*Proc. Natl. Acad. Sci. USA*
Vol. 88, pp. 8987–8990, October 1991
Immunology

# Extensive conservation of α and β chains of the human T-cell antigen receptor recognizing HLA-A2 and influenza A matrix peptide

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*Communicated by David Weatherall, June 10, 1991*

**ABSTRACT** **The major histocompatibility complex class I molecule HLA-A2.1 presents the influenza A virus matrix peptide 57–68 to cytotoxic T lymphocytes in all individuals with this common HLA type and is among the most thoroughly studied immune responses in humans. We have studied the T-cell receptor (TCR) heterogeneity of T cells specific for HLA-A2 and influenza A matrix peptide using the polymerase chain reaction. The usage of $V_\alpha$ and $V_\beta$ sequences seen on these T cells is remarkably conserved as are certain junctional sequences associated with α and β chains. Furthermore, two unrelated HLA-A2 individuals have a similar pattern of TCR usage, implying that this is a predominant response in HLA-A2 populations. Analysis in one individual showed that the conserved TCR $V_\alpha$ and $V_\beta$ genes are minor members of the peripheral blood TCR repertoire. The sequences provide important information on the TCR necessary for the final structural analysis of this ternary complex.**

The presentation of influenza A virus matrix peptide 57–68 to cytotoxic T lymphocytes (CTLs) by HLA-A2 is among the most thoroughly studied immune responses in humans (1, 2). Although HLA-A2 has been characterized at the level of its three-dimensional structure (3, 4) and its interaction with the peptide has been studied in detail by amino acid substitutions of peptide and HLA-A2 (2, 5, 6), less is known about the T-cell receptors (TCRs) that recognize this combination. The α/β TCR heterodimer recognizes peptide antigens in the context of class I and class II major histocompatibility complex (MHC) molecules (reviewed in ref. 7). TCR α and β chains are derived early in ontogeny through the rearrangement of variable ($V_\alpha$ and $V_\beta$), diversity ($D_\beta$), and joining ($J_\alpha$ and $J_\beta$) segments. These, in combination with N region diversity, provide the variation necessary to recognize the wide range of peptide–MHC combinations.

An important aspect of the recognition of antigen by human T cells is whether an *in vivo* response to a peptide–MHC combination is dominated by a single population of TCRs. In the mouse, antigen-specific T-cell clones have often shown limited heterogeneity of α or β sequences but only rarely has conservation been documented in all of the combining elements of the receptor (8–10). Most of these experiments have involved immunization using Freund's adjuvant and have utilized peptides showing substantial homology to self-antigens. In addition, most of the peptides studied have been restricted by class II MHC molecules. TCR characterization in responses to foreign antigens resulting from natural immunization has seldom been described (10, 11). The TCRs used by peptide-specific class I-restricted responses have not previously been studied in humans nor have responses been studied in unrelated individuals sharing the same class I restriction element.

To address this question we have studied the diversity of TCR V, D, and J region sequences for the α and β chains of TCRs obtained from T-cell lines and clones recognizing an influenza A matrix peptide (peptide 57–68) in the context of HLA-A2.

## METHODS

**T-Cell Lines and Clones.** Influenza A-specific T-cell lines were derived from peripheral blood lymphocytes (PBLs) from two unrelated HLA-A2 individuals (JM and FM) and were grown on irradiated autologous B lymphoblasts pulsed with influenza A matrix peptide 57–68. CTLs were generated as described (1). PBLs were cultured in the presence of trace amounts of influenza A/X31 virus for 7 days and then on autologous irradiated (3000 rads; 1 rad = 0.01 Gy) B lymphoblastoid cells (BCLs) that had been incubated in influenza matrix peptide 57–68 at 50 μM in the presence of interleukin 2 (Cetus) at 10 units/ml. Lines were restimulated as above at weekly intervals. The JM CTL line was subcultured after limiting dilution at 100, 10, and 1 cell per well on $10^4$ irradiated, peptide-pulsed autologous BCLs. This technique generated oligoclonal (two clones) lines, 1a8 and 1a11, and the clone 1a6, which was maintained as above. The lysis assay was as described (1). Target cells were labeled with $^{51}Cr$ (Amersham) and dispensed into microtiter wells at $5 \times 10^3$ cells per well; effector cells were added followed by addition of peptide. After 4 hr released chromium was measured and specific lysis was calculated as described (1). Uncloned peptide-specific lines were expanded for at least 3 weeks preceding the analysis of TCR usage.

**PCR Analysis of TCR Genes.** RNA was extracted from each CTL line and 5 μg of total RNA was used in first-strand cDNA synthesis using $C_\alpha$ (5′-GCTCCAGGCCACAGCACT) and $C_\beta$ (5′-GCTCTACCCCAGGCCTCGGCGC) region primers. The reaction products were precipitated three times using 2 M ammonium acetate and 2.5 vol of ethanol and then the 5′ end was tailed with GTPs using terminal deoxytransferase. Five to 10% was used as a template in a PCR reaction using a $C_\alpha$ (5′-GATAGATCTTAGAGTCTCTCAGC) or a $C_\beta$ (5′-CGCGAATTCAGATCTCTGCTTCTGATG) 3′ primer and a 5′ polycytosine primer (5′-CTATCTAGAGAGCTCGCGGC-CGCCCCCCCCCCCCC). PCR conditions were as follows: 95°C for 1 min, 55°C for 2 min, and 72°C for 2 min, repeated for 30 cycles. Both amplification primers incorporated restriction enzyme sites. The amplification products were digested with *Bgl* II and *Not* I and cloned into a modified m13mp18 bacteriophage vector. Up to twenty clones from each ampli-

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Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; CTL, cytotoxic T lymphocyte; PBL, peripheral blood lymphocyte; V, variable; D, diversity; J, joining.

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fication were then sequenced using T7 DNA polymerase. For analysis of peripheral blood TCR repertoire, blood was taken from donor JM and PBLs were separated on a density gradient. Total RNA was extracted and then amplified, cloned, and sequenced as described above. Forty TCR$\alpha$ and 40 TCR$\beta$ sequences that were in correct frame for VJC translation were obtained (aberrant rearrangements were discounted) and assigned to known $V_\alpha$ and $V_\beta$ families (12) or collected into new families ($V_\alpha$17–20 and $V_\beta$22).

## RESULTS AND DISCUSSION

The CTL lines were tested repeatedly for their ability to lyse autologous B-cell targets in the presence of specific peptide and typical results are shown in Fig. 1.

The TCR usage of these lines was determined by modified PCR. Twelve different $\alpha$-chain and nine $\beta$-chain sequences were obtained for comparison from CTLs from both individuals. The predicted amino acid sequences of the 12 TCR $\alpha$ chains are shown in Fig. 2. A predominant $V_\alpha$ chain, $V_\alpha$10.2, was used in 8 of the 12 sequences. This $V_\alpha$ sequence differs by one amino acid residue from $V_\alpha$10.1 (12, 13). The histidine at position 87 (the conserved cysteine at the 3′ end counted as position 90) is replaced by leucine because of a change in codon from CAC to CTC. One $J_\alpha$ sequence is also shared by a large fraction of the influenza-specific sequences, being present in 8 of 12 $J_\alpha$ sequences. This $J_\alpha$ is previously undescribed but has typical features of a $J_\alpha$ segment. Interestingly, the amino acid sequences of the $\alpha$ chains of 1a11a, 1a8b, and B1e are exactly the same, whereas there are two differences in the nucleotide sequences in the junctional region between B1e and the other two, implying strong selection for the protein sequence.

The TCR$\beta$ sequences shown in Fig. 3 also show remarkable similarity. All but one sequence contained $V_\beta$17, and this was exactly the same sequence as previously described (12). Less similarity was seen among the $J_\beta$ sequences, but in N and D region sequences the amino acid motif Ile-Arg-Ser-Ser/Thr was present in four sequences and one complete V-D-J protein sequence was identical in CTLs from both individuals (1a8 and B1b), although there were significant differences at the nucleotide level. Considering the variation in $\beta$-chain N and D region sequences, this result implies strong selection for junctional region and V region sequences by the peptide and HLA-A2.

T-cell sequences were also obtained from CTL lines specific for a different peptide, influenza B nucleoprotein 82–94, again restricted by HLA-A2. None of the sequences seen in the influenza A response was present despite the shared class I restriction element (data not shown).

The strong selection for these $\alpha$ and $\beta$ sequences was confirmed by defining the T-cell receptor repertoire in the peripheral blood of one of the two individuals studied (Fig. 3). These data indicate that $V_\alpha$10.2 and $V_\beta$17 are infrequent in the peripheral blood and these must therefore have been selected strongly to be so prevalent in the influenza-specific clones. Predominant TCR sequences seeing peptide and MHC in the mouse are often derived from the most common V region families in that mouse strain. For example, the $V_\beta$8.2 response to myelin basic protein (1–11) in PLJ mice comes from a $V_\beta$ family that accounts for up to 20% of the total $V_\beta$ repertoire (14, 15). This represents a much less significant selection than that seen in our T cells, where $V_\alpha$10.2 and $V_\beta$17 account for <5% of the peripheral T cells.

One of the lines tested, 1a6, was found to be a single clone; the others were oligoclonal. Different clonal composition was suspected because of differences in fine specificity when tested on peptide analogues or HLA-A2 mutants (unpublished results). The lines were grown on peptide for several weeks before analysis, there was no background activity against uninfected cells, and lysis was high at low

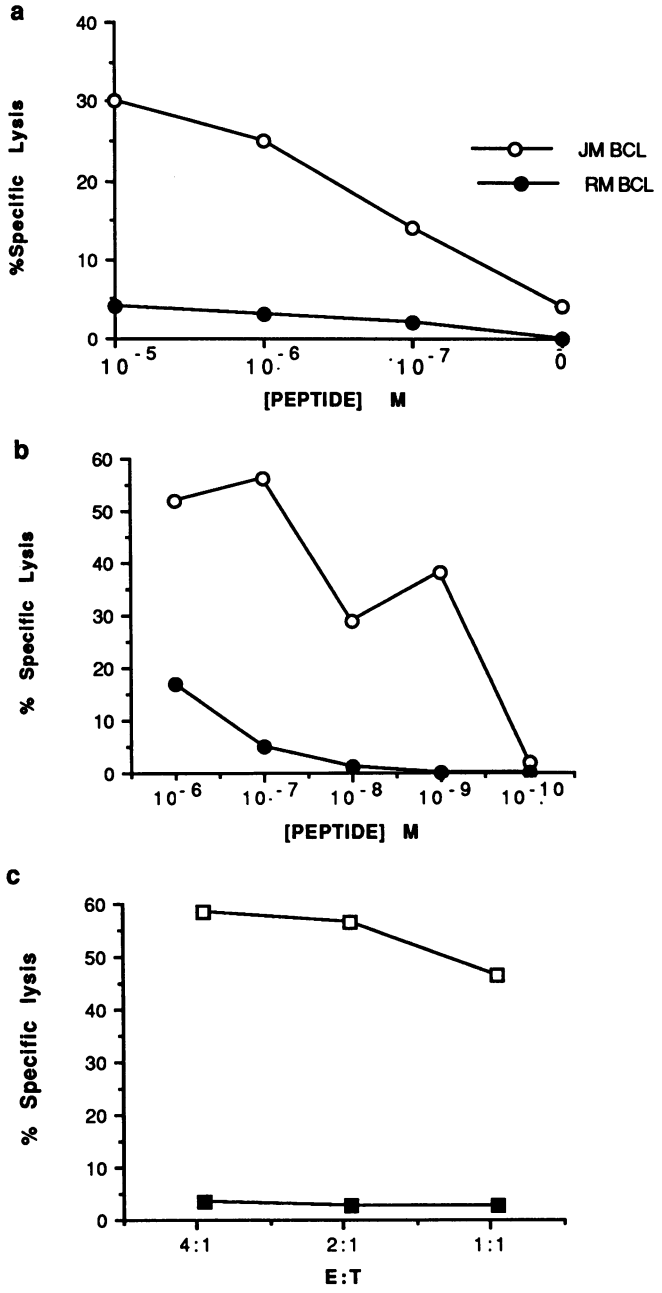


FIG. 1. (*a*) Lysis of HLA-A2-matched target cells (JM BCL) and HLA-mismatched target cells (RM BCL) by the JM CTL line in the presence of influenza A matrix peptide 57–68 at the molar concentrations shown. The effector:target cell ratio was 2:1. (*b*) Lysis of autologous target cells by the CTL clone 1a6 in the presence of influenza matrix peptides 57–68 (○) and 60–68 (●) at the peptide concentrations shown. The effector:target cell ratio was 2:1. Lysis was HLA-A2 restricted (data not shown). (*c*) Lysis of autologous target cells by the FM CTL line in the presence (□) and absence (■) of matrix peptide 57–68, $10^{-7}$ M, at different effector:target (E:T) ratios.

effector:target ratios. Thus, it is highly likely that the TCRs analyzed have the consensus specificity for the A2–matrix peptide combination, although, with the exception of 1A6 and 1A8, $\alpha$ and $\beta$ sequences cannot be paired. The limited sequence variation within these oligoclonal lines from two individuals supports the specificity seen in cellular assays.

The limited heterogeneity of $\alpha$ and $\beta$ chain sequences confirms and extends observations made in the mouse that TCRs recognizing particular peptide–MHC combinations may share V region or J region sequences. A few examples

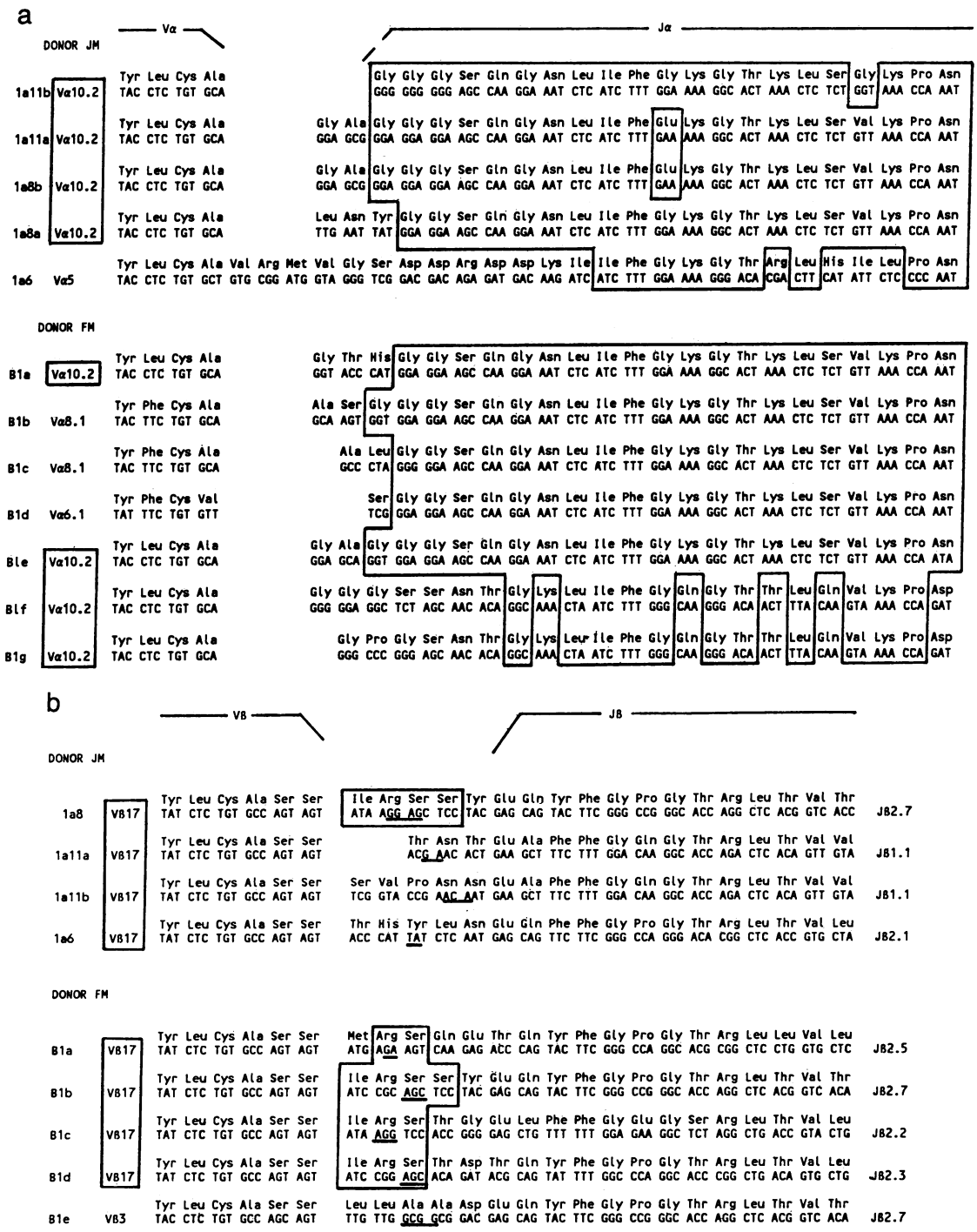


FIG. 2. (*a*) Nucleotide and predicted amino acid sequences of the $VJ_\alpha$ TCR from CTL lines. CTL lines 1a8, 1a6, and 1a11 were from donor JM and line B1 was from donor FM. When more than one sequence was found in a line the individual sequences have been designated with a letter—e.g., 2a, 2b, etc. The 5′ end of the germ-line $J_\alpha$ segments is not known and so the V-J region is split arbitrarily to display possible 5′ N region addition. The boxes indicate areas of amino acid homology. (*b*) Nucleotide and predicted amino acid sequences of the $VDJ_\beta$ TCR from the CTL lines. Regions of amino acid homology in the junctional region are boxed. As $J_\beta$ sequences are highly conserved in the germ line, homology in the $J_\beta$ sequences has not been boxed. Nucleotides that represent possible contributions from the $D_\beta$ gene segment are underlined.

exist where conserved murine $V_\alpha$, $V_\beta$, junctional, and J regions have been seen in antigen-specific T-cell clones (16–19). These examples represent a distinct minority and include the B10A response to cytochrome *c*, the PLJ response to myelin basic protein, and the C57BL response to lymphocytic choriomeningitis virus (LCMV) glycoprotein presented by H-2Dd. Only the LCMV experiment utilized natural immunization with a virus not having homology with self and hence is most comparable with our experiment. In the majority of murine experiments TCR similarity was limited to either $\alpha$ or $\beta$ chains and invariably was demonstrated in clones obtained from single inbred mouse strains. The data presented here provide evidence that the degree of similarity and the extent of selection in these TCR sequences, occurring after a natural infection and in unrelated individuals, are considerably greater than expected. Recent evidence indicates that epitope selection by MHC molecules is much more restricted for peptides presented by class I MHC than for class II (20, 21). Our data show that the TCR usage can also be highly restricted.

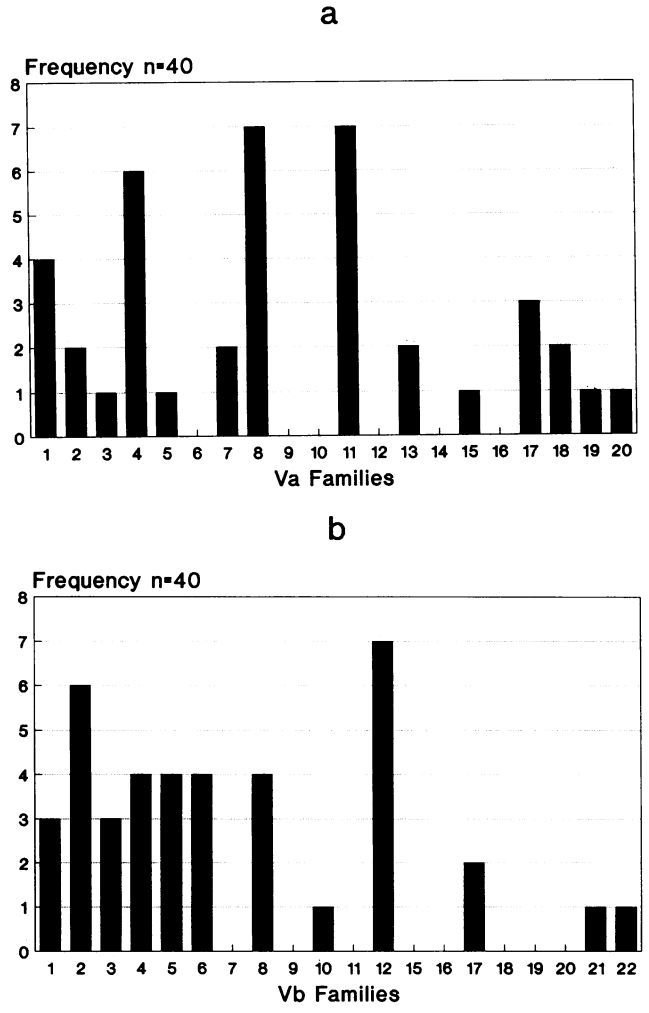


FIG. 3. $V_\alpha$ (*a*) and $V_\beta$ (*b*) TCR repertoire of peripheral blood from donor JM.

The particular combination of influenza matrix peptide with HLA-A2 has been studied in great detail. Three-dimensional data are available on the HLA-A2 structure (3, 4), and HLA-A2 heavy chain has been reconstituted *in vitro* with $\beta_2$-microglobulin by the addition of matrix peptide (22). Important residues of the matrix peptide and HLA-A2 involved in binding and T-cell recognition have also been defined (2, 5, 6). The characterization of the TCRs interacting with this complex and the knowledge that they are highly conserved will considerably assist further characterization of this ternary complex. The data here suggest that there is a single optimum conformation of TCR with peptide:MHC and that sequences in the $V_\alpha$, $V_\beta$, and $J_\alpha$ and the junctional regions of the $\alpha$ and $\beta$ chains are involved in this interaction. It is interesting that there is conservation of $J_\alpha$ segments but not noticeably $J_\beta$. One of the differences between the $\alpha$-chain and $\beta$-chain gene families is that there are many different $J_\alpha$ gene segments, whereas there are only 13 different $J_\beta$ gene segments. The rather limited number of $J_\beta$ gene segments suggests that by itself the $J_\beta$ segment could not contribute greatly to generating diversity for peptide binding. The observation that an influenza B nucleoprotein peptide, peptide 82–94, in the context of HLA-A2 is associated with a completely different set of TCR sequences implies that the peptide and MHC molecule are involved in selecting all components of the TCR. If alloreactive responses involve many self-peptides in a single MHC molecule, then it is likely that the TCR sequences from alloreactive cells will be heterogeneous (23).

A model of the interaction between the MHC and TCR has been proposed in which the TCR V regions interact primarily with the $\alpha$-helices of the MHC and the junctional regions contact peptide (7). The data presented here are consistent with this model, although the conservation of sequences at the membrane proximal end of the $J_\alpha$ region suggests that this region may be more important in antigen–MHC recognition than previously predicted.

We have found extensive conservation of junctional and V region TCR sequences in a human class I-restricted response to a common antigen. Whether or not other human T-cell responses will show such limited heterogeneity, even between unrelated individuals, remains to be established. If so, understanding and perhaps interfering therapeutically with pathological immune responses at this level become a possibility.

**Note Added in Proof.** HLA typing of the two individuals studied (JM and FM) showed them to be very similar. JM: HLA-A2.1, B15,51, C3, and DR4. FM: HLA-A2.1,2.2, B15,49, C3, and DR4. It is possible that thymic selection may have played a role in the selection of the TCR usage recognizing matrix peptide. Analysis of CTL lines from different individuals should help to resolve this question.

We thank Cetus for the gift of interleukin 2. This work was supported by the Wellcome Trust and the Medical Research Council.

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