Associations Between HLA-DRB1 Alleles and Leprosy in an Indonesian Population[1]

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Leprosy is a chronic infectious disease of man caused by an intracellular microorganism, *Mycobacterium leprae.* Clinically, the disease is manifested as a spectrum with two polar forms, tuberculoid (TT) and lepromatous (LL). Between these two polar forms, there are intermediate forms: borderline tuberculoid (BT), midborderline (BB), and borderline lepromatous (BL). However, before clinical manifestations of the disease develop, patients may demonstrate a transitional form called indeterminate (I) which frequently heals spontaneously. The development of the clinical spectrum of the disease is related to the degree of cell-mediated immunity (CMI) and is influenced by genetic factors ([4, 5, 8]). Subclinical infection with *M. leprae* can be demonstrated by the presence of specific antibodies against *M. leprae* antigens in clinically healthy individuals ([1, 2, 6, 13]).

Studies of genetics in leprosy have been reviewed by Fine ([7, 8]). Among the genetic factors, human leukocyte antigens (HLA) have been studied extensively by several investigators ([4, 5, 9, 11, 22–24]). From these studies it became clear that HLA class II rather than HLA class I antigens are associated with disease susceptibility ([22, 23]). Todd, *et al.*([22]) on the basis of their own material in Louisiana and a meta-analysis of pooled reports of cases concluded that only HLA-DR2 and -DQ1 were associated with leprosy, in either tuberculoid or lepromatous forms. Based on his own and many other studies, de Vries ([3]) came to the conclusion that HLA-DR3 is often associated with TT leprosy and HLA-DQ1 universally with LL leprosy; whereas the susceptibility to *M. leprae* infection itself is not controlled by HLA-linked genes.

Leprosy still remains a public health problem in Indonesia. Although, the number of registered cases has decreased significantly since the implementation of multidrug therapy (MDT), the incidence of new cases is apparently unchanged (Hasibuan, Y. Situasi Penyakit Kusta di Indonesia dan Masalah-masalah Yang Dihadapi Dalam Pemberantasannya. *Kumpulan Makalah Kongres Nasional VII Perdoski, Bukittinggi 9–12 Nopember 1992).* The fundamental question of why some people contract leprosy (infection) and others do not still remains unanswered. One possibility is the genetic differences in susceptibility. In Indonesia, the distribution of leprosy is not uniform in either the prevalence or type of the disease. These differences may be due to the effect of many variables, including genetic factors. Since there has been no study thus far on the association between HLA and leprosy or *M. leprae* infection in Indonesia, we have conducted such a study in a Javanese population from Yogyakarta. We have used PCR-SSO DNA typing which is becoming more commonly used worldwide. Based on the earlier reports mentioned before, this study has focused on the HLA-DR and -DQ loci.

MATERIALS AND METHODS
Subjects. Seventy-nine leprosy patients (41 TT/BT, 38 LL/BL) and 50

healthy controls were recruited from Yogyakarta, Indonesia. They were all of the Indonesian/Javanese ethnic group. The diagnosis of leprosy was based on clinical, bacteriological and histopathological examinations, and patients were classified according to Ridley and Jopling ([18]). Blood samples were taken for HLA typing and serological assays and drawn before the start of chemotherapy for leprosy. The healthy controls consisted of 50 nonrelative household contacts (spouses). Another 50 healthy blood donors were used to determine the cut-off point for positivity in serological assays for the detection of anti-*M. leprae* antibodies.

Serological assays. Sera collected from the subjects were tested for specific antibodies against *M. leprae* antigens, i.e., IgM anti-phenolic glycolipid-I (PGL-I) and IgG anti-36-kDa protein antigens, by using an ELISA and an Inhibition ELISA described previously ([14, 20]). The semisynthetic DBSA antigen used in this study for the detection of anti-PGL-I antibodies has been standardized by the World Health Organization (WHO), and this antigen has demonstrated positive responses in the majority of leprosy patients. The 36-kDa protein antigen used in this study identified by Klatser, *et al.* ([15]) has not been studied extensively thus far.

HLA-DRB1/DQA1 typing. For typing of HLA-DR/DQ alleles, we have used oligonucleotide hybridization essentially as previously described ([10]). DNA was extracted from peripheral blood lymphocytes. Amplification of this DNA was performed by the polymerase chain reaction (PCR) using sets of primers specific for DRB1 and DQA1, respectively. Typing for the alleles was carried out by dot blotting the PCR fragments and hybridization with synthetic oligonucleotides ([10]), allowing for low resolution DRB1 and for DQA1 high resolution typing ([12]). Using the available set of primers and SSO probes we could type for 12 HLA-DRB1 and 8 DQA1 specificities.

Statistical analysis. Frequencies of HLA-DRB1 and -DQA1 specificities in leprosy patients and controls were compared using a χ^2-test ([21]). The strength of associations between the HLA specificities and (subclinical) leprosy was expressed as an odds ratio (OR) ([17]). Attributable risk

(AR) or prevented fraction (PF) were calculated using the formula as suggested by Kleinbaum, *et al.* ([16]) and Kramer ([17]); p values were not corrected for multiple comparisions. These computations were performed using the SPSSPC version 3.1 and EPI-Info Statistical Software Packages.

RESULTS AND DISCUSSION

Table 1 presents the frequency distribution of the 12 HLA-DRB1 specificities in cases and controls. DRB1*01 was not found in this population. HLA-DRB1*02 and DRB1*12 showed significant associations with leprosy. For DRB1*02 a significant difference was found only between LL/BL leprosy and the healthy controls. This association had odds ratio (OR) of 2.54. The attributable risk (AR) was calculated to be 41.5%. A significant negative association was found between DRB1*12 and both types of leprosy. The OR of this association was 0.33 for leprosy, 0.37 for TT/BT and 0.33 for LL/BL leprosy. Since the ORs were <1, we calculated the preventive fraction (PF) instead of the AR; these were 65.3%, 63.3% and 58.8%, respectively, for leprosy, LL/BL and TT/BT leprosy. To determine whether this association is secondary to the association with DRB1*02, an analysis was performed on DRB1*02 negative individuals. Also, among DRB1*02 negative individuals the frequency of DRB1*12 was found to be higher in controls compared to both leprosy groups. This suggests that this association is a primary one, and that DRB1*12 is a protective marker for leprosy in this Indonesian population.

As shown in Table 2, eight DQA1* alleles have been identified. A high frequency was found for HLA-DQA1*0601. HLA-DQA1*0101 and DQA1*0102 were distributed in moderate frequency; the remaining alleles were found in low frequency. No significant associations between DQA1* alleles and leprosy or leprosy subtypes were observed.

Next we analyzed whether the presence of certain HLA-DRB1 or DQA alleles was correlated with the levels of IgM anti-PGL-I and IgG anti-*M. leprae* 36-kDa antibodies. This was separately analyzed for the patients and controls because patients had significantly higher antibody levels than did

TABLE 1. *Frequency and odds ratio of HLA-DRB1 specificities in leprosy patients and healthy controls.*

DRB1 Alleles	Controls (n=50) freq.	TT/BT leprosy (n=41)			LL/BL leprosy (n=38)			total leprosy (n=79)		
		freq.	OR (95%CI)	P	freq.	OR (95%CI)	P	freq.	OR (95%CI)	P
01	0	0			0			0		
02	46.0	56.1	1.50 (0.60 - 3.75)	0.340	68.4	2.54 (0.97 - 6.78)	0.037	62.0	1.92 (0.88 - 4.19)	0.075
03	2.0	4.9	2.51 (0.17 - 72.87)	0.586	5.3	2.27 (0.18 - 79.10)	0.576	5.1	2.61 (0.26 - 63.27)	0.648
04	4.0	4.9	1.23 (0.12 - 12.97)	0.756	5.3	1.33 (0.13 - 14.10)	0.814	5.1	1.28 (0.19 - 10.52)	0.881
11	2.0	0	NT		0	NT		0	NT	
12	84.0	65.9	0.37 (0.12 - 1.10)	0.045	63.2	0.33 (0.11 - 0.99)	0.026	64.5	0.33 (0.12 - 0.87)	0.013
13	2.0	7.3	3.87 (0.34 - 100.6)	0.473	0	NT		3.8	1.93 (0.17 - 49.7)	0.958
14	2.0	0	NT		5.3	2.72 (0.18 - 79.10)	0.808	2.5	1.27 (0.09 - 36.48)	0.656
07	14.0	12.2	0.85 (0.21 - 3.35)	0.801	5.3	0.34 (0.05 - 1.97)	0.325	8.9	0.60 (0.17 - 2.06)	0.362
08	2.0	7.3	3.87 (0.34 - 100.6)	0.473	13.2	7.42 (0.78 - 175.8)	0.103	10.1	5.52 (0.66 - 121.4)	0.158
09	4.0	2.4	0.60 (0.02 - 8.92)	0.861	7.9	2.06 (0.26 - 18.78)	0.751	5.1	1.28 (0.19 - 10.52)	0.881
10	0	2.4	NT		2.6	NT		2.5	NT	

freq = frequency.
OR = Odds ratio.
NT = not tested.
P = Chi-square test probability.

TABLE 2.   *Frequency and odds ratio of HLA-DQA1 alleles in leprosy patients and healthy controls.*

DQA1 Alleles	Controls (n=50)	TT/BT leprosy (n=41)			LL/BL leprosy (n=38)			Total leprosy (n=79)		
	freq.	freq.	OR (95%CI)	P	freq.	OR (95%CI)	P	freq.	OR (95%CI)	P
0101	28.6	46.3	2.16 (0.83 - 5.68)	0.083	45.7	2.11 (0.77 - 5.79)	0.108	31.6	2.13 (0.943 - 4.95)	0.051
0102	32.7	29.3	0.85 (0.32 - 2.30)	0.731	34.2	1.08 (0.39 - 2.97)	0.876	30.4	0.95 (0.41 - 2.20)	0.900
0103	6.1	12.2	2.13 (0.40 - 12.20)	0.524	11.4	1.98 (0.34 - 12.16)	0.640	11.3	2.06 (0.47 - 10.2)	0.453
0201	14.3	12.2	0.83 (0.21 - 3.28)	0.983	2.9	0.18 (0.01 - 1.56)	0.166	8.8	0.51 (0.14 - 1.85)	0.255
0301	8.2	9.8	1.22 (0.23 - 6.32)	0.914	11.4	1.45 (0.28 - 7.63)	0.900	10.5	1.32 (0.33 - 5.60)	0.899
0401	8.2	9.8	1.22 (0.23 - 6.32)	0.914	2.9	0.33 (0.01 - 3.40)	0.585	6.6	0.79 (0.17 - 3.75)	0.984
0501	0	2.4	NT		0	NT		1.3	NT	
0601	73.5	61.0	0.56 (0.21 - 1.51)	0.209	60.0	0.54 (0.19 - 1.51)	0.195	58.2	0.54 (0.23 - 1.23)	0.125

freq = frequency.
OR = Odds ratio.
NT = not tested.
P = Chi-square test probability.

the controls. No significant differences in antibody levels were observed for the DRB1*02 and DRB1*12 specificities that were associated with leprosy or leprosy type (data not shown). So these data do not provide evidence for a possible immune response gene effect as the mechanism responsible for this/these association(s) ([3-5]). Some correlations were noted between several other DRB1 and DQA1 specificities and antibody levels but these were not significant after correction for the number of comparisons made.

Finally, we ascertained whether subclinical infection with *M. leprae* as determined by serology in household contacts was associated with HLA-DRB1 or DQA1 specificities. No association was noted between any of these HLA specificities and subclinical infection, neither based on the presence of IgM anti-PGL-I antibodies detected by ELISA (10 out of 50 contacts) nor IgG anti-36-kDa antibodies detected by Inhibition ELISA (15 out of 50 contacts). These numbers are too small to allow a strong conclusion, but they are at least in keeping with the notion that infection with *M. leprae per se* is not controlled by HLA-linked factors ([3, 4, 5, 7, 19]).

The results of this study demonstrate associations between DRB1 alleles and leprosy or leprosy type. DRB1*02 shows a significant association with lepromatous (LL/BL) leprosy but not with tuberculoid (TT/BT) leprosy. This confirms previous studies in Japan ([11]) and India ([19]). In this study we have also found that HLA-DRB1*12 has a negative association with leprosy. This is, indeed, a new finding that needs to be confirmed in future studies. If confirmed, the high frequency of DRB1*12 in this population might be one factor which contributes to the low prevalence rate of leprosy in this population.

SUMMARY

To investigate whether the susceptibility to leprosy (type), subclinical infection with *Mycobacterium leprae* and the antibody response against *M. leprae*-specific antigens are associated with HLA-DR phenotypes sequence-specific oligonucleotide HLA-DRB1 and DQA1 typing and antibody assays have been performed in 79 leprosy patients (41 TT/BT and 38 LL/BL) and 50 healthy controls from a Javanese population in Yogyakarta, Indonesia.

DRB1*02 was associated with LL/BL [odds ratio (OR) 2.54, 95% confidence interval (CI) 0.97–9.78, p = 0.037 and attributable risk (AR) 41.5%] but not with TT/BT leprosy (p > 0.05). HLA-DRB1*12 was negatively associated with leprosy (either LL/BL or TT/BT [OR 0.33–0.35, p < 0.05, prevented fraction (PF) 58.8%–65.3%]. No significant association was found between HLA-DRB1 or DQA1 type, anti-*M. leprae* antibody level and subclinical infection with *M. leprae*.

These data indicate that in this population susceptibility to lepromatous leprosy is associated with HLA-DRB1*02, while resistance to leprosy is associated with HLA-DRB1*12. These associations are not paralleled with associations of the same HLA types with anti-*M. leprae* antibody level. Finally, the results of this study also support the notion that infection with *M. leprae per se* is not associated with HLA-DRB1 or DQA1 alleles.

RESUMEN

Para investigar si la susceptibilidad a la lepra, la infección subclínica, y la respuesta humoral contra los antígenos de *Mycobacterium leprae* están asociadas con determinados fenotipos HLA-DR, se analizaron las secuencias de oligonucleótidos específicas para HLADRB1 y DQA1 y la respuesta humoral contra antígenos de *M. leprae* en 79 pacientes con lepra (41 TT/BT y 38 LL/BL) y en 50 controles sanos de una población Javanesa en Yogyakarta, Indonesia.

El antígeno DRB1*02 estuvo asociado con la lepra LL/BL [grado de predicción de 2.54, 95% de confianza interna (CI) 0.97–9.78, p = 0.037 y riesgo atribuible (RA) de 41.5%] pero no con la lepra TT/BT (p > 0.05). El antígeno HLA-DRB1*12 se encontró negativamente asociado con la lepra en cualquiera de sus tipos [TP 0.33–0.35, p < 0.05, fracción evitada (FE) 58.8–65.3%]. No se encontró asociación significativa entre HLA-DRB1 y el tipo de DQA1, el nivel de anticuerpos anti-*M. leprae*, o la infección subclínica con *M. leprae*.

Estos datos indican que en esta población, la susceptibilidad a la lepra lepromatosa está asociada con el antígeno HLA-DRB1*02, mientras que la resistencia a la enfermedad está asociada con HLA-DRB1*12. Sin embargo, estas asociaciones no están relacionadas con las asociaciones de los mismos tipos HLA y el nivel de anticuerpos contra *M. leprae*. Finalmente, los resultados de este estudio también apoyan la noción de que la infección con *M. leprae* no está asociada per se con los alelos HLA-DRB1 o HLADQA1.

RÉSUMÉ

Pour rechercher si la susceptibilité à la lèpre, l'infection sub-clinique par le *Mycobacterium leprae* et la réponse d'anticorps vis-à-vis d'antigènes spécifiques de *M. leprae* sont associés à des phénotypes HLA-DR, on a réalisé le typage de séquences oligonucléotidiques spécifiques de HLA-DRB1 et DQA1 et des tests d'anticorps chez 79 malades de la lèpre (41 TT/BT et 38 LL/BL) et 50 témoins en bonne santé provenant d'une population javanaise de Yogyakarta, en Indonésie.

DRB1*02 était associé à LL/BL [odds ratio (OR) 2.54, limites de confiance (LC) à 95% 0.97–9.78, p = 0.037 et risque attribuable (RA) 41.5%], mais pas avec TT/BT (p > 0.05). HLA-DRB1*12 était associé négativement avec la lèpre (soit LL/BL, soit TT/BT [OR 0.33–0.35, p < 0.05, fraction attribuable (FA) 58.8%–65.3%]. Aucune association significative n'a été trouvée avec le type HLA-DRB1 ou DQA1, le taux d'anticorps anti-*M. leprae* et l'infection subclinique par M. leprae.

Ces données indiquent que dans cette population la susceptibilité à la lèpre lépromateuse est associée à HLA-DRB1*02, tandis que la résistance à la lèpre est associée à HLA-DRB1*12. Ces associations ne vont pas en parallèle avec des associations des mêmes types HLA avec le taux d'anticorps anti-*M. leprae*. Finalement, les résultats de cette étude supportent également la notion que l'infection par *M. leprae* n'est pas associée par elle-même avec les allèles HLA-DRB1 ou DQA1.

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