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The recognition of 16 mycobacterial Ags by a panel of T cell lines from leprosy patients and healthy exposed individuals from an endemic population was examined within the context of expressed HLA-DR molecules. Although overall no significant differences were found between the frequencies of Ag recognition in the different subject groups, when Ag-specific T cell responses were examined within the context of HLA-DR, a highly significant difference was found in the recognition of the 30/31-kDa Ag. HLA-DR3 appeared to be associated with high T cell responsiveness to the 30/31-kDa Ag in healthy contacts (p = 0.01), but, conversely, with low T cell responsiveness to this Ag in tuberculoid patients (p = 0.005). Within the group of HLA-DR3-positive individuals, differences in 30/31-kDa directed T cell responsiveness were highly significant not only between healthy individuals and tuberculoid patients (p < 0.0001), but also between healthy individuals and lepromatous patients (p = 0.009), and consequently between healthy individuals compared with leprosy patients as a group (p < 0.0001). A dominant HLA-DR3-restricted epitope was recognized by healthy contacts in this population. It has been proposed that secreted Ags may dominate acquired immunity early in infection. The low T cell response to the secreted, immunodominant 30/31-kDa Ag in HLA-DR3-positive leprosy patients in this population may result in retarded macrophage activation and delayed bacillary clearance, which in turn may lead to enhanced Ag load followed by T cell-mediated immunopathology. The Journal of Immunology, 1999, 162: 6912–6918.

Most individuals exposed to Mycobacterium leprae are able to mount a protective T cell-mediated immune response to this bacterium, and only a small proportion of infected individuals develop leprosy. In patients, a spectrum of clinical symptoms can be observed that correlates with the immune response. At one end of the spectrum, tuberculoid leprosy patients typically have a small number of lesions containing few detectable bacteria and mount a strong T cell-mediated immune response. At the other end of the spectrum lepromatous leprosy patients develop disseminated infection and typically display T cell nonresponsiveness to the bacterium. Whereas these observations indicate that the type of response induced following an infection may play a crucial role in what type of disease ensues, the underlying mechanisms are far from clear. The development of lepromatous leprosy, for example, has been associated with the production of Th2-like cytokines (1, 2), but others have disputed this finding and provided evidence for the involvement of suppressor T cells (3, 4). Furthermore, tuberculoid patients as well as healthy exposed individuals mount a strong T cell-mediated response characterized by the production of Th1-like cytokines, which are believed to be essential for clearing the bacterium (1, 5). However, in tuberculoid patients, this Th1-like response is associated with clinical symptoms, whereas in the healthy population no such immunopathology can be detected.

Differences in the antigenic repertoire of M. leprae recognized by patients and healthy exposed individuals has been proposed as another factor that contributes to development of disease or protection. The recent efforts of many laboratories in the identification and characterization of Ags of M. leprae (for a recent review see Ref. 6) has allowed comparative analysis of the antigenic repertoire recognized by both subject groups. However, these studies did not reveal striking differences and indicated that by and large the same set of Ags can be recognized by patients and healthy individuals (7–13). Although the complete antigenic repertoire is perhaps far from identified yet (e.g. 14), these studies suggest that the key to distinguishing protective from disease-inducing immunity does not lie in the recognition of protective or disease-inducing Ags.

In this study we have focused our attention to the role of HLA molecules that, by selecting and presenting antigenic peptides to T cells, may influence the quality of the immune response to M. leprae. Particular HLA class II alleles have been associated with development of tuberculoid or lepromatous leprosy, but generally not with development of disease per se (see for review Refs. 15 and 16). Since this issue has never been addressed within the context of the recognition of individual Ags, we have here studied the association of HLA-DR molecules with the recognition of a panel of protein Ags by T cells from patients and healthy subjects from a region where leprosy is endemic. As found previously, overall no gross differences in the antigenic repertoire recognized by both

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3 Abbreviations used in this paper: HC, healthy control; SI, stimulation index; BT/TT, tuberculoid leprosy; BL/LL, lepromatous leprosy.
Table I. Mycobacterial Ags used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. leprae</td>
<td>Sonicate of M. leprae (batch CD179) derived from armadillo tissue</td>
<td></td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td>Sonicate of M. tuberculosis</td>
<td></td>
</tr>
<tr>
<td>hsp10L</td>
<td>Recombinant M. leprae 10-kDa heat shock Ag</td>
<td>26</td>
</tr>
<tr>
<td>L8</td>
<td>Recombinant fusion protein containing aa 23-112 of M. leprae 15-kDa Ag</td>
<td>21</td>
</tr>
<tr>
<td>hsp16T</td>
<td>Recombinant M. tuberculosis 16-kDa heat shock Ag</td>
<td></td>
</tr>
<tr>
<td>hsp18L</td>
<td>Recombinant M. leprae 18-kDa heat shock Ag</td>
<td>37</td>
</tr>
<tr>
<td>L14</td>
<td>Recombinant fusion protein containing aa 57-287 of the secreted M. leprae 25.5-kDa Ag</td>
<td>35</td>
</tr>
<tr>
<td>30/31 kDa</td>
<td>30/31-kDa Ag 85 Complex Ags purified from M. tuberculosis culture supernatant, contains components A, B, and C.</td>
<td></td>
</tr>
<tr>
<td>36L</td>
<td>Recombinant fusion protein containing 36-kDa M. leprae Ag</td>
<td></td>
</tr>
<tr>
<td>38T</td>
<td>Recombinant 38-kDa M. tuberculosis protein</td>
<td>43</td>
</tr>
<tr>
<td>L2</td>
<td>Recombinant fusion protein containing aa 188-458 of M. leprae 49-kDa thioderodoxin-thioderodoxinreductase Ag</td>
<td></td>
</tr>
<tr>
<td>hsp65T</td>
<td>Recombinant M. tuberculosis 65-kDa-1 heat shock Ag</td>
<td></td>
</tr>
<tr>
<td>hsp65L</td>
<td>Recombinant M. leprae 65-kDa-1 heat shock Ag</td>
<td></td>
</tr>
<tr>
<td>hsp70L</td>
<td>Recombinant M. leprae hsp70 heat shock Ag</td>
<td></td>
</tr>
<tr>
<td>hsp71T</td>
<td>Recombinant M. tuberculosis hsp71 heat shock Ag</td>
<td></td>
</tr>
<tr>
<td>L21</td>
<td>Recombinant fusion protein of 145-kDa containing (part of) undefined M. leprae Ag</td>
<td></td>
</tr>
<tr>
<td>L24</td>
<td>Recombinant fusion protein of 135/145-kDa containing (part of) undefined M. leprae Ag</td>
<td></td>
</tr>
<tr>
<td>L43</td>
<td>Recombinant fusion protein of 140-kDa containing (part of) undefined M. leprae Ag</td>
<td></td>
</tr>
<tr>
<td>P32</td>
<td>Component A of 30/31-kDa Ags purified from M. tuberculosis culture supernatant</td>
<td></td>
</tr>
<tr>
<td>MPT59</td>
<td>Component B of 30/31-kDa Ags purified from M. tuberculosis culture supernatant</td>
<td></td>
</tr>
</tbody>
</table>

subject groups were observed. However, within the context of HLA-DR3, a remarkable difference in the recognition of one of the Ags analyzed (the secreted 30/31-kDa Ag) was found. Whereas this Ag was a dominant target Ag for HLA-DR3-positive tuberculoid patients responding, pointing to an Ag- and HLA-class II allele-specific low responsiveness to this Ag in tuberculoid patients from this ethnic group. These findings confirm and extend our previous studies, where we found that tuberculoid patients display an HLA-DR3-associated T cell low- or nonresponsiveness to M. leprae (15) and indicate that HLA-DR-associated factors can have a profound effect on the Ag-specific T cell response to this bacterium.

Materials and Methods

Donors and HLA typing

Fifty-seven unrelated leprosy patients originating from Surinam, South America, were studied, all of them being members of an ethnic group consisting of Negroid individuals with a predominantly Caucasian admixture. The patients were attending the Dermatology Department of the Academic Medical Centre in Amsterdam, the Dijkzigt Hospital in Rotterdam, and the University Hospital in Paramaribo; they had been classified according to the five-group system described by Ridley and Jopling (45). The diagnosis was based on regular and careful clinical examination, review of the clinical histories, skin-slit smear bacteriology, histopathological examination of skin biopsies, and lepromin testing. The 19 healthy individuals originated from the same ethnic group. Typing for HLA class II specificities was performed with platelet absorbed sera in the two color fluorescence test as described previously (17).

Antigens

The Ags used in this study are listed in Table I. M. leprae sonicates were obtained from R. Rees (London, U.K.); Mycobacterium tuberculosis sonicates from A. Kolk (Amsterdam, The Netherlands), 30/31-kDa (Ag 85 complex A, B, and C) proteins purified from culture filtrate of M. tuberculosis were obtained from C. Abou-Zeid (London, U.K.), Purified P32 (the A component of Ag 85 complex proteins) was obtained from J. de Bruyn (Brussels, Belgium), and purified MPT59 (the B component of the Ag 85 complex proteins) was obtained from S. Nagai (Osaka, Japan). Purified recombinant proteins through the World Health Organization/United Nations Development Program/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases/Immunology of Leprosy Special Program were obtained from J. van Embden (Bilthoven, The Netherlands) (hsp10L, hsp65L, hsp65T, and hsp70L), and from M. Singh (Braunschweig, Germany) (38T and hsp71T). Hsp16T was obtained from J. Ivanyi (London, U.K.), and hsp18L from J. Watson (Auckland, New Zealand). Escherichia coli strains carrying pEX2 containing M. leprae DNA inserts derived from Agt 11 recombinants (L2, L8, L14, L21, L24, L43, and 36L) were established as described (18). Crobetagalactosidase fusion proteins were isolated as described (19). Fusion proteins were approximately 50% pure as estimated from protein profiles from SDS/PAGE gels stained with Coomassie brilliant blue. Synthetic peptides were made on an ABIMED 422 synthesizer (ABIMED, Langenfeld, Germany) using the simultaneous multiple peptide synthesis method. The purity of the peptides was checked on reverse phase C18 HPLC (Lichrospher, 60RP-select B 5 mm, 250 × 4 mm, Merck, Darmstadt, Germany) and was shown to be routinely over 75%.

Generation of T cell lines and T cell clones

Peripheral blood from 76 donors (19 healthy endemic controls with the same ethnic background (HC), 32 tuberculoid leprosy (BT/TT) patients, and 15 lepromatous (BL/LL) leprosy patients) was collected by venapuncture using heparinized vacutainer tubes (Becton Dickinson, Mechelen, Belgium). PBMC cells (1–2 × 10⁶) were stimulated with heparinized vacutainer tubes (Becton Dickinson, Mechelen, Belgium). PBMC cells (1–2 × 10⁶) were centrifuged with Coomassie brilliant blue. Synthetic peptides were made on an ABIMED 422 synthesizer (ABIMED, Langenfeld, Germany) using the simultaneous multiple peptide synthesis method. The purity of the peptides was checked on reverse phase C18 HPLC (Lichrospher, 60RP-select B 5 mm, 250 × 4 mm, Merck, Darmstadt, Germany) and was shown to be routinely over 75%.

T cell proliferation assays

A total of 10⁵ cloned T cells or T cells from T cell lines were cultured in 96-well, flat-bottom microtiter plates together with 5 × 10³ irradiated (20 Gy) HLA-DR-matched PBMC and stimulated with various concentrations of whole mycobacterial sonicates or individual Ags. Sonicates were used at two different concentrations of 1 µg/ml and 10 µg/ml. Purified Ags were used at concentrations of 2 and 20 µg/ml. Semipurified fusion proteins were used at a dilution of 1/50, 1/1,000, and 1/20,000. Synthetic peptides were used at a concentration between 0.1 and 10 µg/ml. Control wells contained either PHA (2 µg/ml) (Wellcome Diagnostics, Dartford, U.K.), crobetagalactosidase (nonfused) proteins, or culture medium alone. Cells were cultured in a total volume of 200 µl of IMDM, supplemented with penicillin (100 µg/ml), streptomycin (100 U/ml), and glutamin (20 µm) (all Life Technologies, Grand Island, NY) and 10% normal human AB serum.
After 60 h, 1 μCi $[^3H]$TdR was added to each well. The cells were harvested 18 h later on a glass fiber filter using a cell harvester. The $[^3H]$TdR incorporation was measured by liquid scintillation counting. The results of the responses to sonicates of M. leprae and M. tuberculosis and to the purified proteins hsp10L, hsp16T, hsp18L, 30T, 38T, hsp65L, hsp65T, hsp70L, and hsp70T are expressed as stimulation indices (SI) as well as by $Δcpm$. SI is the ratio of $[^3H]$TdR incorporation of Ag-stimulated cultures to that of cultures of the same T cell line containing medium alone. $Δcpm$ is the $[^3H]$TdR incorporation of Ag-stimulated cultures minus that of cultures of the same T cell line containing medium alone. Background responses (cultures containing medium alone) varied from 100–200,100 cpm (median value 300 cpm) for HC, from 100–12,100 cpm (median value 200 cpm) for BT/TT patients, and from 100–3,200 cpm (median value 300 cpm) for BL/LL patients. Evaluation of the results of responses to semi-purified fusion protein Ags is based on $[^3H]$TdR incorporation of Ag-stimulated cultures compared with cultures of the same T cell line containing semipurified crotonobacilatoisidase (nonfused) protein. Responses displaying both an SI $≥$ 3 and $Δcpm$ $≥$ 1000 cpm were defined as a positive response. Ten of the 20 lines from HC, 15 of the 52 lines from BT/TT patients, and 7 of 21 lines from BL/LL patients were tested twice or more.

### Statistical analyses

Statistical differences between various groups were tested using the two-sided Fisher’s exact test. In case of groups with zero cases, 0.5 was added to make calculations of odds ratios and two-sided $p$ values possible.

### Results

#### Proliferative responses to M. leprae Ags

To study the antigenic repertoire recognized by T cells from individuals exposed to M. leprae, a panel of 93 M. leprae- or M. tuberculosis-stimulated T cell lines was generated derived from three subject groups: tuberculoid (BT/TT) leprosy patients, lepromatous (BL/LL) leprosy patients, and healthy “exposed” individuals (HC). All individuals originated from Surinam, a leprosy-endemic area. The antigenic repertoire recognized by these lines was examined by studying the proliferative T cell response to a panel of 16 different mycobacterial Ags (Table I). Table II summarizes the responses to these Ags by 20 T cell lines from HC, 52 T cell lines from BT/TT, and 21 T cell lines from BL/LL subjects. In agreement with the reported M. leprae-specific T cell low- or nonresponsiveness in BL/LL patients, these subjects displayed low recognition frequencies (43%) to M. leprae sonicate as compared with HC (95%) and BT/TT subjects (81%). A high frequency of responses (87–100%) to M. tuberculosis sonicate was detected in all three subject groups.

Eleven of the sixteen mycobacterial Ags tested induced proliferative responses in one or more of the subject groups. Of these eleven Ags, four Ags (hsp10, hsp65 T and L, and the secreted 30/31-kDa (Ag 85 complex) Ags) were more frequently recognized (>15%), confirming many previous studies and indicating that these Ags are dominant targets in the immune response to M. leprae and other mycobacteria. The majority of the responses to Ags were found in healthy contacts or tuberculoid patients; in contrast, the recognition frequencies in lepromatous patients were invariably low, except for the 30/31-kDa Ag. When comparing recognition frequencies between healthy contacts and tuberculoid patients, none of the Ags was exclusively recognized by one particular subject groups. Six of the Ags (hsp16T, hsp65T and L, hsp70L, hsp71T, and 30/31-kDa), however, were more frequently recognized by healthy contacts. Only in the cases of 30/31-kDa and hsp71T, however, did these differences reached statistical significance (two-sided $p$ values: 0.02 and 0.01, respectively). In contrast, one Ag, the 15-kDa Ag (L8), which we described previously (21), appeared to induce responses more frequently in tuberculoid patients, but this tendency was not significant (two-sided $p$ value 0.4). Thus, whereas some Ags may be preferentially recognized by healthy contacts or tuberculoid patients, none of them is exclusively recognized by a particular subject group.

When responses of M. tuberculosis-stimulated lines were compared with those of M. leprae-stimulated lines, no significant differences were found in the Ag recognition profiles. Most Ags (except hsp10), however, were recognized more frequently by M. tuberculosis-stimulated lines even when generated from the same individual (data not shown), suggesting a more prominent presence of most Ags in the M. tuberculosis preparation as compared with the M. leprae sonicate.

#### HLA-DR phenotype and recognition of 30/31-kDa Ag

HLA-DR molecules play an important role in the induction of T cell responses to mycobacteria since they are the main molecules that present peptide Ag to CD4 $^+$ T cells. To examine the influence

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**Table II. Recognition of 16 mycobacterial Ags by 93 T cell lines derived from leprosy patients and healthy exposed individuals.**

<table>
<thead>
<tr>
<th>Antigens</th>
<th>HC (n = 20)</th>
<th>BT/TT (n = 52)</th>
<th>BL/LL (n = 21)</th>
<th>Total (n = 93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. leprae sonicate</td>
<td>19/20 (95)</td>
<td>42/52 (81)</td>
<td>9/21 (43)</td>
<td>70/93 (75)</td>
</tr>
<tr>
<td>M. tuberculosis sonicate</td>
<td>20/20 (100)</td>
<td>45/52 (87)</td>
<td>19/21 (90)</td>
<td>86/93 (90)</td>
</tr>
<tr>
<td>hsp10L</td>
<td>2/7 (29)</td>
<td>2/4 (50)</td>
<td>NT</td>
<td>4/11 (36)</td>
</tr>
<tr>
<td>L8 (aa 23–112 of 15 kDa)</td>
<td>1/20 (5)</td>
<td>7/51 (14)</td>
<td>0/21 (0)</td>
<td>8/92 (9)</td>
</tr>
<tr>
<td>L14 (aa 75–287 of 25.5 kDa)</td>
<td>1/12 (8)</td>
<td>2/39 (5)</td>
<td>0/6 (0)</td>
<td>3/57 (6)</td>
</tr>
<tr>
<td>36L</td>
<td>0/9 (0)</td>
<td>0/26 (0)</td>
<td>0/6 (0)</td>
<td>0/41 (0)</td>
</tr>
<tr>
<td>38T</td>
<td>1/11 (9)</td>
<td>4/38 (11)</td>
<td>0/6 (0)</td>
<td>5/55 (9)</td>
</tr>
<tr>
<td>L2 (aa 188–458 of 49 kDa)</td>
<td>0/10 (0)</td>
<td>1/39 (3)</td>
<td>0/6 (0)</td>
<td>1/55 (2)</td>
</tr>
<tr>
<td>hsp65T</td>
<td>7/20 (35)</td>
<td>9/52 (17)</td>
<td>0/21 (0)</td>
<td>16/93 (17)</td>
</tr>
<tr>
<td>hsp65L</td>
<td>7/20 (35)</td>
<td>11/52 (21)</td>
<td>1/21 (5)</td>
<td>19/93 (20)</td>
</tr>
<tr>
<td>hsp70L</td>
<td>1/10 (9)</td>
<td>2/38 (5)</td>
<td>0/12 (0)</td>
<td>3/60 (5)</td>
</tr>
<tr>
<td>hsp71T</td>
<td>4/14 (29)</td>
<td>1/43 (2)</td>
<td>1/21 (5)</td>
<td>6/78 (8)</td>
</tr>
<tr>
<td>L21</td>
<td>0/11 (0)</td>
<td>0/39 (0)</td>
<td>0/6 (0)</td>
<td>0/56 (0)</td>
</tr>
<tr>
<td>L24</td>
<td>0/7 (0)</td>
<td>0/8 (0)</td>
<td>NT</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td>L43</td>
<td>0/7 (13)</td>
<td>0/7 (0)</td>
<td>NT</td>
<td>0/14 (0)</td>
</tr>
</tbody>
</table>

* Number of positive (SI $>3$ and $Δcpm$ $>1000$) proliferative responses per number of cell lines tested for each Ag is indicated (percentage in brackets) for each subject group. The total number (n) of T cell lines for each subject group is indicated.
of HLA-DR molecules on the recognition of specific Ags in different subject groups, most T cell donors in this study (70 of 76) were HLA typed. The most frequently observed DR alleles appeared to be DR2, -3, and -5. Within the group of healthy individuals, a specific and significant association of DR3 with high T cell responses to the 30/31-kDa Ag was found (two-sided \( p < 0.01 \); see Fig. 1B). This association was HLA-DR3-specific (Fig. 1, A and C), as well as Ag-specific, since it was not found in case of any other Ag (as an example, hsp65 is shown in Fig. 1, C-F) or when responses to whole sonicate of \( M. \) leprae were analyzed (not shown). In contrast, in BT/TT patients, a DR3-specific low T cell responsiveness to the 30/31-kDa Ag was found (two-sided \( p \) value = 0.005; see Fig. 1B), whereas no clear association was found in BL/LL.

In additional analyses, the difference in T cell responsiveness between healthy contacts and tuberculoid patients to the 30/31-kDa Ag in the context of HLA-DR3 was highly significant as well; as evident from Fig. 1B, all (9/9) HLA-DR3-positive healthy contacts recognized the 30/31-kDa Ag, whereas only one of 12 of HLA-DR3-positive tuberculoid patients responded (\( p < 0.0001 \)). This difference again was not found when anti-30/31-kDa responses were examined within the context of HLA-DR2 (Fig. 1A), -DR5 (Fig. 1C), -DR1, or -DR4 (not shown), or when responses to other Ags (such as hsp65; Fig. 1, D-F) and whole \( M. \) leprae sonicate were examined (not shown). Moreover, a similar difference was now found in BL/LL patients: five of eight T cell lines from HLA-DR3-positive BL/LL patients did not respond to the 30/31-kDa Ag (\( p = 0.009 \), compared with healthy individuals). Thus, these differences were more significant when leprosy patients were analyzed irrespective of leprosy classification vs healthy individuals (16 of 20 HLA-DR3-positive leprosy patients did not respond; \( p < 0.0001 \)).

Thus, HLA-DR3 is associated with 30/31-kDa-specific high T cell responsiveness in healthy exposed individuals, but with 30/
To further examine the recognition of the DR3-restricted 56–65 epitope in healthy contacts, we examined peptide 56–65 responsiveness among M. tuberculosis-reactive T cell lines from five other HLA-DR3-positive HC subjects from which sufficient T cells were available for further testing. Three of these were previously shown to respond to the peptide p56–65 (46). As shown in Table IV, four out of five of these lines that responded to the 30/31-kDa also responded to the DR3-restricted peptide epitope p56–65. One DR3+ individual that did respond mildly to the 30/31-kDa Ag did not respond to p56–65 (Table IV), suggesting the existence of other DR3-restricted, perhaps subdominant, epitopes. These 30/31-kDa responses could indeed be blocked by the addition of anti-DR but not by anti-DQ or anti-DP Abs (data not shown). The finding that four of five respond to p56–65 indicates that this epitope is an important determinant in the DR3-restricted T cell response in healthy contacts in this population.

### Discussion

In this study we have compared immune T cell responses of 19 healthy exposed individuals and 57 leprosy patients from a Surinam Negroid population to 16-well defined, native or recombinant mycobacterial Ags. The most prominent observation in this study is a significant, HLA-DR3-associated T cell low responsiveness, which was specific for the abundantly secreted mycobacterial 30/31-kDa Ag and which was observed in tuberculoid leprosy patients, whereas the reverse was seen in healthy, HLA-DR3-positive individuals. Moreover, within the group of HLA-DR3-positive individuals, there was a highly significant low T cell responsiveness to the 30/31-kDa Ag in both tuberculoid and lepromatous leprosy patients compared with healthy individuals. These results demonstrate an HLA-DR3-specific-associated and Ag-specific regulation of the antimycobacterial T cell response in leprosy.

In contrast to previous studies that employed peripheral mononuclear cells, we have here used T cell lines because this may help to avoid the stimulation of background T cell proliferation by copurifying E. coli contaminants. Our main aim was to investigate the antigenic repertoire recognized by the healthy exposed population as compared with patients, in particular, in the context of the HLA-DR molecules expressed by these individuals. In analogy to previous studies in the overall population, we found large variations in the frequency by which Ags are recognized. Some Ags (like hsp10, 30/31-kDa, and hsp65) are more “immunodominant” than other Ags, while 4 of the 16 Ags were not recognized at all. These findings agree with other studies that employed peripheral mononuclear cells, T cell clones, or limiting dilution analyses (12, 13, 22). They also support the notion that some Ags play a relatively prominent role in the immune response to M. leprae and other mycobacteria. Factors that promote the Ag’s availability to the immune system, such as secretion from the bacterial cell, timing, and its high level expression inside infected host cells, as well as (genetic) host factors, can all determine whether an Ag will become a prominent immune target. Current evidence (8, 22–27) indicates that hsp10 and hsp65-kDa heat shock Ags, and the secreted 30/31-kDa Ags fulfill at least one of these criteria, and the latter molecules have been proposed as potential candidate Ags in a subunit or DNA vaccines against M. tuberculosis (28, 29).

Several studies have revealed associations of the type of leprosy with the presence of certain HLA-DR molecules (reviewed in Ref. 15), and, in the population studied here, HLA-DR3 was previously reported to be associated with tuberculoid leprosy (15, 16). Here we find a significant difference between both subject groups in the
recognition of the secreted 30/31-kDa Ag: HLA-DR3 was associated with high T cell responsiveness to this Ag in healthy exposed individuals, whereas, conversely, it was associated with low T cell responsiveness to this Ag in tuberculoid patients. Our previous observation that HLA-DR3 is associated with low T cell responsiveness to M. leprae in tuberculoid patients (15, 30), combined with the current study reporting an HLA-DR3-associated low responsiveness to the 30/31-kDa Ag but not to hsp65, suggests that the 30/31-kDa Ag is prominently involved in this MHC allele-specific low responsiveness.

Our study confirms other studies (7–14) that, as far as can be assessed with the Ags studied, there are no Ags that are exclusively recognized by T cells from either healthy contacts or patients. This finding indicates that disease status is not mirrored by a specific repertoire antigenic in healthy contacts or tuberculoid patients. However, some Ags, in particular 30/31-kDa and hsp65, were more frequently recognized by healthy individuals, whereas others such as the 15-kDa Ag induced more frequent responses in tuberculoid patients. The preferential recognition of hsp65 and 30/31-kDa in healthy individuals (9, 12, 25) and of 15-kDa by tuberculoid patients (31) was also found in studies that employed peripheral mononuclear cells. This finding may be a reflection of the fact that development of disease progresses with a quantitative rather than a qualitative change in the antigenic repertoire. It may be that, with continuing exposure to M. leprae, the response to Ags that are dominant early in infection (32, 33), such as 30/31-kDa and hsp65, may become down-regulated, whereas responses to other Ags such as the 15-kDa Ag become more prominent. Since the 30/31-kDa and hsp65 Ag are likely to be important targets for early acquired specific immunity, individuals that develop low responsiveness to other Ags such as the 15-kDa Ag become more prominent. Since the 30/31-kDa and hsp65 Ags are likely to be important targets for early acquired specific immunity, individuals that develop low responsiveness to these Ags may fail to mount an effective immune response to M. leprae, which in turn may lead to disease manifestations such as tuberculoid leprosy. The low T cell responsiveness to the 30/31-kDa Ag in tuberculoid patients in the context of HLA-DR3 in this study serves as a particularly dramatic example. The processes that underlie the DR3-associated nonresponsiveness to the 30/31-kDa Ags in tuberculoid patients are currently unknown. The 30/31-kDa Ag contains an immunodominant DR3-restricted epitope that is recognized by the majority of DR3-positive healthy contacts. Further testing of the recognition of this immunodominant epitope by tuberculoid patients may reveal whether the DR3-associated low responsiveness to the 30/31-kDa Ag in these patients is indeed focused uniquely toward this epitope. This will provide a further basis to elucidate the mechanism of this HLA class II immune response gene regulation, which is associated with the development of tuberculoid leprosy in this population.

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References


