Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11244–11248, December 1992
Immunology

T-cell receptor β-chain gene usage in the T-cell recognition of *Mycobacterium leprae* antigens in one tuberculoid leprosy patient

WIM C. A. VAN SCHOOTEN*†, JONE LONG KO‡§, NIENKE VAN DER STOEP*, JOHN B. A. G. HAANEN*, LULU PICKERING‡, RENÉ R. P. DE VRIES*, AND PETER VAN DEN ELSEN*¶

*Department of Immunohaematology and Blood Bank, University Hospital, Building 1, E3-Q, P.O. Box 9600, 2300 RC Leiden, The Netherlands; and ‡T Cell Sciences, 38 Sidney Street, Cambridge, MA 02139

Communicated by Hugh O. McDevitt, August 14, 1992

ABSTRACT **The β chain of the T-cell antigen receptor present on 20 T-cell clones isolated from a tuberculoid leprosy patient was studied by gene rearrangement and PCR analysis. These T-cell clones all responded to *Mycobacterium leprae*-encoded protein antigens, and 8 of them specifically recognized peptides of the mycobacterial 65-kDa heat shock polypeptide (65hsp). All T-cell clones studied were HLA-DR-restricted (DR2 or -3). In the DR3-restricted group, 7 of 10 used a β-chain variable region Vβ5 gene family member, whereas in the DR2-restricted group, 2 of 10 T-cell clones used a Vβ5 gene segment and 5 used the Vβ18 gene segment. The deduced amino acid sequences of the β chain from 8 T-cell clones have revealed that 3 of 4 DR3-restricted T-cell clones expressed the Vβ5.1 gene segment whereas the fourth DR3-restricted T-cell clone employed a Vβ5 family member not previously described. The Vβ5.1-positive T-cell clones all recognized the same 65hsp peptide from residues 2 to 12. The N-D-N segment (where D is diversity) of the junctional region of these T-cell clones was very similar, despite different β-chain joining gene segments. Of the 4 DR2-restricted T-cell clones investigated, 3 used the Vβ18 gene segment and recognized the 65hsp peptide from residues 418 to 427. In conclusion, within this panel of *M. leprae*-reactive T-cell clones, the DR3-restricted T-cell clones mainly used a Vβ5 gene segment, whereas the DR2-restricted clones employed preferentially the Vβ18 gene segment.**

T cells are stimulated by foreign protein antigens on target cells only when presented by a restricting element that is provided by the major histocompatibility complex (MHC) class I or class II molecules expressed on the antigen-presenting cell. This antigen recognition occurs via the T-cell receptor (TCR) that is embedded in the membrane of the T cell. This multichain chain receptor complex has been well studied and shown to be responsible for both antigen and MHC recognition via two chains of the TCR complex, termed α and β (1). A functional β chain is formed by two successive in-frame rearrangement events involving variable (V), diversity (D), and joining (J) region segments. As is the case with immunoglobulins, the VDJ junctional regions often contain N regions, which are additional amino acids thought to originate from a template-independent addition of nucleotides during the rearrangement process. Several clusters of complementarity-determining regions (CDR1, -2, and -3) can be recognized on the TCR. Modeling studies have suggested that the CDR1 and -2 regions mainly interact with the MHC whereas the CDR3 region interacts with the peptide when it is associated with the MHC molecule (2–4).

Studies on the relationship between the structural elements of the TCR and antigen specificity in animal models have indicated that (*i*) T cell responses may be highly degenerate, using a wide variety of V, D, and J elements (5, 6), (*ii*) responses may be somewhat limited with half the antigen-specific clones using the same combination of Vα and Vβ genes within a given MHC context (7), or (*iii*) responses can be strongly correlated with Vβ usage (8, 9). In humans evidence exists for limited TCR heterogeneity in human autoimmune diseases, such as multiple sclerosis (10, 11). However, no evidence exists to date for limited TCR heterogeneity in the recognition of a small peptide in the context of a single HLA.

In this study, the T-cell response in one tuberculoid leprosy patient against *Mycobacterium leprae* and the immunodominant 65-kDa heat shock polypeptide (65hsp) (12) was used as a model system to define usage of the Vβ region and to study the molecular basis for MHC–peptide–TCR interaction in more detail. *M. leprae* is the causative agent of leprosy, a disease characterized by a close relationship between the cellular immune reaction of the host and outcome of the infection. It is thought that, in most cases, infection with *M. leprae* results in an adequate cellular immune reaction and, hence, no disease manifestion. In a minority of individuals, the cellular immune reaction against *M. leprae* is inappropriate or absent, resulting in either the tuberculoid or lepromatous form of leprosy, respectively (30). The studies related to the T-cell response in leprosy will allow for the development of strategies to control this disease. One obvious target for regulation of the disease would be a TCR idiotype. This approach requires that the disease-related T cells use a restricted set of TCR genes.

In this study, we show that the majority of *M. leprae*-reactive clones from a single patient contain mRNAs that employ V-region gene segments belonging to the Vβ5 or Vβ18 family. Most DR3-restricted clones from the tuberculoid leprosy patient use the Vβ5.1 gene segment and recognize the 65hsp peptide from residues 2 to 12 [65hsp-(2–12)], whereas most DR2-restricted clones from the same patient express the Vβ18 gene segment and recognize the 65hsp peptide from residues 418 to 427 [65hsp-(418–427)]. Despite similar usage of Vβ gene segments, we demonstrate that the junctional region (CDR3) of the TCR β chain of the individual clones differs and might play a pivotal role in peptide recognition. In addition, we show that murine monoclonal antibodies (mAbs) raised against a TCR structure expressing the Vβ5.3 gene segment crossreact with a TCR having a Vβ5.2 gene segment but not with TCRs with a Vβ5.1 or a Vβ5 family member characterized in this paper.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; 65hsp, 65-kDa heat shock polypeptide; V, variable; D, diversity; J, joining; C, constant; mAb, monoclonal antibody; CDR, complementarity-determining region.

†Present address: ImmuLogic Pharmaceutical Corporation, 855 California Avenue, Palo Alto, CA 94304.
§Present address: Cyto Med, 1 Mountain Road, Framingham, MA 01701.
¶To whom reprint requests should be addressed.

MATERIALS AND METHODS

Establishment and Culture of T-Cell Clones. T-cell clones were obtained from tuberculoid leprosy patients as described (13). Approximately 5×10^6 peripheral blood mononuclear cells from tuberculoid leprosy patients were stimulated *in vitro* with *M. leprae* (1 μg/ml). T cells were cultured in Iscove's modified Dulbecco's medium (IMDM; GIBCO) supplemented with streptomycin (100 μg/ml), penicillin (100 μg/ml), and 10% (vol/vol) pooled human serum. After incubation at 37°C and 5% CO_2/95% air for 5 days, T-cell blasts were cloned by limiting dilution at a concentration of 1 cell per 3 wells in the presence of a feeder mixture consisting of 1×10^6 allogeneic peripherial blood mononuclear cells per ml (30-Gy irradiated), 1×10^5 Epstein–Barr virus-transformed autologous B cells (EBV-B cells, 50-Gy irradiated), and sonicated *M. leprae* (1 μg/ml). This suspension was plated in 96-well flat-bottomed microtiter plates (Falcon 3072; Becton Dickinson). Growing cultures were transferred into 24-well tissue culture plates and restimulated with the feeder cell mixture. Antigen specificity of the T-cell clones was determined in proliferation assays as described (12), and the DR restriction was determined with mouse L cells that had been transfected with human HLA-DR genes (31). Clonality of the T-cell clones was confirmed by Southern blot analysis of *Eco*RI- and *Hin*dIII-digested DNA with a β-chain constant region (Cβ)-specific probe (14).

Southern Blot Analysis. DNA from the individual T-cell clones was digested with *Hin*dIII and *Eco*RI separately. Blots were prepared on Zeta-Probe nylon membranes (Bio-Rad) and hybridized by the procedure of Gatti *et al.* (15) or Reed and Mann (16). Finally, four 20-min washes at 42°C were done in 0.1× SSC/0.1% $NaDodSO_4$ (1× SSC is 0.15 M NaCl/0.015 sodium citrate, pH 7.0). First the Southern blots were probed with the Cβ probe (14), then stripped, and rehybridized with the Vβ-specific probes (ref. 17 and J.L.K., unpublished results). To identify the Vβ gene usage, one can superimpose the autoradiograms that were separately probed with the Cβ and Vβ probes. Any unique Vβ rearranged band that can be superimposed over the Cβ uniquely rearranged band is considered to be significant. In other words, it strongly suggests the usage of this particular Vβ gene subfamily. Vβ-specific probes were made by subcloning restriction fragments from V regions of β-chain cDNA clones into an appropriately digested pUC12 plasmid (17). Probes were labeled with all four [α-^{32}P]dNTPs by nick translation (18).

Polymerase Chain Reaction (PCR) and Sequence Analysis. Total RNA was isolated by the guanidine hydrochloride method from T-cell clones restimulated 10 days earlier as described above. Total RNA (5 μg) was converted into cDNA by using oligo(dT) as the primer (Promega). The β chain of the TCR was amplified using Vβ18-, Vβ5-, and Cβ-specific amplimers, each at a final concentration of 20 pmol. The amplimers were chosen on the basis of results obtained in Southern blot analysis of our clones with Vβ-specific DNA probes. The amplimers used were as follows: Vβ5, 5′-CCGGTCGACAGCAAGTGAC(A/T)CTG-3′ (17); Vβ18, 5′-TCTAATATTCATCAATGGCCAGC-3′ (19); Cβ, 5′-TGTGGGAGATCTCTGCTTCTG-3′ (14). Amplifications of the TCR β chain were performed with 2.5 units of *Taq* polymerase (Cetus) in a final volume of 100 μl containing 20 pmol of each amplimer, 50 mM KCl, all four dNTPs (each at 0.5 mM), 10 mM Tris·HCl (pH 8.4), 4 mM $MgCl_2$, and bovine serum albumin (0.06 mg/ml). The TCRβ amplification was performed for 30 cycles. Denaturation was at 95°C for 30 sec, the amplimers were annealed to the cDNA for 30 sec at 55°C, and the elongation step was at 74°C for 1 min. The amplification product was extracted twice with an equal volume of a 1:1:1 mixture of phenol/chloroform/isoamyl alcohol followed by extraction with an equal volume of chloroform/isoamyl alcohol (twice). After ethanol precipitation, the amplified product was digested with *Ssp* I and *Bgl* II (Vβ18) or *Sal* I and *Bgl* II (Vβ5), respectively. The DNA was purified by electrophoresis through a 0.85% low-melting-temperature agarose gel in TAE buffer (18) and ligated into pUC18, followed by transformation of *Escherichia coli* HB101. The nucleotide sequences were determined by the method of Sanger *et al.* (20) and were derived from plasmids of four colonies.

Proliferation Assays. Approximately 1×10^4 T cells and 5×10^4 (40-Gy irradiated) DR-matched allogeneic peripheral blood mononuclear cells as antigen-presenting cells were cultured in 200 μl of IMDM with 10% human serum in the presence or absence of various concentrations of peptides in 96-well flat-bottomed microtiter plates. The cultures were set up in triplicate and incubated as described above for 72 h. Eighteen hours before termination, 1.0 μCi of [^{3}H]thymidine (New England Nuclear; 1 Ci = 37 GBq) was added. The samples were harvested on glass-fiber filters by using a semiautomatic sample harvester. [^{3}H]Thymidine incorporation was measured in a liquid scintillation counter. The results are expressed as the mean of triplicate cultures.

RESULTS

TCR Vβ Gene Segment Usage of *M. leprae*-Reactive Human T-Cell Clones. Twenty *M. leprae*-reactive T-cell clones established from the peripheral blood of tuberculoid leprosy patient R were analyzed on a Southern blot and with PCR amplification using Cβ- and Vβ-specific probes and oligonucleotides. All of these clones were reactive with determinants present in *M. leprae* when presented in the context of HLA-DR3 or -DR2. Seven of 10 samples of the DR3-restricted group used the Vβ5 subfamily, and 5 of the 10 T-cell clones in the DR2-restricted group used the Vβ18 gene

Table 1. Summary of Southern blot and PCR analysis on 20 T-cell clones with Cβ- and Vβ-specific probes and oligonucleotides

T-cell clone	RD	Specificity	Peptide antigen	Vβ (family) usage
R3E10	DR3	D	65hsp-(2–12)	5
R5E10	DR3	D	65hsp-(2–12)	5
R2E4	DR3	D	65hsp-(2–12)	5
R1F9	DR3	D	65hsp-(2–12)	5
R2E5	DR3	D	—	5
R1F3	DR3	A	—	5
R1E4	DR3	A	—	ND
R1E3	DR3	A	—	ND
R1G6	DR3	A	—	ND
Rp151-1	DR3	D	65hsp-(2–12)	5
R3B4	DR2	C	—	5
R2B3	DR2	A	—	18
R2B6-1	DR2	A	65hsp-(418–427)	18
R2B6-5	DR2	D	—	5
R2F9	DR2	B	—	18
R2F10	DR2	A	65hsp-(418–427)	18
Rp171-2	DR2	A	65hsp-(418–427)	18
R2G4	DR2	D	—	ND
R1G5	DR2	A	—	ND
R3E8	DR2	D	—	ND

RD, restriction determinant. Antigen specificity of the T-cell clones: A, T-cell clones recognizing determinants only present in *M. leprae*; B, T-cell clones reactive with determinants present in *M. leprae* and one other mycobacterial strain; C, T-cell clones reactive with several but not all mycobacterial strains; D, T-cell clones crossreactive with at least 17 mycobacterial strains (21, 22). —, Not defined (these clones do not recognize 65hsp); ND, not determined (no hybridization with Vβ5- and Vβ18-specific probes).

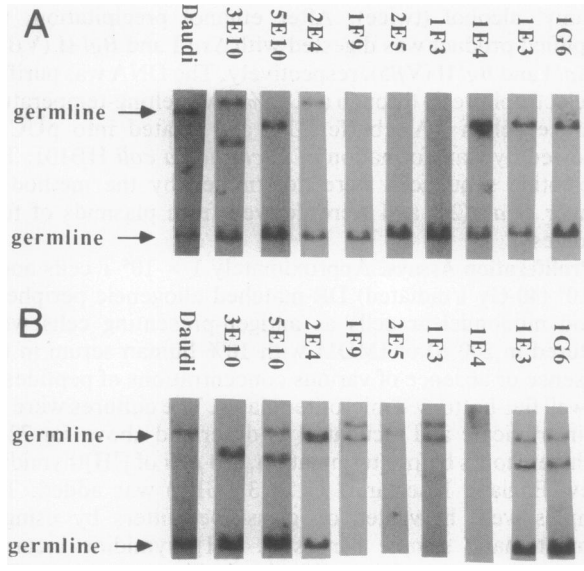

FIG. 1. Southern blot analysis of the *M. leprae*-reactive and DR3-restricted T-cell clones indicated from one tuberculoid leprosy patient. DNA was digested with *Eco*RI (*A*) and *Hin*dIII (*B*) and hybridized with a Cβ probe. Daudi is a B-lymphoblast cell line and represents the germ line.

segment (Table 1). All T-cell clones were clonal in nature (Fig. 1).

Amino Acid Sequences in the VDJ Region. To study TCR Vβ-region usage in more detail, we have determined the deduced amino acid sequence of eight *M. leprae*-reactive T cell clones. The relevant functional properties are depicted in Table 1. Four of these T-cell clones (R3E10, R2E4, R1F9, and R1F3) were HLA-DR3-restricted and, with the exception of one (R1F3), did crossreact with 17 other mycobacteria strains (21, 22). T-cell clones R3E10, R2E4, and R1F9 specifically recognized the peptide 65hsp-(2–12) of the immunodominant mycobacterial 65hsp, whereas R1F3 did not recognize this protein. As can be seen from Fig. 2, specific PCR amplification of the TCR β chain followed by DNA sequence analysis of the PCR-amplified material revealed that these four DR3-restricted T-cell clones used two members of the Vβ5 gene family. Clones R3E10, R2E4, and R1F9 employed the Vβ5.1 gene segment, whereas clone R1F3 expressed a Vβ5 member not previously defined (Vβ5.5). Despite identical usage of the Vβ5.1 gene segment, clones R3E10, R2E4, and R1F9 had distinct Jβ gene segment usage. Interestingly, the N-D-N segment of the junctional regions of R3E10 and R1F9 were very similar; they shared the FGQ amino acid motif in this region. Of note, all three clones, 3E10, R2E4 and 1F9, have a glycine at position 100, five residues to the carboxyl side of the cysteine at position 95 in the β chain, which is in the putative peptide binding site in the TCR. Of the four DR2-restricted T-cell clones, three (R2F9, R2F10, and Rp171-2) used the Vβ18 gene segment. T-cell clones R2F10 and Rp171-2 responded to peptide 65hsp-(418–427) (23), whereas R2F9 did not. Despite identical Vβ and Jβ gene usage, the 65hsp-(418–427)-reactive T-cell clones R2F10 and Rp171-2 differed significantly in their N diversity (Fig. 2). The fourth DR2-restricted T-cell clone, R2B6-5, employed the same Vβ5 gene family member as was used by the DR3-restricted clone R1F3. Fig. 3*A* shows the nucleotide sequence of this Vβ5 region that is expressed by the T-cell clones R1F3 and R2B6-5. Comparison of the deduced amino acid sequence of this Vβ5 region with those of the other Vβ5 gene regions showed that this Vβ5 gene segment has the highest homology with the Vβ5.2 region. It shares 68 out of 78 amino acids with Vβ5.2 as a result of 90% identity at the nucleotide level (Fig. 3*B*).

Reactivity of the T-Cell Clones R2F10 and Rp171-2 with 65hsp-(418–427). Fig. 4 shows the proliferation of T-cell clones R2F10 and Rp171-2 toward the 65hsp-(418–427) peptide. As demonstrated in the previous paragraph, clones R2F10 and Rp171-2 differed only in the N region that contributes to the TCRβ diversity. However, they both recognized the 65hsp-(418–427) in the context of HLA-DR2. T-cell clone R2F10 responded to lower concentrations of this peptide than did Rp171-2. The concentration for half-maximal stimulation was 0.01 μg/ml for R2F10 and was 1 μg/ml for Rp171-2. Furthermore, both clones responded equally to a longer peptide 65hsp-(416–432), suggesting that these clones recognize different epitopes within the 65hsp-(416–432) and have different affinities for the 65hsp-(418–427).

Reactivity of Vβ5$^+$ T-Cell Clones with Vβ5-Family-Reactive Murine mAbs. None of the DR3-restricted or DR2-restricted R2B6-5 T-cell clones was recognized by the murine mAbs (3D6, 1C1, and 65) reactive with the HPB-ALL TCR β chain (24, 25). Since the HPB-ALL cell line expresses the Vβ5.3 gene segment, it is clear from our studies that these mAbs do not crossreact with the Vβ5.1 and Vβ5.5 family members present in this panel of T-cell clones. A DR1-restricted T-cell clone from another tuberculoid leprosy patient using the Vβ5.2 gene was recognized by the anti-Vβ5.3 murine mAbs

T cell clone	V		N-D-N		J
R3E10	5.1	ASS	FGQGS	EAFFGQGTRLTVV	1.1
R2E4	5.1	AS	PRGGRD	YGYTFGSGTRLTVV	1.2
R1F9	5.1	ASS	FGQS	NEQFFGPGTRLTVL	2.1
R1F3	5.5	ASS	LGARA	GELFFGEGSRLTVL	2.2
R2B6-5	5.5	ASS	LAA	NTEAFFGQGTRLTVV	1.1
R2F9	18	CAW	RVAGP	QETQYFGPGTRLLVL	2.5
R2F10	18	CAW	LGGS	NEQFFGPGTRLTVL	2.1
Rp171-2	18	CAW	RQLR	EQFFGPGTRLTVL	2.1
		V region		J region	

FIG. 2. VDJ junctional region amino acid sequence of the TCR β chains from T-cell clones specific for *M. leprae*. The amino acid sequence is shown in the one letter code. Breaks in the amino acid sequence indicate the borders between the known Vβ or Jβ sequence and D and/or N region sequence.

A

```
1                                                                   45
CAGCAAGTGACACTGAGATGCTCTTCTCAGTCTGGGCACAACACT
46                                                                  90
GTGTCCTGGTACCAACAGGCCCTGGGTCAGGGGCCCCAGTTTATC
91                                                                 135
TTTCAGTATTATAGGGAGGAAGAGAATGGCAGAGGAAACTTCCCT
136                                                                180
CCTAGATTCTCAGGTCTCCAGTTCCCTAATTATAGCTCTGAGCTG
181                                                                225
AATGTGAACGCCTTGGAGCTGGACGACTCGGCCCTGTATCTCTGT
226
GCCAGCAGC
```

B

```
                1                 10                  20                  30
Vß5.1   QQVTLSCSPISGHRSVSWYQQTPGQGLQFIFEYFSETQR
Vß5.2   QQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEER
Vß5.3                                          EEES
Vß5.4   QQVTLSCSPISGHRSVSWYQQTPGQGLQFLFEYFSETQR
Vß5.5   QQVTLRCSSQSGHDNVSWYQQALGQGPQFIFQYYREEEN

                40                  50                  60                  70                  78
Vß5.1   NKGNFPGRFSGRQFSNSASEMNVSTLELGDSALYLCASS
Vß5.2   QRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASS
Vß5.3     FPDRFSARQFPNYSSELNVNALLLGDSALYLCASS
Vß5.4   NKGNFPGRFSARQFPNYSSELNVNALLLGDSALYLCASS
Vß5.5   GRGNFPDRFSGLQFPNYSSELNVNALELDDSALYLCASS
```

FIG. 3. (*A*) Nucleotide sequence of the R1F3 and R2B6-5 TCR Vβ chain. This gene has 90% identity with Vβ5.2 gene sequence. The Vβ region sequenced of both T-cell clones was identical. (*B*) Comparison of the amino acid sequences of the Vβ5.1–Vβ5.4 (14, 17, 19) and the Vβ5.5 from the T-cell clones R1F3 and R2B6-5. Amino acids are in one letter code. The Vβ gene segments have been aligned to maximize the homology. Conserved amino acids have been boxed.

(24, 25) (data not shown). These results suggest that these murine mAbs recognize T-cell clones expressing TCRs containing Vβ5.2 and -5.3 but not T-cell clones expressing TCRs containing the Vβ5.1 and -5.5 gene segments.

DISCUSSION

In this study we have analyzed the TCR β chain that is expressed on T-cell clones reactive to the immunodominant *M. leprae* antigen 65hsp or other *M. leprae* antigens. This panel of clones derived from a tuberculoid patient exhibited a limited restriction pattern (i.e., either DR2 or -3). Ten T-cell clones were DR3-restricted, 5 of which reacted with the 65hsp-(2–12) peptide (refs. 21 and 22 and Table 1). Seven of these DR3-restricted T-cell clones had the Vβ5 gene rearranged, of which 3 used the Vβ5.1 gene segment and 1 was a Vβ5 gene segment not previously described, which we tentatively designate as Vβ5.5. Vβ5.5 showed 90% identity at the nucleotide level and 84% identity at the amino acid level with the Vβ5.2 gene (Fig. 3). The Vβ5.5 gene was also used by a DR2-restricted T-cell clone, 2B6-5 (Fig. 2). In the DR2-restricted group, 5 of 10 T-cell clones had the Vβ18 gene rearranged whereas two other DR2-restricted clones had the Vβ5 gene rearranged to a particular Cβ. Three of the DR2-restricted T-cell clones responded to the 65hsp-(418–427) peptide. Although the sample size is limited, these data suggest that in this patient a Vβ5 family member preferentially interacts with HLA-DR3 whereas the DR2-restricted *M. leprae*-reactive T cells preferentially used the Vβ18 gene segment.

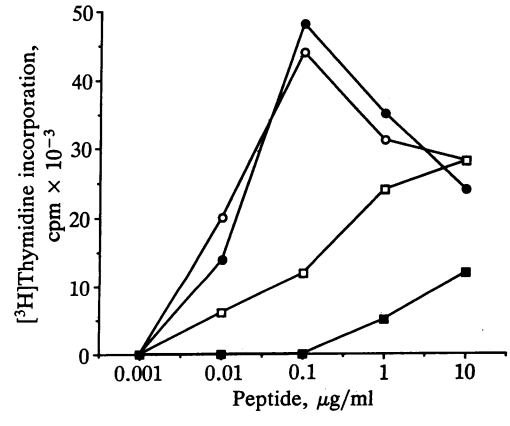

FIG. 4. Proliferation of T-cell clones R2F10 and Rp171-2 in response to the peptides 65hsp-(418–427) and 65hsp-(416–432). Proliferation was measured by incorporation of [3H]thymidine. Data represent the cpm from triplicate determinations (mean). The SEM never exceeded 10% of the mean. □, R2F10 + 65hsp-(418–427); ●, R2F10 + 65hsp-(416–432); ○, Rp171-2 + 65hsp-(418–427); ■, Rp171-2 + 65hsp-(416–432).

Models for the structure of the TCR predict that the site interacting with an antigenic peptide is in CDR3 of the TCR, located in the junctional region. Five DR3-restricted T-cell clones recognized a peptide from 65hsp-(2–12). From three of these five clones, the deduced amino acid sequence was determined. These clones all use the Vβ5.1 gene segment but different Jβ gene segments. However, the N-D-N segment of the junctional region of two of these clones, 3E10 and 1F9, is very similar. Both clones have a FGQ motif in this region. Similar observations have been made for murine T lymphocytes specific for cytochrome, where only restricted usage of TCR β chain V and J elements was observed (26, 27). In addition in cytochrome-specific TCRs, a conserved amino acid in the VDJ junctional region was found. The amino acid at position 100, 5 residues to the carboxyl side of the cysteine of position 95 in the TCR β chain, was found to be critical for peptide recognition (27). Interestingly, all three 65hsp-(2–12)-specific T-cell clones have a glycine at this position. In contrast, the VDJ junctional regions of two 65hsp-(418–427) peptide-specific T-cell clones are very different. The DR2-

restricted 65hsp-(418–427)-specific T-cell clone Rp171-2 has two charged amino acids in the junctional region, whereas R2F10 has only noncharged amino acids. Most likely these two T-cell clones have different fine specificities for the peptide, in conjunction with different affinities for the peptide–HLA-DR2 complex. This notion is supported by the observation that in proliferation assays T-cell clone R2F10 is stimulated to half-maximal proliferation at 0.01 μM and Rp171-2 is stimulated at 1 μM peptide. Furthermore, both clones respond equally well to 65hsp-(416–432), a longer peptide, suggesting that R2F10 has a higher affinity for the HLA-DR2–65hsp-(417–427) complex than Rp171-2.

Another observation made was that none of these T-cell clones was recognized by murine mAbs, that are reactive with the HPB-ALL β chain (24), which expresses the Vβ5.3 gene segment. In addition, a DR1-restricted T-cell clone expressing the Vβ5.2 gene segment did react with these mAbs (data not shown). It is thought (28) that these mAbs, in particular 3D6, recognize an epitope expressed on all Vβ5 family members. Our data suggest that this is not the case. As indicated in Fig. 3, the Vβ5.1, Vβ5.2, Vβ5.3, and Vβ5.5 gene segments differ at a number of positions. We suggest that binding of these mAbs is dependent on the leucine at position 67 in the Vβ5.2, -5.3, and -5.4 genes, because this residue is conserved between Vβ5.2 and -5.3 and is different from the Vβ5.1 and -5.5 gene segments. Both Vβ5.1 and -5.5 gene segments have a glutamic acid at this position and as a consequence are not recognized by 3D6. The change of leucine, a nonpolar residue, to the acidic glutamic acid could easily account for the loss of reactivity of the mAbs (3D6, 65, and 1C1). A similar observation has been made in the lysozyme model in which a glutamine to histidine change resulted in the loss of reactivity of murine mAb, D1.3 (29).

In conclusion, *M. leprae*-reactive T-cell clones derived from leprosy patient R preferentially use Vβ5 and Vβ18 genes in conjunction with HLA-DR3 and HLA-DR2 restriction, respectively. Preliminary data show that also at the polyclonal level the 65hsp-(2–12) peptide selects for Vβ5-bearing T cells in patient R (J.B.A.G.H., unpublished results). This is suggestive of a limited usage of Vβ gene segments that could account for a restricted immune response in *M. leprae* and, in particular, to *M. leprae* 65hsp. Therefore, it is tempting to speculate that regulation of the disease through anti-TCR intervention is feasible in leprosy. Furthermore, we have defined a Vβ5 family member and have shown that murine mAbs made against the Vβ5.3 gene segment product do not crossreact with TCRs expressing the Vβ5.1 and the Vβ5.5 gene segment.

M. leprae was kindly provided by Dr. Rees (CRC, London) and the peptides were provided by Dr. D. C. Anderson (NeoRx Corp., Seattle). We thank Tuna Mutis for providing T-cell clone 1C8, Jeff Powell and Julian Hickling for critical reading of the manuscript, and Prof. Dr. J. J. van Rood for his generous support. This research was supported by grants from the Immunology of Leprosy component of the United Nations Development Programme/World Bank/World Health Organization Special Programme for the Research and Training in Tropical Diseases, from the Dutch Foundation for Medical and Health Research, from the Netherlands Leprosy Relief Association, and the J. A. Cohen Institute for Radiopathology and Radiation protection. P.v.d.E. and N.v.d.S. were supported by Grant H92-90 from the Netherlands Organization for Scientific Research.

1. Dembic, Z., Haas, W., Weiss, S., McCubrey, J., Kiefer, H., von Boehmer, H. & Steinmetz, M. (1986) *Nature (London)* **320**, 232–238.
2. Cothia, C., Boswell, D. R. & Lesk, A. M. (1988) *EMBO J.* **7**, 3745–3755.
3. Claverie, J. M., Prochnicka-Chalafour, A. & Bougueleret, L. (1989) *Immunol. Today* **10**, 10–14.
4. Davis, M. M. & Bjorkmann, P. J. (1988) *Nature (London)* **334**, 395–402.
5. Iwamoto, A., Ohashi, P. S., Pircher, H., Walker, C. L., Michalopoulos, E. E., Rupp, F., Hangartner, H. & Mak, T. (1987) *J. Exp. Med.* **165**, 273–280.
6. Taylor, A. H., Haberman, A. M., Gerhard, W. & Caton, A. J. (1990) *J. Exp. Med.* **172**, 1643–1651.
7. Hochgeschwender, U., Simon, H. G., Weltzien, H. U., Bartles, F., Becker, A. & Epplen, J. T. (1987) *Nature (London)* **326**, 307–310.
8. MacDonald, H. R., Schneider, R., Lees, R. K., Howe, R. C., Acha-Orbea, H., Festenstein, H., Zinkernagle, R. M. & Hengartner, H. (1988) *Nature (London)* **332**, 40–45.
9. Danska, J. S., Livingstone, A. M., Paragas, V., Ishihara, T. & Fathman, C. G. (1990) *J. Exp. Med.* **172**, 27–33.
10. Wuchlerpfennig, K. W., Ofa, K., Endo, N., Seidman, J. G., Rosenzweig, A., Weiner, H. L. & Hafler, D. A. (1990) *Science* **248**, 1016–1019.
11. Oksenberg, J. R., Stuart, S., Begovich, A. B., Bell, R. B., Erlich, H. A., Steinmann, L. & Bernard, C. C. A. (1990) *Nature (London)* **345**, 344–346.
12. van Schooten, W. C. A., Elferink, D. G., van Embden, J., Anderson, D. C. & de Vries, R. R. P. (1989) *Eur. J. Immunol.* **19**, 2075–2079.
13. Haanen, J. B. A. G., Ottenhoff, T. H. M., Voordouw, A., Elferink, B. G., Klatser, R. P., Spits, H. & de Vries, R. R. P. (1986) *Scand. J. Immunol.* **23**, 101–108.
14. Yoshikai, Y., Antoniou, D., Clark, S. P., Yanagi, Y., Sangster, R., van den Elsen, P., Terhorst, C. & Mak, T. W. (1984) *Nature (London)* **312**, 521–524.
15. Gatti, R. A., Concannon, P. & Salser, W. (1984) *BioTechniques* **2**, 148–155.
16. Reed, K. C. & Mann, D. A. (1985) *Nucleic Acids Res.* **13**, 7207–7221.
17. Concannon, P., Pickering, L. A., Kung, P. & Hood, L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6598–6602.
18. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
19. Leiden, J. M. & Strominger, J. L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4456–4460.
20. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
21. Ottenhoff, T. H. M., Klatser, P. R., Ivanyi, J., Elferink, D. G., de Wit, M. Y. L. & de Vries, R. R. P. (1986) *Nature (London)* **319**, 66–68.
22. van Schooten, W. C. A., Ottenhoff, T. H. M., Klatser, P. R., Thole, J., de Vries, R. R. P. & Kolk, A. H. J. (1988) *Eur. J. Immunol.* **18**, 849–854.
23. Anderson, D. C., van Schooten, W. C. A., Barry, M. E., Janson, A. A. M., Buchanan, T. M. & de Vries, R. R. P. (1988) *Science* **242**, 259–261.
24. Boylston, A. W., Borst, J., Yssel, H., Blanchard, D., Spits, H. & de Vries, J. E. (1986) *J. Immunol.* **137**, 741–744.
25. Borst, J., Spits, H., Voordouw, A., de Vries, E., Boylston, A. & de Vries, J. (1987) *Hum. Immunol.* **17**, 426–442.
26. Fink, P. J. L., Matis, L. A., McElligot, D. L., Bookman, M. & Hedrick, S. M. (1986) *Nature (London)* **321**, 219–221.
27. Engel, I. & Hedrik S. M. (1988) *Cell* **54**, 473–484.
28. Lipoldova, M., Boylston, A. W., Yssel, H. & Owen, M. J. (1989) *Immunogenetics* **30**, 162–168.
29. Amit, A. G., Mariuzza, R. A., Phillips, S. E. V. & Poljak, R. J. (1986) *Science* **233**, 747–758.
30. Bloom, B. (1989) *J. Immunol.* **131**, i–x.
31. Wilkinson, D., de Vries, R. R. P., Madrigal, A., Lock, C. B., Morgenstern, J. P., Trowsdale, J. & Altmann, D. M. (1988) *J. Exp. Med.* **167**, 1442–1458.