)
A4	15 (0.109)	12 (0.120)	3 (0.079)	30 (0.134)
A5	30 (0.217)[b]	20 (0.200)[c]	10 (0.263)	69 (0.308)
A5.1	46 (0.333)	33 (0.330)	13 (0.342)	65 (0.290)
A6	7 (0.051)	7 (0.070)		13 (0.058)
A9	41 (0.297)	29 (0.290)	12 (0.316)	47 (0.210)

[a] MICA = Major histocompatibility complex (MHC) class I chain-related A.
[b] RR = 0.62, p = 0.06, leprosy patients overall vs controls.
[c] RR = 0.56, p <0.05, MB patients vs controls.

water for 5 min and electrophoresed in an 8% polyacrylamide gel in 0.5 × TBE at 13 V/cm for 4 hr at room temperature. DNA fragments were detected by using a silver-staining kit according to the manufacturer's instruction.

Haplotype analysis was based on population studies referring to the linkage disequilibria values for two-loci (HLA-B/MICA) in southern Chinese.

Statistical analysis. Statistical analysis for the comparison of the frequencies of the HLA alleles, MICA alleles, and HLA-MICA haplotypes between the patient and the control groups was carried out by a chi-squared test with Yates' correction and relative risk (RR) was calculated following Woolf's formula ([16]). When the p value was less than 0.05, the difference was considered to be significant.

RESULTS

HLA-DR2 DNA typing in leprosy. The antigen frequency of HLA-DR2 was slightly increased in the patients (18.1% in patients vs 12.9% in controls), but it was not statistically significant (RR = 1.63, χ^2 = 2.18). The gene frequencies of DR2-DRB1 alleles, i.e., DRB1*1501 (0.145 vs 0.098), *1502 (0.022 vs 0.005), and *1602 (0.036 vs 0.027), did not show any significant differences between the patients and controls (Table 1).

HLA-B alleles in leprosy. The frequencies of HLA-B alleles are shown in Table 1. In the MB patients, a significantly decreased allele frequency of B46 was observed as compared with healthy controls (0.040 vs 0.129). The calculated RR for B46 was 0.28 (p <0.01). The allele frequency of B46 in the MB patients in comparison with the PB patients was also significantly decreased (0.040 vs 0.185, RR = 0.22, p <0.02). The frequency of B46 was not significantly different between the PB patients and controls.

MICA typing in leprosy. The frequency of the A5 allele showed a decreasing tendency in the patients with leprosy as compared to the controls (0.217 vs 0.308), but the difference was not significant (RR = 0.62, p = 0.06). However, as shown in Table 2, the frequency of the A5 allele was significantly decreased in the MB patients (0.200 vs 0.308, RR = 0.56, p <0.05) but not in the PB patients.

HLA-B/MICA haplotype in leprosy. As shown in Table 3, a negative association between the HLA/MIC haplotype and leprosy was observed for the HLA-B46/A5 haplotype (0.101 vs 0.223, RR = 0.37, p <0.03). Its frequency was significantly decreased in the patients with leprosy, especially in the MB patient group (0.006 vs 0.223, RR = 0.22, p <0.01). In contrast, no significant associations between leprosy and the non-B46/A5, and B46/non-A5 haplotypes were observed in the leprosy patients overall or in any of the clinical subgroups (Table 3).

TABLE 3. *HLA-B/MICA haplotypes in leprosy or MB group and controls.*

HLA/MICA	Leprosy (N = 69)	MB (N = 50)	Control (N = 112)
B46/A5	7 (0.101)[a]	3 (0.060)[b]	26 (0.223)
Non-B46/A5	23 (0.333)	17 (0.340)	43 (0.384)
B46/non-A5	3 (0.044)	3 (0.060)	3 (0.027)

[a] RR = 0.37, p <0.03, patients vs controls.
[b] RR = 0.22, p <0.01, MB patients vs controls.

DISCUSSION

The association between HLA and leprosy in southern Chinese was analyzed at the DNA level for the first time in this study. The results are noteworthy in several aspects. First, there was no significant difference between leprosy patients and controls in the frequencies of the DRB1*1501, *1502 and *1602 alleles encoding for DR2 antigens. This result showed no association of HLA-DR2 and any of its subtypes with leprosy in this southern Chinese population. Although HLA-DR2 association has been suggested by some studies ([2, 6, 8, 12, 14]) in the past, these were positive associations. In this study, HLA-DR2 antigen and its subtypes had a tendency to be increased in the patients, but did not show statistical significance. Our results revealed that HLA-DR2 is the mark allele, but it has no association with this southern Chinese population.

Second, the negative association of HLA-B46 with the southern Chinese leprosy patients was observed to be significant. Although the frequency of HLA-B46 showed an overall decreasing tendency in leprosy patients, only MB patients showed a significant difference. Moreover, MB patients showed a significant decrease in the frequency of the HLA-B46 allele compared to PB patients, suggesting that a gene in linkage disequilibrium with HLA-B46 might control resistance to the MB clinical type and not to the PB clinical type.

Third, the allele frequency of MICA-A5 alleles was significantly decreased in the MB patients compared to the controls. This is the first report investigating MICA gene polymorphism in leprosy. It remains to be elucidated whether the MICA-A5 alleles would be primarily associated with MB leprosy or if the association might only reflect a strong linkage disequilibrium between HLA-B alleles and MICA alleles.

However, the frequency of the B46/A5 haplotype was significantly decreased in the leprosy patients, especially the MB clinical subtype, compared to the controls. In contrast, the frequencies of the non-B46/A5 and B46/non-A5 haplotypes were not significantly different between the patients and controls. These observations suggest that neither HLA-B46 nor MICA-A5 alone was primarily associated with the resistance to leprosy. In other words, the resistance may be controlled by gene(s) other than HLA-B or MICA genes in the HLA class I region.

In summary, this study showed that the resistance to leprosy in a southern Chinese population was significantly associated with the HLA-B46/MICA-A5 haplotype and that DR2 was not significantly associated with leprosy in this population. These results suggest that an HLA-linked disease-control gene for leprosy in southern China may be located near the HLA-B/MICA region and not in the HLA-DR locus.

SUMMARY

According to the World Health Organization recommended multidrug therapy (WHO/MDT), we have carried out this study to investigate the presence of HLA-linked susceptibility or resistance to leprosy in a southern Chinese population. Sixty-nine leprosy patients and 112 healthy controls participated in the study. HLA-DR2 subtypes, HLA-B and MHC Class I chain-related A (MICA) alleles were typed at the DNA level using the polymerase chain reaction-single strand conformation polymorphism method.

The frequencies of HLA-DR2-DRB1 alleles did not show any significant differences between the patient and the control groups, suggesting that the disease susceptibility was not associated with the DR2 subtypes in this southern Chinese population. On the other hand, in the multibacillary (MB) patients significantly decreased allele frequencies of HLA-B46 (0.040 in MB patients vs 0.129 in controls) and MICA-A5 (0.200 vs 0.380) were observed compared with the healthy controls. The calculated relative risk (RR) for B46 was 0.28; for MICA-A5, 0.52. In addition, on haplotype analysis the frequency of the HLA-B46/MICA-A5 haplotype was significantly decreased in the MB patients compared to controls (0.060 vs 0.233, RR = 0.22, $p < 0.01$).

These results suggest that an HLA-linked disease-resistant gene to MB leprosy in southern China is in strong linkage disequilibrium with the HLA-B46/MICA-A5 haplotype. In other words, the resistant gene may be located near the HLA-B/MICA region and not in the HLA-DR locus.

RESUMEN

De acuerdo a los resultados de la poliquimioterapia recomendada por la Organización Mundial de la Salud (PQT/OMS) hemos realizado este estudio para investigar la asociación entre el sistema HLA y la susceptibilidad o resistencia a la lepra en una población del sur de China. En el estudio participaron 69 pacientes con lepra y 112 controles sanos. Se tipificaron los subtipos de HLA-DR2, HLA-B y MHC clase IA (MICA) a nivel de DNA, usando un variante de la reacción en cadena de la DNA polimerasa.

Las frecuencias de los alelos HLA-DR2-DRB1 no mostraron diferencias significativas entre los pacientes y el grupo control, sugiriendo que la susceptibilidad a la enfermedad no esta asociada a los subtipos de DR2 en est población del sur de China. Por otro lado, comparados con los controles sanos, los pacientes multibacilares (MB) mostraron frecuencias significativamente disminuidas de los alelos HLA-B46 (0.040 en MB vs 0.129 en los controles) y MICA-A5 (0.200 vs 0.380). El riesgo relativo (RR) calculado para B46 fue de 0.28 mientras que para MICA-A5 fue de 0.52. Además, la frecuencia del haplotipo HLA-B46/MICA-A5 estuvo significativamente disminuida en los pacientes MB (0.060 en MB vs 0.233 en los controles, RR = 0.22, $p < 0.01$). Estos resultados sugieren que en la población del sur de China estudiada, un gene de resistencia a la enfermedad ligado a HLA está en fuerte desequilibrio de enlace con el haplotip. HLA-B46/MICA-A5. En otras palabras, el gene de resistencia puede estar localizado cerca de la región HLA-B/MICA y no en el locus HLA-DR.

RÉSUMÉ

En suivant les recommendations de l'Organisation Mondiale de la Santé en matière de polychimiothérapie (OMS/PCT), nous avons entrepris de déterminer si il existe un lien entre les antigènes leucocytaires humains (HLA) et la susceptibilité à la lèpre dans une population de Chine du sud. Soixante neuf patients lépreux et 112 contrôles en bonne santé ont participé à l'étude. Les allèles des sous-types de HLA-DR2, de HLA-B et des protéines A liées aux chaînes du complexe majeur d'histocompatibilité de type 1 (MICA) furent typés en utilisant la méthode combinant le réaction de polymérase en chaîne couplée à une analyse du polymorphisme de conformation des chaînes simples d'ADN.

Il n'a pas été observé de différence de fréquences des allèles de HLA-DR2-DRB1 entre le groupe des patients et le groupe des contrôles, suggérant qu'il n'y a pas d'association entre la susceptilité à la maladie et les sous-types DR2 dans cette population de Chine du sud. Par contre, la fréquence des allèles HLA-B46 et MICA-A5 était significativement diminuée chez les patients multibacillaires (MB), comparée aux contrôles en bonne santé (0,040 chez les patients MB contre 0,129 chez les contrôles et 0,200 chez les patients MB contre 0,380 chez les contrôles, respectivement). Le risque relatif (RR) calculé pour B46 était de 0.28; pour MICA-A5, il était de 0.52. De plus, l'analyse des haplotypes révèle une diminution significative de la fréquence de l'haplotype HLA-B46 / MICA-A5 chez les patients MB comparée à celle des contrôles (0,060 contre 0,233, RR = 0,22; $p < 0,01$).

Ces résultats suggèrent que le gène de résistance à la maladie lié à HLA contre la lèpre multibacillaire de Chine méridionale est en fort déséquilibre de lien avec l'haplotype HLA-B46/MICA-A5. En d'autres termes, le gène de résistance pourrait être localisé proche de la région incluant HLA-B/MICA mais pas proche du locus HLA-DR.

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