T Cell Receptor Repertoire for a Viral Epitope in Humans Is Diversified by Tolerance to a Background Major Histocompatibility Complex Antigen

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Summary

Two unusual characteristics of the memory response to the immunodominant Epstein-Barr virus (EBV) epitope FLRGRAYGL, which associates with HLA B8, have provided an unique opportunity to investigate self tolerance and T cell receptor (TCR) plasticity in humans. First, the response is exceptionally restricted, dominated by cytotoxic T lymphocytes (CTL) with identical TCR protein sequences (Argaet, V. P., C. W. Schmidt, S. R. Burrows, S. L. Silins, M. G. Kurilla, D. L. Doolan, A. Suhrbier, D. J. Moss, E. Kieff, T. B. Sculley, and I. S. Misko. 1994. *J. Exp. Med.* 180:2335–2340). Second, CTL expressing this receptor are cross-reactive with the alloantigen HLA B*4402 on uninfected cells (Burrows, S. R., R. Khanna, J. M. Burrows, and D. J. Moss. 1994. *J. Exp. Med.* 179:1155–1161). No CTL using this conserved public TCR could be reactivated from the peripheral blood of EBV exposed individuals expressing both HLA B8 and B*4402, demonstrating the clonal inactivation of potentially self-reactive T cells in humans. A significant FLRGRAYGL-specific response was still apparent, however, and TCR sequence analysis of multiple CTL clones revealed an oligoclonal TCR repertoire for this determinant within these individuals, using diverse V and J gene segments and CDR3 regions. In addition, a significant public TCR component was identified in which several distinct α/β rearrangements are shared by CTL clones from a number of unrelated HLA $B8^+$, $B*4402^+$ donors. The striking dominance of public TCR in the response to this EBV epitope suggests a strong genetic bias in TCR gene recombination. Fine specificity analysis using peptide analogues showed that, of six different antigen receptors for FLRGRAYGL/HLA B8, none associate closely with the peptide's full array of potential TCR contact residues. Whereas the HLA B*4402–cross-reactive receptor binds amino acids toward the COOH terminus of the peptide, others preferentially favor an NH_2-terminal determinant, presumably evading an area that mimics a structure presented on HLA B*4402. Thus, tolerance to a background major histocompatibility antigen can effectively diversify the TCR repertoire for a foreign epitope by deflecting the response away from an immunodominant combination of TCR-binding residues.

T cells that express the $\alpha\beta$ TCR heterodimer recognize immunogenic peptides presented by self-MHC molecules. TCR diversity arises during T cell development in the thymus by rearrangement of variable (TCRAV and TCRBV), diversity (TCRBD), and joining (TCRAJ and TCRBJ) gene segments, as well as N region diversity at the junctional regions (1, 2). The hypervariable complementarity determining region 3 (CDR3)[1] spans the junctional regions and interacts directly with peptide epitopes (3). The mature T cell repertoire expresses only a small proportion of potential T cell specificities resulting from selective processes which include the inactivation of self-reactive T cell clones. This tolerance to self-antigens has been studied mainly in rodents, where clonal deletion during early ontogeny plays a major role. In addition, tolerance is established in the thymus and the periphery by a second mechanism that results in clonal anergy (4, 5). The repertoire of TCR is also powerfully biased by positive selection for self-MHC restriction which, like clonal deletion, involves recognition of self-peptide/MHC complexes in the thymus (6, 7). Similar mechanisms presumably account for the influence of HLA on TCR V segment frequencies and expression levels in peripheral blood (8).

An important effect of self-tolerance is that it can poten-

[1] *Abbreviations used in this paper:* CDR3, complementarity determining region 3; CTLp, CTL precursor; LCL, lymphoblastoid cell line; LDA, limiting dilution analysis.

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tially create holes in the repertoire of TCR for foreign antigens with self homology. This has been demonstrated in mice indirectly by the inability of certain MHC-bound peptides to induce an immune response (9, 10), and more directly where molecular mimicry was shown to be the basis for unresponsiveness to the synthetic polymer, poly (Glu^{50}-Tyr^{50}) (11). In contrast, other evidence has suggested that the murine TCR repertoire has sufficient plasticity to respond to a broad range of antigenic peptides capable of binding to the MHC (12). TCR plasticity has also been investigated using mouse strains with a deficiency of T cells bearing particular TCRBV genes. This may result from either deletion mutations of TCR genes or from tolerance to endogenous superantigens, a group of molecules that bind specific β chains of the TCR essentially independent of either the α chain or the peptide occupying the pocket (13). T cell responses to peptides that normally show restricted TCRBV usage are either crippled (14, 15) or modified (16–18) in mice lacking the relevant β chain. Similar studies have not been possible in humans primarily because endogenous superantigens and TCRBV-specific T cell deletions have not yet been documented in this species. In any case, these results may not reflect the more specific constraints imposed on a TCR repertoire by tolerance to a conventional self-antigen that interacts with both TCR α and β chains.

The immunodominant EBV epitope FLRGRAYGL (19), in association with HLA B8, induces an exceptionally restricted memory response that is dominated by CTL with an invariant public TCR (20, 21) that is cross-reactive with the alloantigen HLA B*4402 (22). Herein, we describe the clonal inactivation of these potentially self-reactive T cells in EBV-$sero^+$ people who are both HLA $B8^+$ and $B*4402^+$. The human TCR repertoire is sufficiently flexible to compensate for this loss, however, and these individuals respond to the peptide with an oligoclonal repertoire that includes a significant public component (i.e., homologous TCR shared by more than one individual). Recognition by these clones of single amino acid–substituted analogues of peptide FLRGRAYGL revealed that tolerance to HLA B*4402 obstructs the normally immunodominant response to a distinct determinant within the nonamer peptide which, in its MHC-associated form, mimics a structure on HLA B*4402.

Materials and Methods

Establishment and Maintenance of Cell Lines. Short-term CTL microcultures were generated by limiting dilution as follows: PBMC were distributed in round-bottomed microtiter plates in growth medium (10% FCS/RPMI 1640) at cell numbers per well below the anti-FLRGRAYGL CTLp frequency estimates for each donor (see Fig. 3), e.g., 10^3 cells per well for HLA $B*4402^-$ individuals and 3×10^3 cells per well for HLA $B*4402^+$ individuals. Approximately 5×10^4 γ-irradiated (2,000 rads) autologous PBMC, which had been preincubated with peptide FLRGRAYGL (1 μM for 1 h), were added to each well to give a total volume of 100 μl. Cultures were fed on days 4 and 7 with 50 μl of medium supplemented with 20 U of rIL-2 (23, 24) and 25% (vol/vol) supernatant from MLA-144 cultures (TIB-201; American Type Culture Collection, Rockville, MD). On day 10, each CTL microculture was split into three replicates which were used as effectors in a standard 5-h ^{51}Cr release assay.

CTL clones were generated by agar cloning as follows: 2×10^6 PBMC were stimulated in 2 ml of growth medium with autologous cells presenting the epitope FLRGRAYGL. These stimulators were either PBMC precoated with the peptide FLRGRAYGL (1 μM for 1 h) or the γ-irradiated (8,000 rads) lymphoblastoid cell line (LCL) (responder/stimulator ratio = 5:1 or 50:1, respectively). After 3 d, cells were dispersed and seeded in 0.35% agarose (Seaplaque; FMC BioProducts, Rockland, ME) containing 55% RPMI 1640, 20% FCS, 25% supernatant from MLA-144 cultures, and 30 U/ml rIL-2. Colonies were harvested after an additional 3–4 d and were amplified in culture with biweekly restimulation with rIL-2, MLA-144 supernatant, and the γ-irradiated (8,000 rads) autologous LCL. CTL clone LC13 has been described previously (20, 25).

PHA blasts were generated by stimulating PBMC with PHA (CSL, Melbourne, Australia), and after 3 d, growth medium containing MLA-144 supernatant and rIL-2 was added. PHA blasts were propagated with biweekly replacement of rIL-2 and MLA-144 supernatant (PHA-free) for up to 8 wk. LCL were established by exogenous transformation of peripheral B cells (26) with EBV derived from the IARC-BL74 cell line, which encodes the epitope FLRGRAYGL (27). All cell lines were regularly screened for mycoplasma contamination. Blood donors used in this study were healthy laboratory staff members selected for particular HLA alleles.

Cytotoxicity Assay. CTL clones were tested in duplicate for cytotoxicity in the standard 5-h chromium release assay (E/T ratio of 1:1). Briefly, CTL were assayed against ^{51}Cr-labeled PHA-blast targets that were pretreated with the peptide FLRGRAYGL (1 μM for 1 h) and were either washed or left untreated. In a variation of this method, used when screening the large number of FLRGRAYGL analogues, peptides were added directly to ^{51}Cr-labeled targets and remained present throughout the assay. A beta scintillation counter (Topcount Microplate; Packard Instrument Co., Meriden, CT) was used to measure ^{51}Cr levels in assay supernatant samples. The mean spontaneous lysis for target cells in culture medium was <20%, and the variation about the mean specific lysis was <5%. Peptides were synthesized by Chiron Mimotopes (Chiron Corp., Emeryville, CA) using pin technology (28). Recognition by the CTL clone LC13 of monosubstituted analogues of the peptide FLRGRAYGL, which had a β-alanine-diketopiperazine group at the COOH-termini, has been reported previously (19). Since these peptides were found to be much less active than corresponding peptides synthesized with free acid COOH termini (data not shown), all peptides used in this study were made with unblocked COOH and NH_2 termini. Toxicity testing of all peptides was performed before screening by adding each peptide to PHA blasts in the absence of CTL effectors.

Amplification and Sequencing of Rearranged TCRA and TCRB. Fresh or frozen samples of CTL clones were obtained for analysis. Cells were washed three times in PBS before freezing and/or processing. Poly A^+ RNA was extracted from $1–5 \times 10^6$ CTL using an mRNA purification kit (QuickPrep Micro; Pharmacia P-L Biochemicals Inc., Milwaukee, WI). Antisense TCRAC and TCRBC primers were used to generate first-strand cDNA from 500 ng poly A^+ RNA, and an anchor oligonucleotide, amino-blocked at the 3′ end, was ligated to the cDNA synthesis products. Nested TCRAC and TCRBC primers, as well as a primer complementary to the anchor, were then used to amplify specific TCRA- and TCRB-rearranged sequences (20).

Nucleotide sequence analysis of recovered DNA fragments was performed using a PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit and a 373A DNA sequencer (Applied Biosystems, Inc., Foster City, CA) according to the protocols described by the manufacturer. Each sequence presented in this study was obtained from two separate PCRs. In the event that nucleotide sequence analysis of a PCR product revealed more than one specific sequence, the product was subsequently ligated into the pGEM-T Vector System (Promega Corp., Madison, WI). The nucleotide sequence of at least six clones was determined for each ligation.

For some T cell clones, Vβ family–specific PCR was performed according to the method described by Panzara et al. (29) using 0.5 μl anchor-ligated cDNA (diluted 1/100) and 10 pmol of each a 5′ sense primer, specific for each of the 20 known Vβ families, and a 3′ antisense primer specific for the Cβ region. Amplifications were performed in 25-μl reaction volumes consisting of 200 μM dNTPs, 20 mM $MgCl_2$, and 1.25 U of Taq polymerase (Ampli-Taq) using a GeneAmp PCR 9600 system (all from Perkin-Elmer Cetus Corp., Norwalk, CT). The PCR conditions consisted of denaturation at 95°C for 15 s, annealing at 55°C for 40 s, and extension at 72°C for 40 s for 35 cycles, followed by a 5-min final extension at 72°C. β-actin cDNA was also amplified as a control for cDNA integrity (30). PCR products were excised from 2.5% ($^1/_2$ × Tris-buffered EDTA) NuSieve GTG agarose gels (FMC BioProducts) and purified using a QIAEX gel extraction kit (Qiagen Inc., Chatsworth, CA).

Semiquantitative PCR Analysis of FLRGRAYGL-specific TCR-β Gene Expression in Bulk T Cell Cultures. PBMC from the EBV-sero$^+$ donors RL, TF, CF, LC, IM, and EBV-sero$^-$ donor PGP (HLA A1,A24,B8,B14,DR1,3) were stimulated with autologous PBMC precoated with peptide FLRGRAYGL (1 μM for 1 h) (responder/stimulator ratio = 5:1). After 7 d, total RNA was extracted from 5 × 10^6 cells using a total RNA isolation kit (RNagents; Promega), and first-strand cDNA was synthesized using 5 μg of total RNA and 10 pmol of an antisense TCRBC gene–specific primer (C_{b1}; 5′-TATCTGGAGTCATTGAGGGCGGGCA-3′) according to the above mentioned procedure. 200 ng of first-strand cDNA was used as a template for PCR amplification using a ^{32}P-labeled TCRBV6 family–specific primer (5′-GGCCTG-AGGGATCCGTCTC-3′) and a degenerate primer complementary to the CDR3 region of the conserved β chain (β_{CDR3}; 5′-GTACTGCTC(G,A)TA(A,G,C,T)GC-3′). β-actin cDNA was amplified in parallel (30) for use as a standard in the calculation of the relative abundance of the product obtained with the TCR-BV6-β_{CDR3} primer set. The amplification schedule was 94°C for 20 s, 60°C for 20 s, and 72°C for 20 s. Amplifications were performed in 25-μl reaction volumes, and samples were removed after 35 cycles. The amplified products were resolved on a 6% acrylamide gel and exposed to a storage phosphor screen (Molecular Dynamics, Inc., Sunnyvale, CA). Relative quantification of the amplicons was performed using a phosphorimager and ImageQuant software version 3.3 (Molecular Dynamics). Sizes of bands were estimated by comparison of their mobilities relative to the migration of a ^{32}P-labeled 1-kb DNA ladder (GIBCO BRL, Gaithersburg, MD).

Limiting Dilution Analysis (LDA). PBMC were distributed in graded numbers from 10^3 to 10^4 cells per well in round-bottomed microtiter plates. Approximately 5 × 10^4 γ-irradiated (2,000 rads) autologous PBMC, which had been preincubated with FLRGRAYGL (1 μM for 1 h), were added to each well to give a total volume of 100 μl. Cultures were fed on days 4 and 7 with 50 μl of medium supplemented with 20 U rIL-2 and 25% supernatant from MLA-144 cultures. On day 10, each CTL microculture was split into two replicates which were used as effectors in a standard 5-h ^{51}Cr release assay against HLA B8$^+$ PHA blasts (SC PHA blasts: HLA A1,A31,B8,B51,DR3,DR4) precoated with peptide FLRGRAYGL (1 μM for 1 h) or left uncoated (control). 36 replicates were used at each concentration in each experiment. Wells were scored as positive when the percent specific chromium release exceeded the mean release from control wells by 3 SDs. LDA was performed by the method of maximum likelihood estimation (31). Data from all experiments were compatible with the hypothesis of single-hit kinetics ($P > 0.4$), and precursor estimates are given with 95% confidence limits.

Results

Clonal Inactivation of Potentially Self-reactive T Cells in Humans. Multiple CTL microcultures were raised by limiting dilution from six HLA B8$^+$, EBV-sero$^+$ individuals after in vitro stimulation with the EBV epitope FLRGRAYGL. Three of these donors (CF, RL, and TF) also express HLA B*4402, the antigen that mimics the viral epitope in the context of HLA B8, while the other three donors (LC, IM, and SC) do not. Each microculture was assayed separately for CTL activity against HLA B8$^+$ PHA blast target cells (SC PHA blasts: HLA A1,A31,B8,B51,DR3,DR4) with and without peptide FLRGRAYGL, as well as HLA B*4402$^+$ PHA blasts (SJ PHA blasts: HLA A2,A3,B7,B*4402,DR1,−). All CTL microcultures from donors LC, IM, and SC that recognized the EBV peptide cross-reacted with the alloantigen HLA B*4402 (Fig. 1 *A*). These results support earlier evidence that the memory response to FLRGRAYGL is often remarkably restricted (20), and suggested that individuals who are both HLA B8$^+$ and B*4402$^+$ may not respond to the epitope because of self-tolerance. As shown in Fig. 1 *B*, however, donors CF, RL, and TF did respond to the epitope, but with a repertoire of CTL that did not lyse HLA B*4402$^+$ target cells significantly. This suggests that the T cell clonotype that usually dominates the memory response for FLRGRAYGL is inactivated by mechanisms of self tolerance in HLA B*4402$^+$ individuals.

To investigate this apparent clonal inactivation more directly, PCR was used to search for the conserved, B*4402 cross-reactive TCR in bulk T cell cultures from EBV-sero$^+$, HLA B8$^+$ individuals who were either positive or negative for B*4402, after stimulation with the peptide FLRGRAYGL. A similarly generated bulk T cell population from an EBV-sero$^-$ donor was included as a negative control. All T cell cultures from EBV-exposed individuals were strongly reactive to HLA B8$^+$ PHA blasts preincubated with peptide FLRGRAYGL (data not shown). To monitor for the conserved β chain of the cross-reactive receptor in the stimulated cultures, we used semiquantitative PCR using a TCRBV6 family primer (TCRBV6) and a degenerate primer complementary to the TCRB CDR3 region of the conserved receptor for FLRGRAYGL (β_{CDR3}) (20). Comparison of the normalized PCR profiles using a phosphorimager revealed that a DNA band with the expected mobility of an amplified product encoding the con-

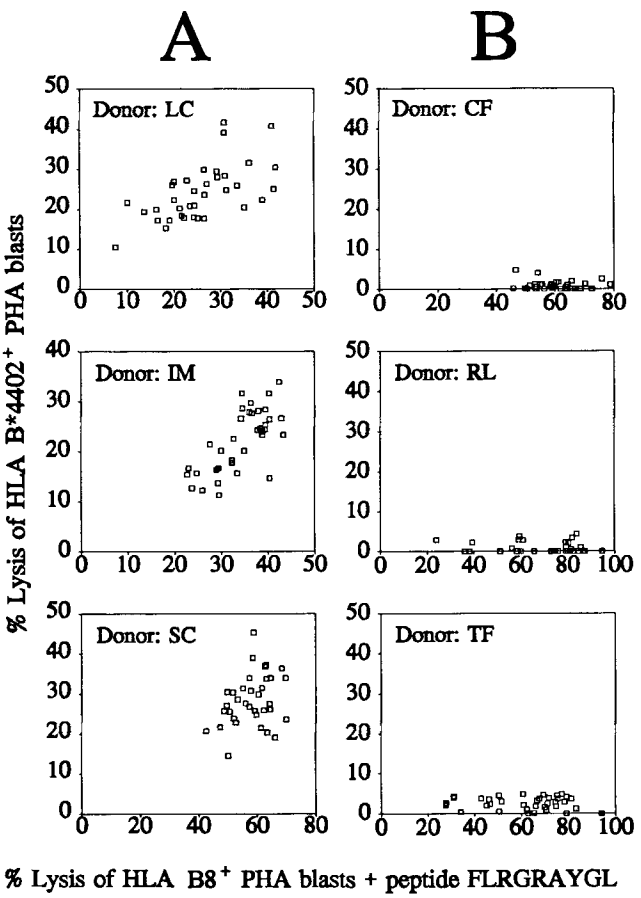

Figure 1. Cross-reactivity with alloantigen HLA B*4402 by CTL microcultures raised against FLRGRAYGL/HLA B8. Multiple CTL microcultures from three HLA $B8^+$, $B*4402^-$ (Fig. 1 *A*) and three HLA $B8^+$, $B*4402^+$ (Fig. 1 *B*) individuals were tested for lysis of SJ PHA blasts (HLA $B*4402^+$; *vertical axis*), and SC PHA blasts preincubated with peptide FLRGRAYGL (HLA $B8^+$; *horizontal axis*). SC PHA blasts were also used as targets without peptide presensitization (data not shown); these were lysed by <5% of CTL microcultures, and data from these are not included. Data from CTL microcultures that failed to significantly lyse any targets are also excluded. HLA types of the six donors are as follows: *LC*, HLA A1,−,B8,B18,DR3,DR11; *IM*, HLA A1,A11,B8,B51,DR3,DR7;*SC*, HLA A1,A31,B8,B51,DR3,DR4; *CF*, HLA A1,A2,B8,B*4402,DR3, DR4; *RL*, HLA A1,A2,B8,B*4402; and *TF*, HLA A1,A2,B8,B*4402, DR3,DR4.

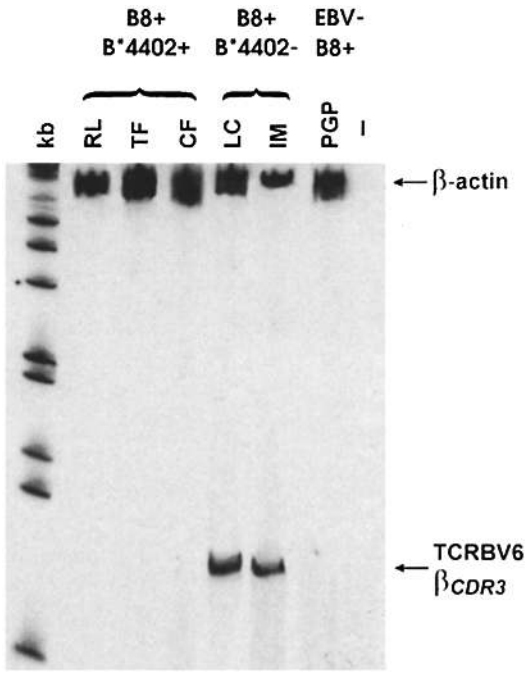

Figure 2. Detection of the conserved β chain of the TCR with dual reactivity for FLRGRAYGL/HLA B8 and B*4402. Semiquantitative RT-PCR analysis for the conserved β chain of the TCR with dual reactivity for FLRGRAYGL/HLA B8 and B*4402 in bulk FLRGRAYGL-stimulated T cell cultures from six individuals. These were donors LC, IM ($EBV\text{-}sero^+$, $HLA\ B8^+$, $B*4402^-$), CF, RL, TF ($EBV\text{-}sero^+$, HLA $B8^+$, $B*4402^+$), and PGP ($EBV\text{-}sero^-$, HLA $B8^+$, $B*4402^-$). β-actin cDNA was coamplified (30) for use as a standard in the calculation of the relative abundance of the product obtained with the TCRBV6-β_{CDR3} primer set (data not shown). The relative mobilities of these amplicons are indicated by arrows.

served TCR β chain could be detected in T cell populations from HLA $B*4402^-$, $EBV\text{-}sero^+$ donors, but not from either the $EBV\text{-}sero^-$ donor or HLA $B*4402^+$, $EBV\text{-}sero^+$ donors (Fig. 2). Direct sequencing of the PCR products showed that only one nucleotide sequence was amplified from donors LC and IM, and these were identical to those found in CTL clones raised previously from these donors (20). These data, together with the functional assays, demonstrate the clonal inactivation of potentially self-reactive T cells in humans.

To quantitate the influence of HLA B*4402 expression on the memory response to FLRGRAYGL, we compared estimates of CTL precursor (CTLp) frequencies for this peptide between the six $EBV\text{-}sero^+$ individuals examined above using LDA. As shown in Fig. 3, CTLp frequencies for three HLA $B*4402^+$ donors (1 per 6,290, 7,620, and 7,560 PBMC) were less than those estimated for three $B*4402^-$ donors (1 per 1,940, 1,880, and 2,760 PBMC). No CTLp for the peptide were detected in PBMC from three $EBV\text{-}sero^-$, HLA $B8^+$ donors (one of whom was also $B*4402^+$), included as negative controls (data not shown). Thus inactivation of the usually dominant clonotype appears to restrain, but not prevent, this T cell response to a viral epitope.

TCR Repertoire for a Viral Epitope is Diversified by Self-MHC Tolerance. We have previously shown that identical TCR protein sequences are used by clones for peptide FLRGRAYGL from each of four HLA $B*4402^-$ unrelated virus carriers (20). To investigate the TCR repertoire used for this epitope in the absence of this "prototypical" receptor, 10 $CD8^+$ CTL clones that recognize FLRGRAYGL were raised from donor CF (HLA A1,A2,B8,B*4402, DR3,DR4) using agar cloning. None were found to be cross-reactive with HLA B*4402 (data not shown). TCRA-(V-J-C) and TCRB(V-D-J-C) rearrangements expressed by these clones were identified using a modification of the single-strand ligation to single-stranded cDNA technique (SLIC) (32, 33), as previously described (20), followed by direct sequencing of SLIC-generated PCR products. Align-

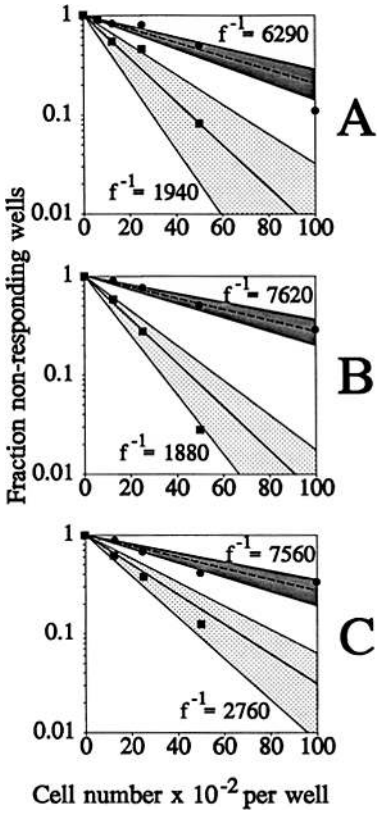

Figure 3. CTLp frequencies for EBV epitope FLRGRAYGL in HLA B*4402$^-$ versus B*4402$^+$ individuals. Using limiting dilution analysis, the frequencies of CTLp for peptide FLRGRAYGL were estimated in PBMC from six donors. These were HLA B8$^+$, B*4402$^-$ donors LC, IM, and SC (light shading in *A*, *B*, and *C*, respectively) and HLA B8$^+$, B*4402$^+$ donors CF, RL, and TF (dark shading in *A*, *B*, and *C*, respectively). Reciprocal values of responder frequencies (f^{-1}) are indicated. The shaded areas indicate 95% confidence limits.

ment of junctional TCRA and TCRB nucleotide and polypeptide sequences (Fig. 4) revealed that each clone expresses one of five distinct TCR-αβ heterodimers. Also shown in Fig. 4, for comparison, are the TCR junctional regions of the CTL clone LC13 (20, 25), isolated from a B*4402$^-$ individual, with dual reactivity to FLRGRAYGL/HLA B8 and the alloantigen HLA B*4402.

Sequence comparison of the different TCR for FLRGRAYGL/HLA B8 revealed little apparent homology within the CDR3 regions and, with the exception of clones CF6/34/40 and CF9, no conservation of TCRV or TCRJ segments. Clones CF6/34/40 and CF9, however, express very similar TCRV and TCRAJ genetic elements (TCRDV-104S2J17S3 and TCRBV21S4), and they differ only in TCRBJ usage and CDR3 sequence in both chains. Apart from a few conserved amino acids shared between the nongermline encoded regions of the CDR3 loops in both chains of these TCR, there is no apparent conservation of CDR3 amino acid composition. Interestingly, the clones from donor CF were raised from three different bleeds during a 12-month period (bleed 1, CF3; bleed 2, CF4, 6, 9, 24, 36, and 42; bleed 3, CF8, 34 and 40). The isolation of T cells using identical TCR from bleeds several months apart indicates antigen-driven clonal expansion in this memory CTL response.

To further investigate the TCR repertoire for FLRGRAYGL/HLA B8 in the absence of the prototypical receptor, CTL clones were established from another four HLA B8$^+$, B*4402$^+$, EBV-sero$^+$ unrelated individuals (RL, TF, MH, and PP). As with donor CF, these individuals use a number of different TCR-α/β combinations in their response to the immunodominant FLRGRAYGL petpide (Fig. 5). 3 out of 10 CTL clones (RL10/45 and RL16) were shown to have two in-phase α chain rearrangements. The clonality of these T cell lines was confirmed by Vβ family–specific PCR and nucleotide sequencing, which detected a single in-frame β chain (data not shown). In addition, nonproductively rearranged α and β chain transcripts were detected for clones PP31 and RL10/45, respectively (data not shown).

Comparison of the TCR sequences from all HLA B8$^+$, B*4402$^+$ donors revealed a surprising number of highly conserved TCR uses by different individuals (Figs. 4 and 5). At least one clone from each of the five donors was shown to express a TCR-β chain or -α/β heterodimer with strong homology to a TCR used by at least one other donor. The most striking examples are clones RL16, TF1, and PP7, isolated from three different people, which express identical TCR except for a single conserved amino acid substitution in the β chain CDR3 region of clone RL16. In another example, the TCR of clones CF8 and RL10/45 share identical β protein chains and express α chains encoded by the same TCRAV gene (TCRAV7S4), each of which has recombined with different members of the TCRAJ17 gene family. The CDR3 regions of these α chains are both nine residues in length, and six of these residues are identical at the same positions.

In a third example of TCR conservation, clones CF6/34/40, PP22, and PP31 all express receptors encoded by TCRBV21S4 rearranged to TCRBJ2S3. The β chain CDR3 region of the CF clones differs from that of the PP clones by a single conserved amino acid substitution. With respect to α chain rearrangements, clones CF6/34/40 and PP22 use different TCRV gene segments. The respective CDR3 regions, however, are similar because of rearrangement, without N region additions, of the different TCRV gene segments with a common TCRAJ segment (TCRAJ17S3). In contrast, the productively rearranged α chain of clone PP31 bears no homology to the α chains of either CF6/34/40 or PP22. Finally, β chain homology is also observed between clones CF9 and MH12. Both clones use TCRBV21S4 and TCRBJ2S2, and their CDR3 regions are identical in length, sharing 8 out of 12 residues at corresponding positions. The α chains of each of these clones, however, show no TCR V, J, or CDR3 homology.

Overall, these data illustrate the dramatic influence of HLA B*4402 coexpression on the CTL response to this HLA B8–restricted EBV epitope. In contrast to B*4402$^-$ individuals, who have been shown to use an essentially monoclonal repertoire, individuals that express B*4402 mount an oligoclonal response, and use diverse V and J gene segments

A

CTL Clone	TCR V Gene	FW	CDR3	FW	TCR J Gene
CF3 CF4	AV2S1	C A V tgt gcc gtg	N W S G N T P L aac tgg tca gga aac aca cct ctt	V F G gtc ttt gga	AJ9S14
CF24 CF36 CF42	AV1S1	C A V tgt gcc gtg	N A R E R D D K I aat gcg cga gag aga gat gac aag atc	I F G atc ttt gga	AJ9S4
CF8*	AV7S4	C A V tgt gct gtg	R D Q G G G N K L aga gat cag gga gga gga aac aaa ctc	T F G acc ttt ggg	AJ17S9
CF6* CF34* CF40*	DV104S2*a	C A M tgt gca atg	R E D T G N Q F aga gag gac acc ggt aac cag ttc	Y F G tat ttt ggg	AJ17S3
CF9	DV104S2*b	C A M tgt gca atg	R A P N Q F aga gca cca aac cag ttc	Y F G tat ttt ggg	AJ17S3
LC13*	AV4S1	C I L tgc atc ctg	P L A G G T S Y G K L ccc ctt gct ggt ggt act agc tat gga aag ctg	T F G aca ttt gga	AJ14S3

B

CTL Clone	TCR V Gene	FW	CDR3	FW	TCR J Gene	TCR C Gene
CF3 CF4	BV8S6	C A S tgt gct agt	G P P L R G N Y G Y ggt ccc cct ctc cgg gga aac tat ggc tac	T F G acc ttc ggt	BJ1S2	BC1
CF24 CF36 CF42	BV18S1	C A S tgt gcc agc	S P R V S G G V Y E Q tca cca cgg gtg agc gga ggg gtc tac gag cag	Y F G tac ttc ggg	BJ2S7	BC2
CF8*	BV7S5	C A S tgc gcc agc	S H G T S G I L E T Q agc cat ggg act agc ggg att ttg gag acc cag	Y F G tac ttc ggg	BJ2S5	BC2
CF6* CF34* CF40*	BV21S4	C A S tgt gcc agc	S F T W T S G G A T D T Q agc ttc acc tgg act agc gga ggg gcc aca gat acg cag	Y F G tat ttt ggc	BJ2S3	BC2
CF9*	BV21S4	C A S tgt gcc agc	S L F P T G S T A G E L agc tta ttc ccg aca ggg agt acg gcc ggg gag ctg	F F G ttt ttt gga	BJ2S2	BC2
LC13*	BV6S3a	C A S tgt gcc agc	S L G Q A Y E Q agc tta ggg cag gcc tac gag cag	Y F G tac ttc ggg	BJ2S7	BC2

Figure 4. V-(D)-J junctional region sequences of α and β chains from CTL clones that recognize FLRGRAYGL/HLA B8 from the B*4402$^+$ donor, CF, and the B*4402$^-$ donor, LC. TCRA (*A*) and TCRB (*B*) junctional region sequences of 10 CTL clones that recognize FLRGRAYGL/HLA B8 isolated from an HLA B8$^+$, B*4402$^+$ donor, CF. The nucleotide sequences are presented, and the one-letter code designating the translated amino acid is shown above the first nucleotide in each codon. CTL clones are listed on the vertical axis and those expressing identical TCR sequences are grouped together. For comparison, the TCR junctional regions of a CTL clone from HLA B*4402$^-$ donor LC (LC13), which express the prototypical receptor for FLRGRAYGL, are also shown. TCRV gene segments are classified according to family designations outlined by Clark et al. (34). TCRAJ genetic elements are assigned according to the nomenclature described by Moss et al. (35). Designations for TCRBJ and TCRBC elements follow that of Toyonaga et al. (36). For each clone, the deduced amino acid sequence of the CDR3-equivalent loop, defined according to Chothia et al. (37), is shown putatively supported by two framework branches (*FW*). The TCRV and TCRJ segments are consistent with previously published sequences, except for the TCRAV sequence of clone CF8. This new TCRAV segment (assigned AV7S4) shows strong homology with TCRAV7 family sequences both at the nucleotide and predicted amino acid level. TCRJ germline sequences are underlined and in normal print. TCRBD1 and TCRBD2 germline sequences are italicized and underlined. The asterisk indicates a public TCR chain that is the same or structurally similar to a corresponding chain from a different individual (see Fig. 5). These α/β chain sequences are available from EMBL/Genbank under accession numbers Z49957, Z49924 (CF3/4); Z49956, Z49923 (CF24/36/42); Z49903, Z49922 (CF8); Z49955, Z49921 (CF6/34/40); and Z49954, Z49920 (CF9), respectively.

and CDR3 regions. Within these oligoclonal repertoires, we have identified several additional public TCR for FLRGRAYGL/HLA B8, wherein distinct α/β rearrangements are shared by CTL clones from a number of unrelated individuals. These data strongly suggest genetic bias in TCR gene rearrangement. In some cases, the identical α or β chains of these public TCR were generated by different rearrangement events wherein codon usage varies only within N regions, also suggesting peptide-driven TCR selection.

*Tolerance to HLA B*4402 Obstructs the Normally Immunodominant Response to a Distinct Determinant within the Nonamer Peptide.* To investigate the relationship between these TCR structural differences and ligand recognition, the fine specificity of clones representing six different TCR sequences was determined using a set of 171 monosubstituted peptide analogues in which each residue within FLRGRAYGL was sequentially replaced with all other genetically coded amino acids. Peptides in which an amino acid had been deleted al-

A

CTL Clone	TCR V Gene	FW	CDR3	FW	TCR J Gene
RL10* RL45*	AV7S4	C A V tgt gct gtg	R D Q T G A N N L aga gat caa act ggg gca aac aac ctc	F F G ttc ttt ggg	AJ17S5
	AV6S1	C A S tgt gcg tcc	Q G G K L cag gga gga aag ctt	I F G atc ttc gga	AJ14S2
RL42	AV2S8	C V V tgt gtg gtt	R A G K L agg gca gga aag ctt	I F G atc ttc gga	AJ14S2
RL16*	AV27S1	C A V tgt gcc gtc	L F G N E K L ctt ttt gga aat gag aaa tta	T F G acc ttt ggg	AJ17S8
	AV1S4	C A V tgt gct gtg	S L A A G S S N T G K L agc ctc gcc gcg ggc tct agc aac aca ggc aaa cta	I F G atc ttt ggg	AJ14S1
TF9	AV1S10	C V V tgt gtt gtg	S S A G G F K T agt tcg gct gga ggc ttc aaa act	I F G atc ttt gga	AJ1S4
TF1*	AV27S1	C A V tgt gcc gtc	L F G N E K L cta ttt gga aat gag aaa tta	T F G acc ttt ggg	AJ17S8
MH12	AV3S1	C A T tgt gct acg	D N S W G K L gac aac agc tgg ggg aaa ttg	Q F G cag ttt gga	AJ1S9
PP7*	AV27S1	C A V tgt gcc gtg	L F G N E K L cta ttt gga aat gag aaa tta	T F G acc ttt ggg	AJ17S8
PP22*	AV2S7	C T M tgt acg atg	K S H T G N Q F aaa agt cac acc ggt aac cag ttc	Y F G tat ttt ggg	AJ17S3
PP31	AV4S1	C C V tgc tgt gta	L L H P E R Q D D N Y G Q N F cta ctg cat cct gag aga caa gac gat aac tat ggt cag aat ttt	V F G gtc ttt ggt	AJ13S2

B

CTL Clone	TCR V Gene	FW	CDR3	FW	TCR J Gene	TCR C Gene
RL10* RL45*	BV7S5	C A S tgc gcc agc	S H G T S G I L E T Q agc cat ggg act agc ggg ata ttg gag acc cag	Y F G tac ttc ggg	BJ2S5	BC2
RL42	BV12S4	C A S tgt gcc agc	G Q G N F D I Q ggt cag ggt aac ttc gac att cag	Y F G tac ttc ggc	BJ2S4	BC2
RL16*	BV7S1B	C A S tgc gcc agc	S Q G L S I S S Y E Q agc caa gga ttg tcg atc agc tcc tac gag cag	Y F G tac ttc ggg	BJ2S7	BC2
TF9	BV21S4	C A S tgt gcc agc	S T N S L G E R E Y Y E Q agc aca aat tct ctc ggg gag cgg gag tac tac gag cag	Y F G tac ttc ggg	BJ2S7	BC2
TF1*	BV7S1B	C A S tgc gcc agc	S Q G L A I S S Y E Q agc cag gga cta gcg ata agc tcc tac gag cag	Y F G tac ttc ggg	BJ2S7	BC2
MH12*	BV21S4	C A S tgt gcc agc	S L T T T G S N T G E L agc tta acg acg aca gga tcg aac acc ggg gag ctg	F F G ttt ttt gga	BJ2S2	BC2
PP7*	BV7S1B	C A S tgc gcc agc	S Q G L A I S S Y E Q agc caa ggt cta gcg att agc tcc tac gag cag	Y F G tac ttc ggg	BJ2S7	BC2
PP22*	BV21S4	C A S tgt gcc agc	S F S W T S G G A T D T Q agc ttt tct tgg act agc ggg ggg gcg aca gat acg cag	Y F G tat ttt ggc	BJ2S3	BC2
PP31*	BV21S4	C A S tgt gcc agc	S F S W T S G G A T D T Q agc ttt tct tgg act agc ggg ggg gcg aca gat acg cag	Y F G tat ttt ggc	BJ2S3	BC2

Figure 5. V-(D)-J junctional region sequences of α and β chains from CTL clones that recognize FLRGRAYGL/HLA B8 from the B*4402$^+$ individuals RL, TF, MH, and PP. TCRA (*A*) and TCRB (*B*) junctional region sequences of 10 CTL clones that recognize FLRGRAYGL/HLA B8, isolated from four HLA B8$^+$, B*4402$^+$ donors, are presented. Clones RL10, RL45, RL42, and RL16 are from the donor RL; clones TF9 and TF1 are from the donor TF; clone MH12 is from the donor MH; and clones PP7, PP22, and PP31 are from the donor PP. TCR V, J, and BC gene segments and CDR3 region loops are presented and assigned as outlined in the legend of Fig. 4. These α/β chain sequences are available from EMBL/Genbank under accession numbers Z49945, Z49953, Z49947 (RL10/45); Z49958, Z49864 (RL42); Z49952, Z49951, Z49930 (RL16); Z49959, Z49929 (TF9); Z49950, Z49928 (TF1); Z49949, Z49927 (MH12); Z49948, Z49926 (PP7); Z49964, Z49925 (PP22); and Z49946, Z49925 (PP31), respectively.

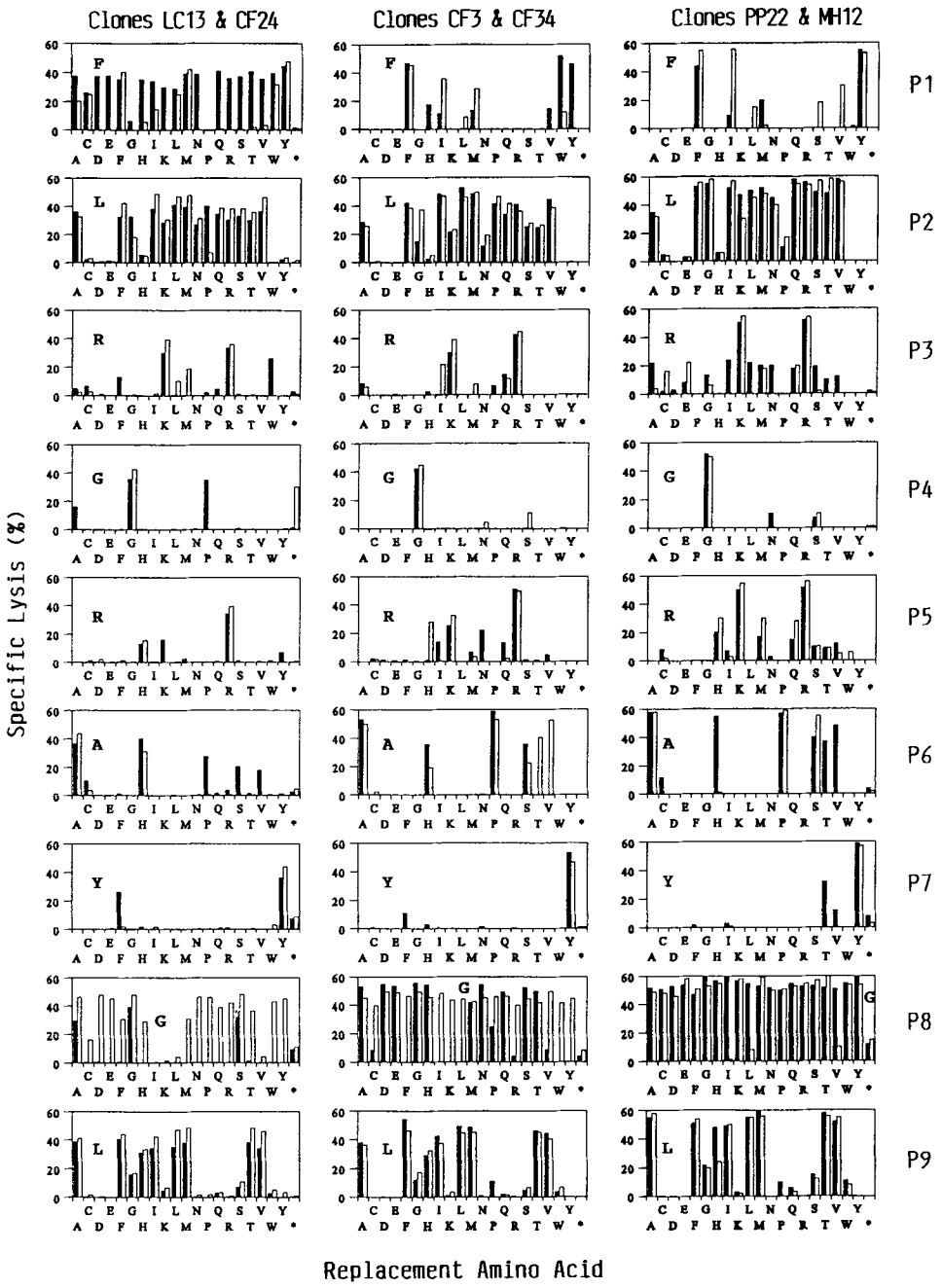

Figure 6. Recognition by CTL clones using different TCR of monosubstituted analogues of the peptide FLRGRAYGL. Fine specificity analysis of CTL clones using six different TCR (LC13, CF3, and PP22, *solid bars*; CF24, CF34, and MH12, *open bars*). Every one of the 20 genetically coded amino acids, as well as a deletion (*), were tested in each of the nine locations within the parent sequence FLRGRAYGL. The letter within each graph represents the parent residue being replaced; the horizontal axis lists the residue replacing the parent residue. P1–P9 at the right of the figure denote the position of the amino acid substitution relative to the parent peptide. Target cells were SC PHA blasts, the peptide concentration was 100 nM, and the E/T was 1:1.

together were also included (represented by asterisks). Each peptide was tested for its capacity to sensitize HLA $B8^+$ PHA blasts to lysis by the clones (Fig. 6). Only one concentration of peptide (100 nM) was used because of the number of peptides and clones tested. It should be noted that dose–response curves for peptide FLRGRAYGL vary little between clones expressing the different TCR (50% maximal activity observed at peptide levels between 7 and 16 nM), and all clones recognize endogenous antigen presented on LCLs (data not shown).

The minimal antigenic determinant for all TCR is the nonamer peptide FLRGRAYGL since truncation at the NH_2- or COOH-terminal ends results in loss of recognition. The side chain of Phe at position 1 (P1), however, does not appear to associate specifically with either HLA B8 or the TCR that cross-reacts with HLA B*4402 (used by CTL clone LC13), since all but two amino acids (Gly and Pro) can be tolerated at this position without significant detrimental effects on recognition by this TCR. This implies that occupancy of P1 is a requirement for optimal hydrogen bonding between the NH_2 terminus of the peptide main chain and HLA B8 (38). The intolerance of Pro at P1 is probably caused by the disruption of this hydrogen bond network because of the nature of its side chain, which is covalently attached to the peptide NH_2 terminus (39). The overall proportion of substitutions that leads to loss of anti-

genic activity (defined as lysis levels below 10%) was shown to vary only slightly between the different clones, ranging from 56.7% for PP22 to 67.8% for CF24.

TCR sequence variation between the clones is reflected in unique fine specificity patterns. The previously defined peptide binding motif for HLA B8 (x-x-K/R-x-K/R-x-x-(x)-L/I) proposes that amino acid at P3, P5, and P9 are important MHC anchor residues (40). In good agreement with this motif, analogues of the EBV peptide with Lys at P3 or P5 are almost as active as the parent peptide. The effect of other substitutions at these positions differs slightly between the clones, suggesting that some may induce conformational changes in the peptide which indirectly affect the interaction with some TCR (41). The high replaceability of Leu at P9 appears to conflict with the HLA B8–specific motif. Since this is based on sequence homology between defined HLA B8 restricted viral epitopes rather than synthetic peptide binding, however, our data support the view that COOH-terminal amino acid selection of endogenously processed peptides is influenced by mechanisms upstream of class I binding, such as antigen proteolysis or peptide transport (42). Certain destabilizing amino acid substitutions at positions other than the primary anchor residues may also inhibit MHC binding (43).

Recent studies using x-ray crystallography of peptide presentation by several MHC class I alleles have shown that four or five peptide side chains are directed out from the binding cleft of the MHC and are therefore accessible to TCR (39, 44–49). Since the side chains of amino acids at P3, P5, and P9 of FLRGRAYGL have major roles in anchoring the peptide to HLA B8, these are unlikely to be potential TCR contact residues. In addition, Fig. 6 illustrates that Leu at P2 does not appear to associate specifically with any of the TCR, a result that is consistent with the known conformations of peptides bound to other MHC alleles in which P2 is buried deep in the cleft. Thus, residues at P1, P4, P6, P7, and P8 of the peptide are the most likely to be accessible to TCR.

As has been observed in larger class II–associated determinants (50), a hierarchy in the importance of individual residues in contacting the TCR is evident. Whereas several are important for all T cell responses (P4, P6, and P7, where the majority of replacements impaired recognition by all clones), the fine specificity for secondary TCR contact residues is unique for each receptor. Of the six different TCR examined, none associate closely with the full set of potential TCR contact residues of the peptide. The most variation between the different TCR in fine specificity pattern is at P1 and P8, where either the side chain of Phe or the main chain of Gly (which lacks a side chain) is more important for recogntion by each clone. Whereas clones CF24, CF3, CF34, PP22, and MH12 bind Phe at P1 more stringently, LC13 interacts with amino acids toward the COOH terminus of the peptide, including Gly at P8. There are very few significant differences between CF34 and PP22 in their recognition of peptide analogues. This is not surprising since they express almost identical β chains and similar α chains. Both can tolerate any substitution at P8, but only PP22 can accommodate the polar Tyr residue at P1, suggesting that the TCR α chains of these clones lie over the NH_2 terminus of bound peptide. A similar orientation has been proposed for TCR that are specific for other peptides (3, 51).

The fine specificities of several other clones using identical TCR to LC13, CF24, CF3, and CF34 were also determined and found to be analogous with data presented in Fig. 6 (data not shown). CTL clone CF8, expressing a seventh distinct TCR, was also screened for recognition of the peptide analogues. In contrast to other clones tested from HLA B*4402$^+$ individuals, this clone displays a similar fine specificity pattern to clone LC13 with just two major exceptions (data not shown). At P8, the requirement for the native residue (Gly) is absolute for clone CF8, while clone LC13 can tolerate two conservative substitutions (Ala and Ser).

Discussion

By exploiting the unusual characteristics of the memory CTL response to an antigenic determinant of EBV, we have demonstrated, for the first time in humans, how the TCR repertoire for a foreign epitope is influenced markedly by an MHC allele that is not involved in presenting the epitope. Self tolerance to a conventional antigen, in the form of a background MHC protein, leads to the diversification of a memory response that is commonly dominated by a single public TCR.

TCR Repertoire Diversity. Many factors may contribute to variation in the size of antigen-selected TCR repertoires. These include antigen-dependent and -independent events. One proposal is that the degree of diversity in TCR usage may be critically dependent on structural constraints imposed by particular peptide/MHC combinations, and that the available receptors that can make a "close-fit" interaction is dependent on the target structure (52). Another proposal suggests that the diversity of T cell responses is often restricted by tolerance to self-proteins that are homologous, but not identical, to foreign antigens (53). In this model, antigenic peptides that are similar to self-determinants and bound to the same restriction element will stimulate a limited response. When considered in the context of data presented herein, the highly restricted TCR repertoire used for the memory response to peptide FLRGRAYGL in the absence of HLA B*4402 (20) cannot be explained simply by either of these proposals. Rather, it is likely to reflect selective T cell maturation and expansion in the periphery as a result of chronic immune stimulation. According to the balance of growth model (54), a T cell clone that best responds to a foreign peptide could, with time, dominate over other clones that have also been stimulated during the primary response. EBV is a gamma herpes virus that is thought to persist as a latent infection in B cells. Thus, marginal antigen concentrations on infected B cells after convalescence could stimulate preferentially a CTL clone with the highest proliferative capacity. Consistent with this model, we have demonstrated that, when T cells using a dominant TCR are inactivated by mechanisms of self-tolerance, a new TCR hierachy is established such that CTL expressing alternative TCR become

apparent. This hierachy is most likely a result of quantitative differences in signaling through the TCR upon binding to the peptide/MHC complex. Recent reports have suggested that TCR signaling is influenced, not only by the affinity/avidity of the TCR–ligand interaction, but also by conformational changes in the TCR (55).

The data presented herein suggest that the memory response to peptide FLRGRAYGL can be influenced, not only by self-tolerance and peripheral T cell selection, but also by bias at the TCR gene recombination level. Individuals expressing both HLA B8 and B*4402 mount an oligoclonal T cell response to the EBV peptide. Comparison of TCR sequences from five such individuals revealed an unexpected number of public or shared TCR. Indeed, >60% of clones from the unrelated donors express public receptors with highly homologous β or α/β rearrangements; a surprising observation given the enormous potential diversity of the TCR repertoire. Theoretically, if it is assumed that the processes of V, D, and J combinatorial joining, exonucleolytic nibbling, and N nucleotide insertions operate randomly, then the potential number of available α/β specificities range from 10^{15} to 10^{20} (2, 56). Despite this potential and the fact that the number of lymphocytes present at any time in the adult human is $\sim 10^{12}$, we have described the recurrence of identical TCR-α and/or -β chains in unrelated individuals. Moreover, this public TCR response predominates in the β chain of the heterodimer, which has the greater potential for diversity ($\sim 5 \times 10^{12}$), since it contains two D regions and it has the added potential of N additions at both the V-D and D-J junctions (56). Notably, several of these identical α and/or β rearrangements were generated by complex recombination events involving exonucleolytic trimming and addition of nongermline-encoded N nucleotides. Taken together, these observations provide evidence for strong genetic bias in TCR recombination. Indeed, several studies have suggested nonrandomness in the TCR recombination process (57, 58).

Recently, a public TCR Vβ rearrangement has been reported in the human T cell response to the influenza peptide, M58-66 (59). In this study, $BV17^+$ T cells were shown to dominate in 13 individuals, and identical TCR-β protein chains were identified in clones from five individuals. A public Vβ TCR has also been documented in the murine response to hen egg lysozyme, where a single Vβ rearrangement dominates the TCR repertoire (21). This dominant public Vβ rearrangement is exclusively germine encoded. By comparing these mice with transgenic mice low in hen egg lysozyme, the authors further showed a private TCR component for the same epitope that was specific to each mouse. These private TCR were unlike the dominant public TCR in that they involved nongermline-encoded N additions in their Vβ rearrangements. The distinction between these previous studies and the present report is that we have identified both α and β chain conservation for multiple TCR directed against a single antigenic determinant. The unprecedented level of TCR conservation we have documented suggests that the occurrence of T cells expressing public receptors may be frequent in memory responses to certain peptides, especially in immunological environments where antigen persists. Furthermore, our study shows that the public arm of an immune response can consist of multiple TCR that display diverse CDR3, V, and J gene usage in both the α and β chains.

The present report contests the conclusions of a previous analysis of the murine T cell response to a 12-mer peptide of sperm whale myoglobin that binds MHC class II (15). Their study demonstrated a strict association between recognition of distinct determinants within the peptide and TCRBV usage, concluding that T cell responses that appear diverse may be restricted when viewed from the perspective of individual determinants. Although we agree that TCR diversity is, in part, a reflection of the recognition of distinct determinants within a bound peptide, our TCR structure/function analysis (Figs. 4–6) also revealed that the same determinant of exposed residues within peptide FLRGRAYGL can be recognized by receptors with no apparent common structural motifs within their CDR3 regions. The diversity of CDR3, V, and J gene usage in both the α and β chains of TCR for the EBV peptide FLRGRAYGL provides an excellent example of the remarkable plasticity of the TCR repertoire.

TCR Cross-reactivity. Our data (Fig. 6), together with previous reports (51, 60), demonstrate that simple class I–associated nonamer peptides may generate multiple determinants when associated with a single MHC molecule, as has been demonstrated for longer peptides that bind class II (15, 41, 50, 61). A feature of many T cell clones used in these studies and in our investigation is that their TCR interact stringently with either amino acids toward the COOH- or the NH_2-terminus of the peptide. A core region is important for all T cell responses, whereas secondary TCR contact residues often vary between distinct receptors. Thus, a peptide's full array of potential TCR contact residues is rarely used by individual clones. It is unlikely that this curious feature is caused by the inability of α and β chain CDR3 loops to span the 2.5-nm groove length of class I MHC since modeling of TCR structure, based on immunoglobulin folds, suggests they can (37). Alternatively, T cells that fail to engage amino acids at both ends of a peptide are perhaps more likely to be both positively selected in the thymus and responsive to a nonself antigen, given recent evidence that positive selection is dependent on particular endogenous peptide/MHC complexes (6, 7).

It can be calculated, based on the number of amino acids encoded in a higher vertebrate genome ($\sim 3 \times 10^7$ to 10^8), that peptides not less than eight to nine residues in length are required for adequate self versus nonself discrimination (62). Since many T cells fail to "view" the full-length of bound peptides, it is not surprising that individual TCR bearing dual specificities are a common occurrence (63). These may have an important role in the overall functioning of the immune system. For example, the impact of a point mutation within a foreign peptide epitope will be reduced if the TCR repertoire includes receptors that do not interact

with the altered peptide position. In addition, broadly reactive T cells may aid primary (64) and memory responses (65), where memory T cells for one pathogen are reactivated by a different infectious agent.

The limited specificity of self-MHC–restricted T cells is also the basis of the alloresponse and its associated clinical problems. This is supported by numerous reports of T cell clones with dual specificity for an allo-MHC molecule and a nominal antigen complexed with self-MHC (63). The T cell clonotype expressing the prototypical TCR for peptide FLRGRAYGL is an example, and we have shown that the potent memory response to this peptide that persists after primary EBV exposure augments the alloresponse to HLA B*4402 (22). It is now clear that, in most cases, T cell recognition of alloantigens involves both the allo-MHC molecule and its associated peptide ligand (66). It seems possible, based on the fine specificity analysis (Fig. 6), that the dual reactivity of the prototypical receptor for FLRGRAYGL (see clone LC13) is related to its failure to bind specifically to the NH_2-terminal amino acid of the peptide. Perhaps a determinant toward the COOH terminus of the EBV peptide resembles a peptide presented on HLA B*4402. Supporting this concept, most of the clones we have examined from B*4402$^+$ donors interact relatively stringently with Phe at P1. An exception, however, is clone CF8, which recognizes the same residues of FLRGRAYGL as the cross-reactive clone, although slightly more specifically. The TCR of clone CF8 may avoid self-reactivity by binding MHC residues that differ between HLA B8 and B*4402. Alternatively, differences in the stringency of interaction with MHC bound peptides may influence cross-reactivity with the alloantigen. Future studies aimed at defining the relevant HLA B*4402-associated peptide may clarify the molecular basis of this cross-reactivity.

We have described the inactivation of a T cell clonotype with self-reactive potential. If, for some reason, these T cells were to slip through the mechanisms that maintain self-tolerance, an HLA- and viral-associated autoimmune disease could result. Many autoimmune conditions follow an immune response to a foreign antigen, leading to the speculation that T cells with dual reactivity for microbial and host determinants are often involved (67). The characterization of such determinants is of major importance, particularly in light of recent demonstrations that antigenic peptide analogues can act as powerful and specific inhibitors of T cell activation, and could therefore be used for antigen-specific immunointervention (68). Consistent with the molecular mimicry model are studies indicating that induced autoimmunity can be driven by T cells with quite restricted repertoires (69). Susceptibility to almost all human autoimmune disorders is also strongly influenced by genetic factors, particularly class I and II HLA alleles. In some cases, more than one MHC molecule may contribute to disease. For example, the extended haplotype HLA A1,B8,DR3 is linked with an increased risk of developing insulin-dependent diabetes mellitus, myasthenia gravis, SLE, celiac disease, and Sjögren's syndrome (67). Interestingly, Sjögren's syndrome is also associated with EBV (70), raising the possibility that CTL activated against peptide FLRGRAYGL could contribute to its pathogenesis in some cases.

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