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HLA Antigens and Neural Reversal Reactions in Ethiopian Borderline Tuberculoid Leprosy Patients[1]

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Type 1 upgrading or reversal reactions (RR) in borderline tuberculoid (BT) leprosy patients are known to be associated with a rapid increase in cell-mediated immune (CMI) reactivity ([3, 13]). Clinical and histological changes show a shift toward the tuberculoid pole of the spectrum ([13]). Peripheral blood lymphocytes from such RR patients respond much stronger to antigens of *Mycobacterium leprae* in the *in vitro* lymphocyte stimulation test than those of BT patients not in RR ([4, 5]). RR is most frequently seen within the first year of antileprosy treatment ([3, 6]). A very common complication of RR is acute neuritis leading to irreversible nerve damage. This constitutes the main cause of chronic disabilities affecting roughly 25% of all leprosy patients.

So far, the mechanism of the development of RR is poorly understood. Because there is no evidence for external etiological factors, genetic factors may play a role in determining whether or not such reactions will develop ([3, 6]). Genetic markers predicting a predisposition to RR would not only be of clinical importance but would also provide insight into the mechanism of the development of RR.

The HLA region encodes immune-response (Ir) and immune-suppressor (Is) genes, the products of which are assumed to be the HLA class I and class II molecules ([11]). Such HLA Ir and Is genes are involved in the regulation of immune responses, probably as restriction elements for T cells ([9]). Interindividual variability for these genes as a consequence of the polymorphism within the HLA region may lead to interindividual differences in susceptibility to or expression of disease caused by invading microorganisms. Multicase leprosy family studies in which the segregation of HLA haplotypes was analyzed have clearly demonstrated that HLA-linked (Ir) genes are important factors in determining the type of leprosy that develops in susceptible individuals upon infection ([8, 10, 18, 22, 25]). Besides family studies, several population studies have revealed associations between HLA—mainly class II—antigens and tuberculoid or lepromatous leprosy ([10, 16, 19, 21]). Moreover, HLA-linked (Ir) genes control the type of response to *M. leprae* antigens both in *in vivo* skin tests ([8, 12, 20]) and in *in vitro* T-cell proliferation studies ([9, 21] and unpublished data). Taken together, these studies suggest that the HLA region encodes Ir genes for *M. leprae* that determine the type of leprosy as well as the type of skin-test responsiveness which develops upon encountering *M. leprae*. Since a rapid increase in CMI is a prominent feature of RR ([3, 5, 6]), it follows from the mentioned role of HLA in the regulation of the immune response against *M. leprae* that HLA Ir genes might well control the development of RR. Therefore, we have investigated whether HLA class I (A, B, C) or class II (DR, DQ) alleles are associated with the occurrence of RR and/or skin-test responsiveness to *M. leprae* in BT patients.

MATERIALS AND METHODS

Patients and controls. Sixty-one, unrelated, borderline tuberculoid leprosy patients were selected for the study. All patients were Ethiopian highlanders belonging to the Amhara, Oromo, Tigray, and Gurage language groups. They were clinically classified as borderline tuberculoid (BT) leprosy patients according to the five-group system of Ridley and Jopling ([14]). They all had a history of at least 3 years since the start of antileprosy treatment. All patients were randomly

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drawn from a group completing a 6-month course of multidrug (dapsone, rifampin, and clofazimine) therapy, and being released from treatment. Thirty-three patients had experienced one or more episodes of RR; the remaining had remained free of RR.

The assignment of RR was based entirely on past and/or present signs of neuritis, since the degree of skin inflammation is nearly impossible to determine in retrospect. RR occurring with skin involvement only is rare in Ethiopia ([1]). Acute neuritis episodes were characterized by swelling, pain, and tenderness of one or more peripheral nerves, often accompanied by a loss of sensory (anesthesia) and/or motor (paresis) functions, that were tested in each case.

With the exception of one RR patient, all patients had been skin tested with human lepromin: a volume of 0.1 ml of lepromin (4×10^7 acid-fast bacilli/ml) was injected intradermally into the forearm. The skin reactions were read at 3 weeks by experienced leprologists, and expressed as mm of induration representing the mean of two perpendicular diameters. All lepromin skin tests were determined before HLA typing was performed. In the RR-positive group, the skin testing was done when the patients were free of active RR symptoms. All patients were negative for acid-fast bacilli in slit-skin smears from the time of first diagnosis.

There were no differences in the duration or type of treatment between these two groups. The majority of acute RR episodes took place before or during the first year of treatment. As a control group, 39 healthy unreleated individuals originating from the same leprosy-endemic area and the same ethnic group were HLA typed.

HLA typing. For HLA typing, mononuclear cells were isolated on Ficoll-Isopaque from 25 ml defibrinated peripheral blood. The cells were suspended in medium containing 10% (v/v) dimethyl sulfoxide (DMSO), divided into aliquots of $6–8 \times 10^6$ cells and put into 1 ml polypropylene tubes (Nunc, Roskilde, Denmark). For freezing, the ampoules were stored first at −70°C and then in liquid nitrogen (−196°C). After freezing, the cells were transported to Leiden, The Netherlands.

HLA-A, HLA-B, and HLA-C typing was performed using the standard NIH microlymphocytotoxicity technique with a set of 120 well-defined sera ([23]). Eighty platelet-absorbed sera, recognizing the known HLA-DR groups, were used in the two-color fluorescence test for the typing of DR specificities on B cells ([24]). In addition, the sera used recognized the supertypic HLA-DRw52, HLA-DRw53, and HLA-DQ specificities ([15]).

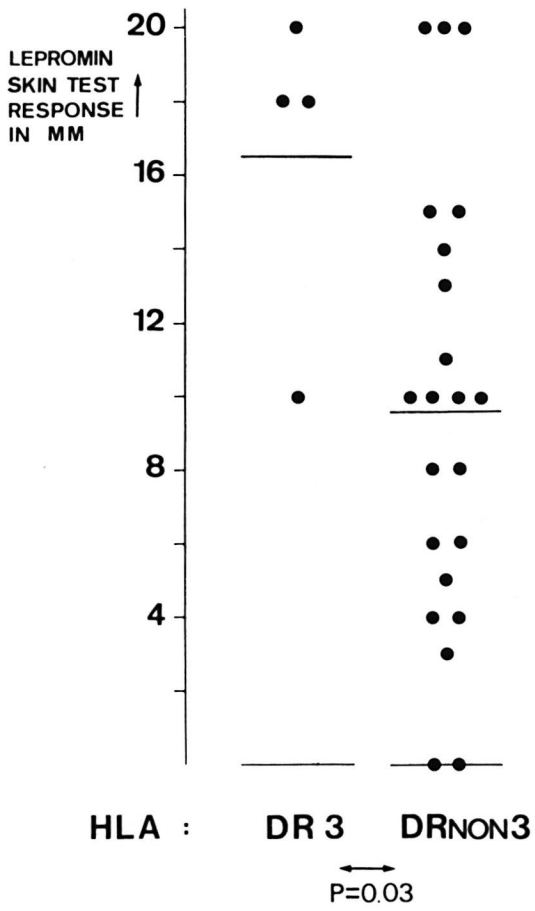
Statistical analysis. Associations between HLA phenotypes and either BT or the development of RR were tested by 2×2 contingency tables. Relative risks and their significances were calculated according to Woolf's method as modified by Haldane ([17]). The significance of the differences in the distribution of the lepromin skin-test results for HLA class II antigens in BT patients with RR, BT patients without RR, or BT patients irrespective of RR were determined by the nonparametric Mann-Whitney rank sum test (SPSS-X computer package).

RESULTS

Of the 28 BT patients without RR, 25 could be typed for HLA-A, HLA-B, and HLA-C; 27 for HLA-DR; and 26 for HLA-DQ. For the 33 patients with a history of RR, these numbers were 28, 28, and 26, respectively, and for the 39 healthy controls, 33, 33, and 33. The remaining samples did not contain sufficient living cells required for HLA typing.

The phenotype frequencies of the HLA class I and class II antigens did not show significant differences among the three different groups of individuals tested (data not shown). In The Table, results are shown for HLA-DR3 since for this particular antigen differences were observed in lepromin response (see below).

The results of the lepromin skin tests in both groups of patients indicated that HLA-DR3 was associated with high lepromin skin-test responsiveness in RR-positive BT patients ($p = 0.03$) (The Figure). In this case, no correction was necessary for the number of HLA antigens tested because this result confirmed earlier findings of our group ([19, 20, 22]). In BT patients without RR no differences were observed. No significant differences were observed in the case of the other DR and the DQ antigens. Interesting, however, in light of earlier results ([8, 9]) might



THE FIGURE. Differences in distribution of the lepromin skin-test results between HLA-DR3 positive and HLA-DR3 negative (non 3) BT patients with RR. No significant differences were observed for the other HLA-DR specificities. No significant differences existed in the group of BT patients without a history of RR (p value derived by Mann-Whitney rank sum test; mean skin test diameters shown.)

be that DQw1 tended to be associated with low lepromin skin-test responsiveness in these same patients.

DISCUSSION

We have studied the influence of HLA antigens on RR (i.e., acute neuritis episodes) in BT leprosy since RR is an important immunopathological phenomenon in BT patients that can result in profound and irreversible nerve damage. Whereas HLA class I or class II antigens were not found to be associated with the development of RR in

THE TABLE. *HLA-DR3 in BT patients with or without RR as compared to healthy controls.*

	BT patients		Healthy controls
	With RR	Without RR	
HLA-DR3	4	4	5
HLA-DR non 3	24	21	28

BT patients or with susceptibility to BT per se, the data presented in this study show that HLA-DR3 is associated with high responsiveness to intradermally injected *M. leprae* antigens in BT patients with RR.

A rapid increase of CMI is one of the most characteristic features in RR ([3–6, 13]). Since HLA Ir genes are important factors in determining the type of leprosy that develops upon infection ([8–11, 18, 22]) as well as the type of cellular immune reactivity against *M. leprae* antigens *in vivo* and *in vitro,* we had expected to find an association between HLA antigens and the development of RR. However, the data obtained in this study show that this is not the case. Although this study cannot formally rule out that HLA-linked genes predispose to the development of RR, since family studies have not been performed for RR, the lack of an association between HLA and BT or RR distinguishes BT and the occurrence of RR from polar tuberculoid and lepromatous leprosy and might, therefore, reflect immunological or pathogenetical differences between BT and/or RR and these polar forms.

This assumption might provide a second lead to account for the lack of an association between HLA and RR. Although *M. leprae* displays a remarkable predilection for nerves and, thus, cellular immunity toward the bacillus may be related directly to nerve damage, as has been suggested earlier ([3–5]), it remains to be established if the increased CMI during RR is specific for antigens of *M. leprae.* It has been reported, for instance, that a nonspecific increase in immunoglobulin production ([2]) as well as a nonspecific increase in natural killer (NK) cell reactivity ([7]) occurs during RR. If the increased cellular immune reactivity during RR were nonspecific, it would be impossible to detect an influence of HLA-linked Ir genes since

such genes control responsiveness against mycobacterial antigens in an antigen-specific manner ([9, 11, 19]). One can, for instance, envisage that when triggering of an inflammatory process in the presence of *M. leprae* takes place, the latter might act as an adjuvant for the induction of CMI. Such an adjuvant effect on the induction of CMI might well be antigen nonspecific and would, therefore, not be expected to be under HLA-linked Ir gene control. Future studies addressing the question of whether or not the increase in CMI during RR is specific for *M. leprae* antigens may provide an answer to this question.

Previous studies of our group have shown that an HLA-DR3-linked Ir gene is associated with a) the development of tuberculoid leprosy and protection against lepromatous leprosy ([19, 22]), b) protection against skin-test nonresponsiveness against mycobacterial antigens ([20]), and c) high T-cell responsiveness against *M. leprae* and *M. tuberculosis* antigens in healthy individuals ([9] and unpublished data). These three observations suggest that HLA-DR3 acts as a (high) responder allele against *M. leprae* and presumably other mycobacteria. In the second part of this study, these findings are confirmed and extended since DR3 was found to be associated with high skin-test responsiveness against *M. leprae* antigens in RR patients. The fact that DR3 is associated with high skin-test responsiveness during RR but not with RR itself may suggest that increased cellular immune reactivity against *M. leprae*-specific antigens might follow rather than cause the development of RR.

SUMMARY

Reversal reactions (RR) or acute neuritis episodes are frequently observed in borderline tuberculoid (BT) leprosy patients during the first year of treatment, and are associated with a rapid increase in cell-mediated immunity. Because HLA-linked genes have been shown to be an important factor in determining the type of leprosy that develops in susceptible individuals and because HLA molecules regulate cellular interactions in the immune system, we have investigated whether RR are associated with HLA antigens in Ethiopian patients. The data reported here indicate that this is not the case: no significant differences in the distribution of HLA class I and class II antigens were observed among three groups: 28 BT patients with a history of RR, 27 BT patients with no history of RR, and 33 healthy individuals. In contrast to these negative results, we observed that HLA-DR3 was associated with high skin-test responsiveness against *Mycobacterium leprae* antigens among RR patients. Since DR3 was not associated with RR per se, the observed DR3-associated high responsiveness to *M. leprae* may not be primarily related to the development of RR.

RESUMEN

Las reacciones reversas (RR) o episodios agudos de neuritis se observan frecuentemente en los pacientes con lepra tuberculoide-intermedia (BT) durante el primer año de tratamiento, y están asociadas con un rápido incremento en la respuesta inmune celular. Dado que algunos genes ligados al HLA juegan un importante papel en la determinación del tipo de lepra que se desarrolla en los individuos susceptibles y puesto que ciertas moléculas del HLA regulan las interacciones celulares en el sistema inmune, en este trabajo se ha investigado si las RR están asociadas con antígenos HLA en pacientes de Etiopia. Los resultados indicaron que este no es el caso. No se observaron diferencias significativas en la distribución de antígenos HLA clase 1 y clase II entre 3 grupos estudiados: 28 pacientes BT con antecedentes de RR, 27 pacientes BT sin antecedentes de RR, y 33 individuos sanos. En contraste con estos resultados negativos, observamos que el HLA-DR3 estuvo asociado con una marcada reactividad en piel a los antígenos del *Mycobacterium leprae* entre los pacientes con RR. Puesto que el DR3 no estuvo asociado con la RR per se, la marcada reactividad al *M. leprae* asociada al DR3 no parece estar relacionada con el desarrollo de la RR.

RÉSUMÉ

On observe souvent des réactions réverses (RR) ou des épisodes aigüs de névrite chez des malades atteints de lèpre dimorphe tuberculoïde (BT), au cours de la première année de traitement. Ces phénomènes sont associés avec un renforcement rapide de l'immunité à médiation cellulaire. On sait par ailleurs que des gènes du système HLA jouent un rôle important dans la détermination du type de lèpre qui se développe chez un individu susceptible; par ailleurs, des molècules HLA règlent les interactions cellulaires du sytème immunitaire. On a dès lors étudié si les réactions réverses étaient associées avec des antigènes HLA. Cette étude a été menée chez des malades éthiopiens. Les données

rapportées indiquent que ceci n'est pas le cas. Aucune différence significative n'a été observée dans la distribution des classes I et II des antigènes HLA dans les trois groupes étudiés, à savoir 28 malades BT ayant des antécédents de RR, 27 malades BT sans tels antécédents, et 33 sujets en bonne santé. En opposition avec ces résultats négatifs, on a observé que HLA-DR3 était associé avec une capacité élevée de réponses cutanées aux antigènes de *Mycobacterium leprae* chez les malades atteints de réaction reverse. Puisque DR3 n'est pas associée avec la réaction réverse en tant que telle, la capacité de réponse élevée à *M. leprae* associée à DR3, telle qu'elle a été observée, ne peut pas être mise en relation causale avec le développement de la réaction réverse.

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