Tumor Necrosis Factor Promoter Polymorphism and Susceptibility to Lepromatous Leprosy


Genetically determined differences in immune responses to environmental agents may underlie susceptibility to many autoimmune and infectious diseases. Leprosy provides an example of a polarity in the type of immune response made to an infectious agent, and there is evidence that the major histocompatibility complex is genetically linked to leprosy type. It was found that HLA-DR2 is associated with both tuberculoid and lepromatous types of leprosy; however, a variant at position −308 of the promoter of the neighboring tumor necrosis factor (TNF) gene was increased in frequency in lepromatous (odds ratio = 3.0, \( P = .02 \)) but not tuberculoid leprosy. Some studies have found higher serum levels of TNF in lepromatous than tuberculoid leprosy, and high TNF levels are found in malaria and leishmaniasis, which are also associated with this TNF allele. It is speculated that this association reflects genetic variability in cytokine production, which influences the immune response to and clinical outcome of leprosy.

Leprosy presents perhaps the most striking clinical example of a dichotomous immune response. Patients with tuberculoid leprosy display a strong cellular immune response, in that skin lesions contain well-organized tuberculoid granuloma with very few bacilli in the macrophages. In contrast, lepromatous leprosy patients have bacilli-laden macrophages, with a stronger humoral immune response but a weak cell-mediated immune response. Family studies have suggested that part of this variation in response to *Mycobacterium leprae* is genetically determined, and early linkage studies have indicated that the major histocompatibility antigens might influence leprosy type [1, 2]. Numerous case-control studies of HLA and leprosy in Asian populations have reported a consistent HLA-DR2 association that is found both with tuberculoid and lepromatous types of leprosy [3]. However, it is unknown whether clinical outcome is modified by major histocompatibility complex (MHC) class II genes directly or by a nearby genetic locus. An effect of the MHC on leprosy type in family linkage studies that is not observed in HLA association studies could be explained by another non-HLA gene in the MHC region.

Located within one megabase of the MHC class II region is the gene for tumor necrosis factor (TNF). TNF may mediate host defense both by stimulating effector mechanisms that kill mycobacteria and by promoting granuloma formation, but high TNF concentrations can cause immunopathology, including direct damage to myelin and oligodendrocytes [4]. It is thus possible that the clinical outcome of leprosy is affected by the propensity of the host to produce TNF in response to *M. leprae*. It has been found that a variant allele of the TNF promoter region is associated with susceptibility to cerebral malaria, a disease in which high TNF levels are believed to play a causal role [5]. The variant allele, referred to as TNF2, is a single base transition located at nt −308 relative to the transcription start site of the TNF gene. We carried out a case-control study to determine the relationship between this allele and the two polar forms of leprosy in an Indian population.

**Methods**

**Patients and controls.** Both cases and controls were adults resident in the Calcutta area. Cases were recruited on presentation to the Leprosy Clinic at the School of Tropical Medicine. Each patient was examined by an experienced physician who assessed the type of leprosy on the basis of the appearance and distribution of skin lesions, the nature of anesthesia, slit-skin smear examinations, and the presence of thickened peripheral nerves. Following slit-skin smear tests at three or more sites (including ear lobe, nostril, and skin lesions), the clinical diagnosis was reassessed by a panel of 3 clinical leprologists. A diagnosis of lepromatous leprosy required the presence of generalized skin lesions with a large number of acid-fast bacilli on the smear test; tuberculoid leprosy was diagnosed by a small number of smear-negative, well-defined lesions with dry surfaces. Borderline cases were excluded from this study. Following confirmation of the diagnosis, a venous sample of blood was collected in tubes containing EDTA. Control samples were collected from adult volunteers attending the Swasti Blood Bank in Calcutta. DNA was extracted promptly and stored at \(-20\)°C.
We examined whether this result might relate to disease associations with MHC class II type since most earlier studies have associated MHC class II antigens with leprosy. Although TNF2 was in linkage disequilibrium with HLA-DR3 (OR = 10.0, P < .00001), the proportion of subjects with HLA-DR3 was similar in the lepromatous patients (7.2%) and controls (7.4%). As previously reported from this region [3] and as found in our study of common HLA-DR antigens (unpublished data), HLA-DR2 was associated with both lepromatous and tuberculoid leprosy (OR = 1.95, 95% CI = 1.2–3.1, P = .003). However, HLA-DR2 was not in linkage disequilibrium with TNF2; in fact, stratification for HLA-DR2 served to augment rather than to diminish the association of the TNF2 allele with lepromatous leprosy (OR = 3.3, 95% CI = 1.3–10.0, P = .008 by stratified Mantel-Haenszel test). In other words, the observed disease associations with TNF2 and the association with MHC class II type appear to be entirely independent, despite the proximity of the two loci.

Discussion

The effect of this polymorphism on TNF production at the cellular level remains to be proven. The results of reporter gene studies are equivocal: some have shown that it acts to up-regulate TNF transcription [7], while others have not [8, 9]. However associations with this allele have been noted for other infections in which excessive TNF production has been postulated to be involved, including cerebral malaria [5], mucocutaneous leishmaniasis [10], and fatal meningococcal disease [11]. Thus one interpretation of these data would be that a predisposition to overproduce TNF favors the development of lepromatous leprosy. It is difficult to quantitate such a predisposition by immunologic measurement while the disease process is under way. Although lepromatous leprosy has been associated with high serum TNF levels [12, 13], this could simply reflect the high bacillary load, while reports of low levels of TNF production by peripheral blood mononuclear cells from lepromatous leprosy patients [14] might represent refractoriness due to chronic overstimulation in vivo. The clearest example of TNF overproduction is in erythema nodosum leprosum, a reactional state that occurs in a significant proportion of patients undergoing treatment for lepromatous leprosy. Both serum TNF levels and in vitro TNF responses are very high, and the clinical symptoms are alleviated by thalidomide, which specifically blocks TNF production [15]. It would therefore be of particular interest to determine the frequency of the TNF2 allele among the subset of lepromatous leprosy patients who develop this condition.

The present findings add to a growing body of evidence that the level of TNF production affects the clinical expression of mycobacterial infection, and they reinforce the need for a better understanding of the relationship between cytokine gene regulation and infectious disease.

HLA associations have been defined for many autoimmune and infectious diseases, but different associations may be found.

Table 1. Frequencies of TNF2 genotypes in leprosy patients and controls.

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<th>TNF2 type</th>
<th>Allele frequency (%)</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Heterozygotes</td>
</tr>
<tr>
<td>Lepromatous leprosy</td>
<td>121</td>
<td>11</td>
</tr>
<tr>
<td>Tuberculoid leprosy</td>
<td>107</td>
<td>6</td>
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<tr>
<td>Controls</td>
<td>160</td>
<td>9</td>
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The study population comprised 121 patients with lepromatous leprosy (median age, 33 years), 107 patients with tuberculoid leprosy (median age, 30 years), and 160 controls (median age, 34 years). There were 6 women in the tuberculoid group, 10 in the lepromatous group, and none in the control group. Patients and controls were well matched for ethnic group. Both the patient and control groups consisted of individuals belonging to the Hindu, Muslim, or Christian religions. Among cases of lepromatous leprosy, 85% were Hindus (43% Shudras, 30% Kaisthas, 12% Brahmins), 12% were Muslims, and 3% others. The respective proportions were 83% (39%, 35%, 9%), 14%, and 3% for those with tuberculoid leprosy, and 80% (40%, 29%, 11%), 17%, and 3% for controls.

Laboratory analysis. DNA was extracted by salting-out of the cellular proteins by dehydration and precipitation with a saturated sodium chloride solution [6]. Both the TNF2 allele and the common HLA class II alleles were amplified by polymerase chain reaction (PCR), followed by hybridization of the product with allele-specific oligonucleotides [5]. The accuracy of the TNF2 typing method was confirmed by DNA sequencing of the PCR product for 10 subjects with different genotypes. Analysis for TNF alleles was carried out using oligonucleotides end-labeled with digoxigenin–dideoxyuridine triphosphate according to the manufacturer’s protocol (Boehringer Mannheim, Lewes, UK), while HLA class II alleles were analyzed using radioactively ([γ-32P]-adenosine triphosphate) labeled probes.

Statistical analysis. The frequencies of alleles in the clinical groups were compared using χ2 analysis. The Mantel-Haenszel test was used for stratified analysis, employing the Statcalc program (Epi Info, version 5.0; USD, Stone Mountain, GA).

Results

Results are presented in table 1. Genotype frequencies were found not to deviate significantly from Hardy-Weinberg equilibrium for controls. The frequency of the TNF2 allele was significantly higher in the lepromatous group than in the control group (P = .03, χ2 test with Yates’s correction). Both heterozygotes and homozygotes contributed to this effect. After we corrected for ethnic group by a stratified Mantel-Haenszel test, the TNF2 allele was associated with a weighted relative risk of 2.5 for lepromatous leprosy, with a 95% confidence interval (95% CI) of 1.1–6.5 (since leprosy is relatively uncommon, these values can be estimated from the weighted odds ratio [OR]; P = .03). By contrast, allele frequencies in the tuberculoid leprosy patients were similar to those in controls.
in different populations. It has frequently been speculated, but never demonstrated, that this heterogeneity might result from a primary association with some other linked non-HLA gene in the MHC. Here we have shown that an MHC effect on leprosy type, first identified 20 years ago [1], may be a compound effect that involves HLA-DR and polymorphisms in the region of the TNF gene.

Acknowledgment

We are grateful to V.N. Bhatia Serological Institute, Calcutta, for providing facilities for DNA extraction.

References