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A *Mycobacterium leprae*–Specific Human T Cell Epitope Cross-Reactive with an HLA-DR2 Peptide

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***Mycobacterium leprae* induces T cell reactivity and protective immunity in the majority of exposed individuals, but the minority that develop leprosy exhibit various types of immunopathology. Thus, the definition of epitopes on *M. leprae* antigens that are recognized by T cells from different individuals might result in the development of an effective vaccine against leprosy. A sequence from the 65-kD protein of this organism was recognized by two HLA-DR2–restricted, *M. leprae*–specific helper T cell clones that were derived from a tuberculoid leprosy patient. Synthetic peptides were used to define this epitope as Leu-Gln-Ala-Ala-Pro-Ala-Leu-Asp-Lys-Leu. A similar peptide that was derived from the third hypervariable region of the HLA-DR2 chain, Glu-Gln-Ala-Arg-Ala-Ala-Val-Asp-Thr-Tyr, also activated the same clones. The unexpected cross-reactivity of this *M. leprae*–specific DR2-restricted T cell epitope with a DR2 peptide may have to be considered in the design of subunit vaccines against leprosy.**

LEPROSY CONTINUES TO BE AN important endemic disease in most countries of Asia, Africa, and Latin America, with 10 to 12 million estimated cases worldwide (*1*). A problem is that resistance develops to the widely used drug dapsone. A promising alternative to this and other more expensive combined drug therapies is the possibility of an effective vaccine against leprosy. Most people can respond to infection by *Mycobacterium leprae* with both a humoral and cellular immune response. The latter, however, seems to control the outcome of an infection. Victims of lepromatous leprosy, the most severe form of the disease, have a specific anergy to *M. leprae* in their cellular immune response, which allows unlimited multiplication and spread of this bacillus. However, victims of tuberculoid leprosy suffer from an enhanced cellular immune response which can result in nerve destruction (*1*). It will be important for vaccine development to understand this difference in response. We are, therefore, defining specific interactions of *M. leprae* with the immune system and have begun to catalog *M. leprae* T cell epitopes. One important *M. leprae* antigen is the 65-kD protein, a prominent mycobacterial protein similar in sequence to the heat shock proteins (*2*). This highly immunogenic protein contains an *M. leprae*–specific B cell epitope (*3*) and a number of T cell epitopes (*4, 5*).

The HLA-DR2–restricted (*6*) *M. leprae* reactive T cell clone 2F10 appeared to be nearly *M. leprae*–specific. At low mycobacterial lysate concentrations 2F10 reacted only with Dharmendra lepromin and armadillo-derived *M. leprae* (Fig. 1); at higher concentrations it reacted weakly with *M. vaccae*. It did not react with 18 other species of mycobacteria, including *M. tuberculosis* (7). This particular clone was selected because it proliferated in the presence of the 65-kD protein of *M. leprae* [produced in *Escherichia coli* (*8*)], but not the *M. tuberculosis* Bacille Calmette Guerin (BCG) 65-kD protein [produced in *E. coli* (*9*)] (Table 1). Thus this clone recognizes an *M. leprae*–specific epitope on the 65-kD protein.

To further define the epitope, we synthesized peptides from *M. leprae*–specific regions of the 65-kD sequence. The only positive peptide, peptide 17, contains residues NH_2-terminal to, and including the epitope defined by, the *M. leprae*–specific mouse monoclonal antibody (MAb) IIIE9 (*3*) (Table 2). Peptide 17 almost fully activates clone 2F10 at a concentration of 0.35 n*M*, a half-stimulating concentration considerably lower than the 1 to 100 μ*M* range of many stimulatory peptides for helper T cells (*10, 11*). The activation of this clone is inhibited by the mouse MAb IIIE9, which recognizes a linear *M. leprae*-specific epitope on the 65-kD protein (*3, 12*), but not by F67-13, another mouse MAb to the 65-kD protein (*3*). Thus, peptide-induced T cell proliferation is inhibited by specific antibody, as has been previously demonstrated in a few cases (*13–15*).

We used different synthetic peptides to determine the exact location of the epitope recognized by 2F10 (Fig. 2). Data from

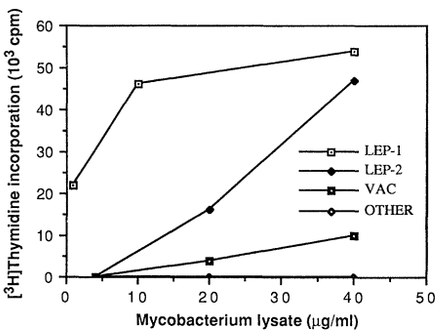

Fig. 1. Mycobacterial species-specificity of the helper T cell clone 2F10. Mycobacterial antigens were presented via autologous antigen presenting cells to 2F10. Details of the proliferation assay are as described (7). LEP-1 is Dharmendra lepromin, LEP-2 is armadillo-derived *M. leprae*, VAC is *M. vaccae*. "Other" represents mycobacteria giving no response: *M. kansasii*, *M. tuberculosis*, *M. avium*, *M. africanum*; *M. scrofulaceum*; *M. tuberculosis* strains H37Rv and H37Ra; *M. nonchromogenicum*; *M. gordonae*; *M. bovis*; *M. fortuitum*; *M. bovis BCG*; *M. duvalii*; *M. smegmatis*; *M. lepraemurium*; *M. lufu*; *M. avium/intracellulare*; and armadillo-derived aspecific mycobacteria.

Table 1. Specificity of the helper T cell clones 2F10 and 2B6. Antigen presentation, cloning, expansion, and proliferation assays were performed as described (7). Clones 2F10 or 2B6 (1×10^4 cells) were incubated with autologous mononuclear cells (5×10^4). Medium or bacterial lysates (10 μg/ml) were included in the assay. Each experiment was performed on three independent replicate cultures.

Addition to assay*	[^{3}H]Thymidine incorporation (cpm)	
	2F10	2B6
Medium	303 ± 139	277 ± 55
Y4178	26,907 ± 807	24,087 ± 2,408
1402	500 ± 80	132 ± 446
pEX II	392 ± 50	150 ± 22
CD75	53,343 ± 1,600	82,830 ± 3,313

*Y4178, *E. coli* expressing the gene for the *M. leprae* 65-kD protein (*8*); 1402, *E. coli* expressing the gene for the *M. bovis* BCG 65-kD protein (*9*); pEX II, *E. coli* expressing the control plasmid; CD75, armadillo-derived *M. leprae*.

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peptides 17, 83, and 22 (Fig. 2A) indicate that the NH_2-terminus is a leucine. Similar data from peptides 17, 77, 80, and 81 indicate that the COOH-terminus is also a leucine. These experiments with NH_2- and COOH-terminal truncations of peptide 17 suggest that LQAAPALDKL (*16*) is the minimal peptide strongly stimulating clone 2F10, although peptides as long as the 24-residue peptide 17 are somewhat more potent. The minimal epitope is within the size range (8 to 12 amino acids) of other minimal T cell stimulatory peptides (*17*). Omission of leucine at either terminus causes a considerable loss of potency (Fig. 2B). This peptide overlaps the *M. leprae*–specific IIIE9 epitope, KLKLTGDEA, by two residues at its COOH-terminus.

Table 2. Antigen-induced proliferation of 2F10. The MAb IIIE9 and the MAb F67-13 react with the 65-kD protein. The essay was done as described in Table 1. Each experiment was performed on three independent replicate cultures.

Addition to assay*	[^{3}H]Thymidine incorporation (cpm)
Medium	212 ± 23
Peptide 17	16,463 ± 5,762
Peptide 17 + IIIE9	160 ± 68
Peptide 17 + F67–13	11,465 ± 4,127

*The MAbs were added as 1/300 dilutions of ascites at the start of the assay. Peptide 17 (1 ng/ml) was added to all experiments except medium alone.

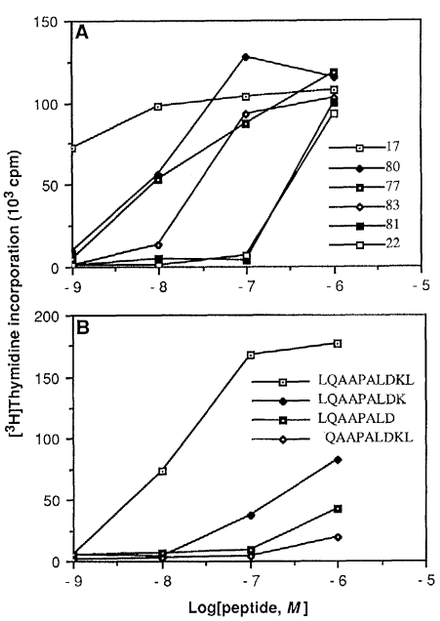

Fig. 2. (**A** and **B**) Use of synthetic peptides to locate the epitope of 2F10 in the 65-kD protein sequence. Antigen presentation assay was as described in Table 1. The peptide sequences are in single-letter code. The sequences in (A) are peptide 17, TLLQAAPALDKLKLTGDEATGANI; 77, TLLQAAPALDKLKLTGDE; 80, TLLQAAPALDKL; 81, TLLQAAPALD; 83, LQAAPALDKLKLTGDEATGANIC; 22, QAAPALDKLKLTGDEATGANIC. Peptides were synthesized in the amide form, using solid phase peptide synthesis methodology (*12, 21, 22*) and checked by analytical reversed-phase high-performance liquid chromatography and amino acid analysis (*12*).

Because it has been suggested that peptides restricted by a given murine Ia molecule show similarity to portions of that particular protein (*18*), we searched for possible similarities between the DR-2 restricted *M. leprae* peptide LQAAPALDKL and HLA–class II molecules. This search revealed a similarity with the third hypervariable region of the DR2 B3 chain. A synthesized peptide from this region also stimulated the T cell clone 2F10, although about 100 times less efficiently (Fig. 3). Another DR2-restricted clone (2B6) from the same patient, which responds to the same epitope, also reacted to this DR2 peptide, which was approximately as potent as the *M. leprae*–specific peptide (Fig. 3). This stimulation was not due to mitogenicity of the peptide itself, since other T cell clones, including DR-2 restricted clones from the same patient, did not respond to this DR2 peptide. Presentation of the *M. leprae* peptide requires the B1 chain of the DR2 molecule (*6*). Thus a peptide of the DR2 B3 chain is presented via the A and B1 chains of the DR2 molecule.

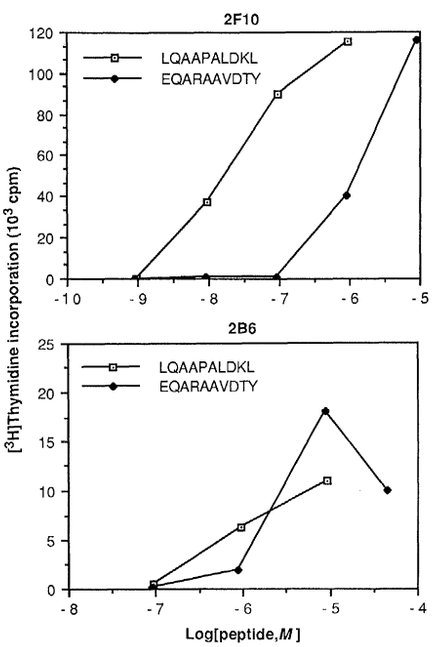

Fig. 3. Comparison of the stimulatory effects of an *M. leprae* peptide and an HLA-DR B3 peptide on T cell clones 2F10 (**top**) and 2B6 (**bottom**). Peptide EQARAAVDTY is from the third hypervariable region of the B3 chain of the DR2 molecule. Clones 2F10 (1×10^4 cells) or 2B6 (1×10^4 cells) and the DR-matched antigen presenting cells (5×10^4) were incubated with varying concentrations of each peptide as shown, and were assayed as described (7).

We have defined an *M. leprae*–specific HLA-DR2 restricted human T cell epitope on a 65-kD protein of the bacillus that is cross-reactive with a DR2-peptide. Because this epitope is *M. leprae*–specific and situated on an immunogenic molecule (*19*) it is interesting not only for the development of a vaccine, but also for *M. leprae*–specific diagnostic tests, such as skin tests.

At least two aspects of our data need further attention before such applications can be considered. First, the T cell recognition of this epitope seems to be under strict Ir gene control like other epitopes on the 65-kD protein (*20*). Thus, this particular epitope may only be recognized by T cells from individuals carrying the DR2 allele (less than one third of most populations). Second, the cross-reactivity with an HLA-DR2 peptide may have in vivo relevance. The CD-4–positive T cell clones 2F10 and 2B6 that define the *M. leprae* peptide and its cross-reactivity were generated from a patient with the tuberculoid form of leprosy, which is associated with HLA-DR2 in some populations (*21, 22*). It is generally assumed that the increased cellular immune response to *M. leprae* observed in these patients is responsible for the immunopathology that results in nerve destruction (*1*). Thus the cross-reactivity between the *M. leprae*–specific and DR2 peptide may have a role in the pathogenesis and immunopathology observed in tuberculoid leprosy, rather than in protection against it.

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Unusual Immunoglobulin Gene Rearrangement Leads to Replacement of Recombinational Signal Sequences

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An unexpected immunoglobulin gene rearrangement, signal sequence replacement, was observed in which the recombinational signal sequences of a V_H gene segment are fused intact to the 5′ end of a DJ_H element. Nucleotides are not lost from the signal sequences, but they may be lost from the DJ_H coding sequence. Signal sequence replacement may result from the alternative resolution of an intermediate in V_H-to-DJ_H recombination. This type of rearrangement provides a means to alter the targeting of immunoglobulin gene segments and suggests a mechanism for the occurrence of V_H-J_H junctions in vivo. Signal sequence replacement may represent an additional pathway for the generation of antibody diversity.

THE VARIABLE REGIONS OF IMMUNOGLOBULIN (Ig) heavy chains are encoded in three separate DNA segments, V_H, D, and J_H, that are brought together by a series of site-specific recombinational events (*1*). In the unrearranged state these segments are associated with recombinational signal sequences, conserved heptamer and nonamer elements that are separated by a spacer region (*2, 3*). The spacer regions fall, on the basis of length, into two classes of 12 and 23 base pairs (bp); recombination normally occurs only between gene segments carrying spacers of different lengths (*2*). In the Ig heavy chain system, V_H and J_H segments are accompanied by 23-bp spacers, while D segments carry a 12-bp spacer at either end (*2, 3*). We now report a type of Ig gene rearrangement in which the recombinational signal sequences at the 5′ flank of a D gene segment are replaced by the recombinational signals of a V_H segment. This reaction provides a means by which the targeting of Ig gene segments may be altered, and suggests an additional mechanism for generation of immunological diversity.

We previously assayed the joining of V_H and DJ_H segments that were stably integrated into the genomes of B-lymphoid cell lines by retroviral transduction (*4*). The assay relies on direct observation of the products of recombination; because the assay does not depend on biological selection for rearrangement, it places few constraints on the structures of the recombinants. We used a similar assay here. The substrate for recombination, pLJHCR-2 (Fig. 1A), was constructed in the retroviral vector pDOL$^-$ (*5*), and contains three murine Ig gene segments: V_H, DJ_H, and J_H (Fig. 1A). Between the V_H and DJ_H segments lies the *gpt* gene of *Escherichia coli*. The Ig gene segments are arranged so that joining of V_H to DJ_H or to J_H results in an inversion of the intervening DNA (Fig. 1B).

The pLJHCR-2 construct was packaged in the helper cell line ψ-2 (*6*) and the recombinant retrovirus was used to infect the B-progenitor cell line HAFTL-1 (*7, 8*), which undergoes continuing D-to-J_H joining during propagation in culture (*8*). Derivatives of HAFTL-1 that contained integrated provirus were selected in G418.

Rearranged and unrearranged substrates can be distinguished by digestion with Kpn I and hybridization to probes specific for *gpt* or *neo* sequences. Digestion of unrearranged proviral DNA creates a 2.4-kb fragment that hybridizes to a *gpt*-specific probe, and a 4.0-kb fragment that hybridizes to a *neo*-specific probe (Fig. 1A). Proviral DNA that has undergone V_H-to-DJ_H joining yields a 4.8-kb fragment that hybridizes to both probes (Fig. 1B). This assay was used to detect rearrangement of the integrated substrate in the HAFTL-1 cell line. Of 34 clones assayed, 21 yielded a 4.8-kb *neo*-containing fragment that also hybridized to the *gpt* probe, consistent with V_H-to-DJ_H joining within the substrate [for example, clone a-15, Fig. 2, A and B, lanes d, and (*9*)]. This

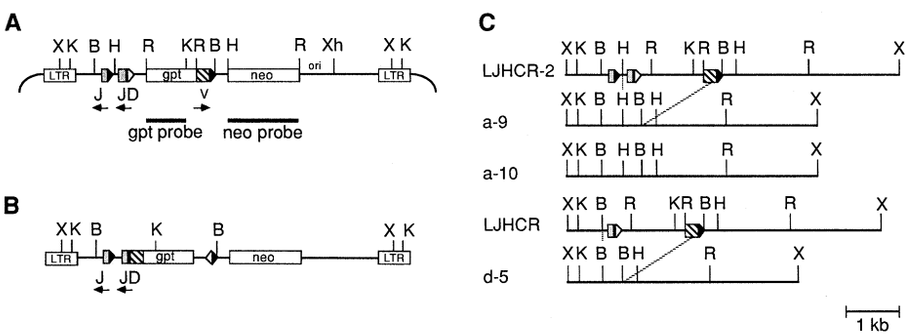

Fig. 1. (**A**) Structure of the pLJHCR-2 substrate for recombination. The retroviral portion of plasmid pLJHCR-2 is similar to pLJHCR (*4*), except for the insertion of a 407-bp Hind III–Bam HI fragment containing an unrearranged J_H2 gene segment. The Moloney murine leukemia virus long terminal repeats (LTRs), the *neo* gene, and the *gpt* gene are indicated by open boxes. The pBR322 origin of replication is indicated. The D sequence is indicated by a filled box; the J_H sequence by a shaded box, and the V_H sequence by a hatched box. Recombinational signal sequences carrying 23 and 12-bp spacers are indicated by filled and open triangles, respectively. The sense orientations of the Ig coding sequences are indicated by arrows. Sequences surrounding the retroviral LTR (about 7300 bp) are not included. Restriction enzymes are abbreviated as follows: X, Xba I; K, Kpn I; B, Bam HI; H, Hind III; R, Eco RI; and Xh, Xho I. (**B**) Structure of the LJHCR-2 substrate following V_H-to-DJ_H joining. Sequence elements and restriction sites are indicated as in (A). Rearrangement of V_H to J_H would yield internal Kpn I fragments of about 1.2 and 5.2 kb. (**C**) Restriction maps of the recombined substrates from cell clones a-9, a-10, and d-5. Top line, the LJHCR-2 restriction map from the Xba I site in the 5′ LTR to the Xba I site in the 3′ LTR. Second and third lines, retroviral DNA from cell clones a-9 and a-10. Fourth line, the LJHCR restriction map from the 5′ Xba I site to the 3′ Xba I site (*4*). Fifth line, retroviral DNA recovered from cell clone d-5. Restriction sites, Ig gene segments, and recombinational signal sequences are indicated as in (A). Recovery of rearranged proviral sequences from clones of infected lymphoid cells was performed as described (*13*).

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