Evidence for cooperation between TCR V region and junctional sequences in determining a dominant cytotoxic T lymphocyte response to herpes simplex virus glycoprotein B

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Abstract

TCR repertoire availability has the potential to influence the immune response to foreign antigens. Here we have analysed how changes in V region availability influence the H-2b-restricted cytotoxic T lymphocyte (CTL) response to a dominant peptide determinant derived from the herpes simplex virus glycoprotein B (gB). We have previously shown that C57BL/6 mice mount a gB-specific, Kb-restricted CTL response which is dominated by a TCRBV10$^+$ population and a TCRBV8S1$^+$ subpopulation, both containing highly conserved CDR3 elements. We find that this dominant gB-specific CTL pool is lost in C57/L mice which have a different TCRBV haplotype. A population of CTL with diverse TCRBV and junctional sequence usage, which otherwise represents a minor subset in the gB-specific response, appears to emerge as a consequence of this TCRBV gene variation. The loss of preferential V region-encoded complementarity determining regions (CDR) 1– and/or CDR2–ligand interactions in this emerging population also results in a change in CDR3 sequence usage and a corresponding focusing of an otherwise promiscuous pattern of cross-reactivity with a panel of $gB_{498–505}$ substitution analogues. This suggests that the difference between the two distinct TCR populations is the relative contributions of the CDR towards ligand recognition. Therefore, preferential V region–ligand interaction, at the expense of CDR3 peptide recognition, appears to control the dominant TCR selection in the C57BL/6 response to this peptide determinant.

Introduction

Cytotoxic T lymphocytes (CTL) recognize antigens in the form of short peptide fragments bound within the groove of MHC class I molecules (1). Recognition is mediated by a clonotypic cell surface heterodimer receptor (TCR) composed of $\alpha$ and $\beta$ polypeptide chains encoded by variable (V), diversity (D, for the $\beta$ chain only), joining (J) and constant (C) elements (2,3). This heterodimer adopts an Ig-like fold consisting of conserved framework regions and hypervariable loops that closely resemble the complementarity determining regions (CDR) of antibody molecules (4–8). The third hypervariable loops (CDR3) are found at the most variable portion of the TCR at the V–(D)–J junctions. CDR3 diversification occurs through the random joining of the V, D and J elements, and the loss or template-independent addition of nucleotides at the junctions (9). The recently published crystal structure of the $\alpha\beta$TCR shows that the CDR3 loops play a prominent role in peptide interaction and straddle the central region of the peptide (7,8). As previously proposed (9–11), the CDR1 and CDR2 loops, which are entirely germline, V region encoded, contact the relatively invariant $\alpha$ helices that flank the MHC–peptide binding cleft. However, the crystal structure has revealed that the CDR1 and CDR2 loops also contact the terminal regions of the peptide, with the CDR1 and CDR2 loops from the $\alpha$ and $\beta$ chains contacting the N- and C-terminal residues respectively of the MHC-bound peptide (7,8).

Diversity in the TCR repertoire generated through germline, junctional and combinatorial mechanisms provides an individual with the variation necessary for the recognition of a
wide range of peptide–MHC combinations. However, host genetic variation within a population can also influence the TCR repertoire and, potentially, the immune response to foreign antigens. Positive selection for self-MHC restriction has been shown to alter TCR V gene segment usage and expression levels in the periphery (12–15). Similarly, tolerance to self-antigens through thymic deletion and clonal anergy results in the loss of potentially harmful T cell specificities and also T cell specificities to foreign antigens with self homology. For example, the highly conserved, cross-reactive CTL subset specific for the HLA B8-restricted immunodominant EBNA-3 determinant of the Epstein–Barr virus (EBV) is absent in humans expressing the background MHC antigen HLA B^*4002 and the TCR repertoire is diversified in these individuals (16). Allelic polymorphism in the TCR has also been shown to lead to the loss of specific immune responses to simple antigenic peptides which are dominated by T cells bearing particular TCRBV genes (17).

Here, we have analysed what effect allelic polymorphism has on the CTL response to a complex infectious agent, herpes simplex virus type 1 (HSV-1). Previous studies have shown that up to 80% of the response to HSV-1 in C57BL/6 mice is directed to a H-2b-restricted peptide determinant derived from glycoprotein B (gB), depending on the site of lymphocyte isolation and the time after initial viral inoculation (18). Approximately 60% of the gB-specific TCR in C57BL/6 mice contain the TCRBV10 element while 20% use the TCRBV681 sequence (19), despite little bias in the TCR plates (Greiner, Frickenhausen, Germany) using 10^5 irradiated (12,000 rad) MC57gB cells and 5 × 10^3 irradiated (3000 rad) C57BL/6 spleen cells in 2 ml RPMI 1640 medium containing 10% FCS, 2 mM glutamine, 5 × 10^3 M 2-mercaptoethanol and antibiotics (complete RPMI) in wells of a 24-well tissue culture plate (Costar, Cambridge, MA). The cells were re-stimulated a further two times as before with the addition of 15 U/ml IL-2. T cell lines were cloned by limiting dilution in 96-well flat-bottom microtitre plates (Greiner, Frickenhausen, Germany) using 1 × 10^4 irradiated (12,000 rad) MC57gB cells and 5 × 10^5 irradiated (3000 rad) C57BL/6 spleenocytes in 0.2 ml of complete RPMI containing 15 U/ml IL-2.

All CTL lines and clones were shown to be specific for the major determinant of the HSV-1 gB antigen, gB_498–505, by testing their response to peptide-pulsed EL4 (K b) cells and also T cell specificities to foreign antigens with self-antigens through thymic deletion and clonal anergy (single letter amino acid code) was synthesized using an Applied Biosystems model 431A synthesizer (ABI, Foster City, CA) and kindly provided by Dr J. Fecondo (Swinburne University of Technology, Hawthorn, Australia). The gB peptide analogues with selected amino acid substitutions at positions 4, 6 and 7 were purchased from Chiron Mimotopes (Clayton, Australia).

Derivation of gB-specific CTL lines and clones

Polyclonal CTL lines were derived from mice infected in the hind footpad with 4 × 10^5 p.f.u. of HSV-1 as previously described (20). Briefly, draining popliteal lymph node cells were isolated 5 days after infection and stimulated once in vitro with 1 × 10^6 irradiated (12,000 rad) MC57gB cells and 5 × 10^4 irradiated (3000 rad) C57BL/6 spleenocytes in 2 ml RPMI 1640 containing 10% FCS, 2 mM glutamine, 5 × 10^3 M 2-mercaptoethanol and antibiotics (complete RPMI) in wells of a 24-well tissue culture plate (Costar, Cambridge, MA). The cells were re-stimulated a further two times as before with the addition of 15 U/ml IL-2. T cell lines were cloned by limiting dilution in 96-well flat-bottom microtitre plates (Greiner, Frickenhausen, Germany) using 1 × 10^4 irradiated (12,000 rad) MC57gB cells and 5 × 10^5 irradiated (3000 rad) C57BL/6 spleenocytes in 0.2 ml of complete RPMI containing 15 U/ml IL-2.

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Methods

Mice, viruses and cell lines

C57BL/6, C57/L, C3H/He, 129/SV and BALB/c.H-2b mice were purchased from the Central Animal Facility at Monash University, Clayton, Australia. The McIntryre strain of HSV-1 (ATCC VR-539) was grown and titrated in VERO cells. The gB-expressing H-2b transfectant MC57gB (22) was propagated in DMEM containing 10% FCS, 4 mM glutamine, 5 × 10^{-5} M 2-mercaptoethanol, antibiotics and 400 µg/ml neomycin.

Peptide synthesis

The gB_498–505 determinant with the sequence SSIEFARL (single letter amino acid code) was synthesized using an Applied Biosystems model 431A synthesizer (ABI, Foster City, CA) and kindly provided by Dr J. Fecondo (Swinburne University of Technology, Hawthorn, Australia). The gB peptide analogues with selected amino acid substitutions at positions 4, 6 and 7 were purchased from Chiron Mimotopes (Clayton, Australia).

Frequency analysis of gB-specific CTL precursors

Limiting dilution analyses were performed as previously described (18). Draining popliteal lymph node cells were isolated from mice infected in the hind footpad with 4 × 10^5 p.f.u. of HSV-1 5 days earlier. Graded numbers of lymphocytes in 0.1 ml complete RPMI were plated into round-bottom, 96-well plates (Greiner) with 16 replicates for each lymphocyte concentration. To each well was added 0.1 ml complete RPMI containing 10^5 irradiated (3000 rad) C57BL/6 spleenocytes as filler cells, 5 U recombinant IL-2, 10% (v/v) supernatant from concanavalin A-stimulated rat spleen cells and 50 mM α-methyl mannoside (Sigma, St Louis, MO). The cultures were incubated for 5 days and were then assessed for lytic activity. A standard CTL lysis assay was performed using ^51Cr (150
μCi)-labeled EL4 cells in the presence of 10⁻⁶ M gB₄₉₈–₅₀₅ peptide as the targets. Positive wells were scored if cytotoxicity exceeded the spontaneous release (wells containing filler cells but no responder cells) + 3SD. The fraction of negative wells was plotted against starting lymphocyte density according to a Poisson distribution to derive the CTL precursor values, at 37% negative wells (23).

Analysis of recognition of gB peptide analogues varying at TCR contact residues

CTL lysis by gB-specific CTL lines and clones was assessed in a 4 h release assay using ¹¹⁵⁷⁸⁸⁹⁰⁵Cr (150 μCi)-labeled EL4 cells in the presence or absence of the gB₄₉₈–₅₀₅ peptide (SSIEFARL) and peptide analogues with amino acid substitutions at positions 4, 6 and 7 as follows: position 4 Glu to Ala, Arg, Asp, Asn, Ile, Ser, Tyr; position 6 Ala to Arg, Asp, Asn, Gly, Ile, Ser, Tyr; position 7 Arg to Ala, Asp, Asn, His, Ile, Lys, Ser, Tyr. The peptides were used at final concentrations of 10⁻⁶ M with a fixed E:T ratio of 3:1. Results are expressed as a percentage of specific lysis of each peptide analogue relative to lysis of the gB₄₉₈–₅₀₅ peptide with spontaneous lysis <20%.

Anti-CD8 inhibition of CTL lysis

Anti-CD8 inhibition of CTL lysis was performed as previously described (24). Each CTL clone, at a concentration that would result in 50–90% specific target lysis, was incubated in microtitre wells in the presence of a titrated amount of anti-CD8 antibody (PharMingen, San Diego, CA) for 30 min at 37°C. A standard CTL lysis assay was subsequently performed using ¹¹⁵⁷⁸⁸⁹⁰⁵Cr (150 μCi)-labeled EL4 cells pulsed with 10⁻⁶ M gB₄₉₈–₅₀₅ peptide. The dilution of anti-CD8 antibody which resulted in 50% inhibition of maximal specific lysis was calculated for each CTL clone. Spontaneous lysis was <10%.

Flow cytometry

CTL populations were analysed for their TCRBV region distribution on day 5 following the third stimulation. CTL were double-stained with a panel of anti-TCRV antibodies; TCRBV2, B20.6 (25), TCRBV8S1/S2/S3, F23.1 (26), TCRBV8S2, F23.2 (27), TCRBV8S1/S2, KJ16 (28), TCRBV10, KT10 (29), TCRBV14, 14.2 (13) and a FITC-labeled anti-CD8 antibody (Caltag, South San Francisco, CA). The primary TCR-specific antibodies were visualized using biotinylated isotype-specific antibodies (Caltag) and phycoerythrin-labeled streptavidin (Tago, Burlingame, CA). A minimum of 10,000 events were collected using a FACSScan flow cytometer (Becton Dickinson, Mountain View, CA).

TCR sequencing

Total cellular RNA was prepared from 10⁷ cells by guanidinium thiocyanate–phenol–chloroform extraction (30). For CTL lines, single-stranded cDNA synthesis was carried out using 0.1 μg RNA with a primer specific for the murine TCR β chain (TCRBβa: 5′-CCAGAAGCTAGAGAGACC-3′) and 8 U Superscript II reverse transcriptase (Gibco/BRL, Grand Island, NY). The cDNA was then purified and tailed with 2 mM dCTP and 10 U TdT according to manufacturer’s instructions for the 5′ RACE System for Rapid Amplification of cDNA Ends (Gibco/BRL). The dC-tailed cDNA was amplified by PCR using 20% of the cDNA, 20 pmol of primers, 0.2 mM dNTP and 1.5 U Taq polymerase (Gibco/BRL). The primers used for PCR amplification were the anchor primer provided in the 5′ RACE System kit (Gibco/BRL) and a second murine TCRBV primer (5′-GCAAGAGCAAGGGTGGACC-3′) located 5′ to TCRBVα. Amplification involved a single cycle of 95°C for 5 min, 55°C for 1 min and 72°C for 2 min, followed by 30 cycles of 95°C for 1 min, 55°C for 30 s and 72°C for 2 min, and one final cycle of 95°C for 1 min, 55°C for 1 min and 72°C for 7 min using a DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT). The PCR product (1, 4 and 7 μl of reaction mixture) was ligated into the plasmid pGEM-T (Promega, Madison, WI) according to manufacturer’s instructions. Escherichia coli (DH5α; Gibco/BRL) was transformed with the ligation products and isolates from each PCR product were chosen for TCR sequence analysis by PCR amplification using the abridged universal anchor primer from the 5′ RACE System kit (version 2.0) (Gibco/BRL) and a third TCRBV primer (TCRBβc, 5′-CCTGGGTTGAGTCACATTTCTC-3′). After amplification, the PCR products were purified by isopropanol precipitation and ~50 fmol of the purified product sequencing using the TCRBVb primer. Sequencing was performed by double-stranded cycle sequencing (Gibco/BRL) according to the manufacturer’s instructions and the sequence separated on an 8 M urea/6% acrylamide gel. Sequences were named according to Arden et al. (31).

For CTL clones, single-stranded cDNA synthesis was carried out using 2 μg RNA with oligo(dT)₁₅ (0.5 μg) and 200 U MuLV reverse transcriptase (Gibco/BRL). PCR amplification was performed in 100 μl using 2% of the cDNA, 20 pmol of primers, 0.2 mM dNTP and 1.5 U Taq polymerase. The primers used for PCR amplification were the TCRBVα primers and a panel of TCRBV family-specific primers described by Casanova et al. (32). Amplification involved a single cycle of 95°C for 5 min, 55°C for 1 min and 72°C for 2 min, followed by 30 cycles of 95°C for 1 min, 55°C for 30 s and 72°C for 2 min, and one final cycle of 95°C for 1 min, 55°C for 1 min and 72°C for 7 min using a DNA Thermal Cycler. Specific PCR products (3 μl of reaction mixture) were ligated into the plasmid pGEM-T according to the manufacturer’s instructions. E. coli (DH5α) was transformed with the ligation products and isolates from each PCR product were chosen for TCR sequence analysis by PCR amplification using the TCRBVb primer and the appropriate TCRBV-specific primer. After amplification, the PCR products were purified by isopropanol precipitation and ~50 fmol of the purified product sequence used the TCRBVb primer as described above.

Results

TCRBV polymorphism results in the absence of V region dominance in the gB-specific CTL response

The gB-specific, K⁺-restricted CTL response in C57BL/6 mice is dominated by receptors expressing TCRBV10 and TCRBV8S1 gene elements (19). We set out to examine what effect host genetic variation has on the CTL response to the HSV-1 gB determinant. Polyclonal gB-specific CTL lines were derived from two mice of each of the following H-2b-restricted strains: C57BL/6, C3H/He, 129/Sv, BALB/c, H-2b and C57L.

TCRBV availability and antigen-dependent TCR selection

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Mice were infected in the footpad with HSV-1 and, 5 days later, draining lymph node cells were isolated and cultured over 3 weeks with MC57gB cell transfectants expressing HSV-1 gB<sub>498-613</sub>. At the end of this culture period the CTL lines were shown to be specific for the MC57gB transfectant and the gB<sub>498-605</sub> peptide by a CTL lysis assay. All CTL lines recognized and lysed target cells at similar E:T ratios (data not shown). The resultant CTL were then double stained with a limited panel of anti-TCRBV antibodies specific for BV2, BV8, BV10S1A1 and BV14 together with a FITC-labelled anti-CD8 antibody and analysed with a FACScan flow cytometer (Table 1). All mouse strains except C57/L were found to show a similar pattern of TCR V region bias in response to the HSV-1 gB determinant involving the preferential expression of the BV10S1A1 gene element with a minor population expressing BV8S1. This pattern of TCR V region dominance appeared to be lost in the gB-specific CTL lines derived from C57/L mice. This mouse strain has a different allele of the S3 ([F23.1 (26)], TCRBV8S2 ([F23.2 (27)], TCRBV8S1/S2 ([KJ16 (28)], TCRBV10S1A1 ([KT10 (29)] and TCRBV14 ([14.2 (13)]. The anti-TCRBV region analysis provided only a limited insight into the change in the gB-selected TCR repertoire. The complete TCR β chain usage of gB-specific CTL lines derived from C57/L mice was determined by sequencing following 5’ RACE. The predicted amino acid sequences of the in-frame transcripts derived by this method and the frequency with which each appeared in each of the CTL lines are listed in Fig. 1. TCRBV gene usage in these lines was quite diverse with the variable expression of nine different elements including TCRBV8 and TCRBV14 as observed in Table 1. Unlike the response in C57BL/6 mice, where consistently ~60% of all gB-specific CTL lines expressed TCRBV10S1A1 sequence results in the less efficient recognition, CTL clones were derived from two of the gB-specific CTL lines. Thus, the TCRBV polymorphism results in a detectable, albeit small, reduction in the HSV-1 gB-specific CTLp frequency (Table 2). Approximately 1 in 2700 lymph node cells showed a difference in TCRBV usage suggests that it is the polymorphism in the TCRBV genes rather than the background genes which is responsible for the change in TCR expression.

### TCRBV Polymorphism Results in a Dramatic Alteration in the Overall Pattern of TCR Sequence Usage in Response to the gB Determinant

The anti-TCRBV region analysis provided only a limited insight into the change in the gB-selected TCR repertoire. The complete TCR β chain usage of gB-specific CTL lines derived from C57/L mice was determined by sequencing following 5’ RACE. The predicted amino acid sequences of the in-frame transcripts derived by this method and the frequency with which each appeared in each of the CTL lines are listed in Fig. 1. TCRBV gene usage in these lines was quite diverse with the variable expression of nine different elements including TCRBV2 and TCRBV14 as observed in Table 1. Unlike the response in C57BL/6 mice, where consistently ~60% of all gB-specific CTL lines expressed TCRBV10S1A1 (19), the TCRBV10S1A2 gene element usage in the four C57/L-derived gB-specific CTL lines shown in Fig. 1 averaged 11% with two lines providing no TCRBV10<sup>+</sup> transcripts. This suggests that one or more of the six amino acid differences in the C57/L TCRBV10S1A2 sequence results in the less efficient recognition of the K<sup>b</sup>-gB<sub>498-505</sub> complex.

An overall alteration in the pattern of TCR sequence usage was observed in gB-specific CTL derived from C57/L mice as the β chain junctional regions were also extremely diverse. In contrast, 98% of TCRBV10-expressing and 57% of TCRBV8S1-expressing gB-specific CTL in C57BL/6 mice have previously been found to contain conserved junctional motifs in positions 3 and 4 of the TCR β chain CDR3 which were could lead to differences in the fine specificity of gB peptide recognition. CTL clones were derived from two of the gB-specific C57/L CTL lines (lines 2 and 4). These clones were compared with TCRBV10- and TCRBV8S1-expressing, gB-specific CTL clones previously derived from C57BL/6 CTL lines (20) for the recognition of the gB<sub>498-505</sub> peptide and 22 peptide analogues containing single amino acid substitutions

### Table 1. TCRBV Gene Element Usage in HSV-1 gB-Specific Polyclonal CTL Lines Derived from Various H-2<sup>b</sup>-Restricted Mouse Strains

<table>
<thead>
<tr>
<th>CTL line</th>
<th>Percent of cells showing TCRBV gene usage</th>
<th>CD8&lt;sup&gt;+&lt;/sup&gt; cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV2</td>
<td>BV6&lt;sup&gt;+&lt;/sup&gt;</td>
<td>BV10</td>
</tr>
<tr>
<td>C57/L</td>
<td>33</td>
<td>–</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>C3H/He</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>129/sv</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>BALB/c.H-2b</td>
<td>1</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>5</sup>Five different strains of H-2<sup>b</sup>-restricted mice were inoculated with HSV-1 and the draining lymph nodes were removed 5 days later. Lymph node cells were stimulated in vitro three times with the gB transfectant MC57gB. CTL lines were double-stained with an anti-CD8 antibody and murine anti-TCRBV [B20.6 (25)], TCRBV8S1/S2/ S3 [F23.1 (26)], TCRBV8S2 [F23.2 (27)], TCRBV8S1/S2 [KJ16 (28)], TCRBV10S1A1 [KT10 (29)] and TCRBV14 [14.2 (13)]. Values are the percentages of cells which stained positive.

<sup>6</sup>These percentages refer to cells expressing TCRBV8S1 as deduced from the three anti-TCRBV8 antibodies listed above.

<sup>7</sup>There is no antibody available for the detection of the TCRBV10S1A2 gene product in the C57/L TCR repertoire.

### TCRBV Polymorphism Results in a Reduction in gB-Specific CTL Precursors (CTLp) Frequency

As the TCRBV gene polymorphism in C57/L mice leads to a loss of the dominant TCRBV10- and BV8S1-expressing gB-specific CTL pool found in C57BL/6 mice, we investigated whether there was a concomitant decrease in the number of gB-specific CTLp in the C57/L mice. The draining lymph node cells from two C57/L and two C57BL/6 mice infected 5 days earlier with HSV-1 were expanded under limiting dilution culture conditions for 5 days and the gB-specific CTLp frequencies determined (Table 2). Approximately 1 in 2700 lymph node cells recognized the gB<sub>498-505</sub> peptide in C57BL/6 mice while the gB-specific CTLp frequency in C57/L mice was ~1 in 4800 lymph node cells. Thus the TCR polymorphism results in a detectable, albeit small, reduction in the HSV-1 gB-specific CTLp frequency 5 days after primary infection.

### TCRBV Polymorphism Alters Determinant Fine Specificity without Changing TCR-Ligand Affinity

Differences in TCR primary structure, particularly in the CDR3 region, have been shown to lead to differences in the fine specificity pattern of TCR toward peptide-MHC (35,36). To investigate whether the structural differences in the TCR of the gB-specific CTL derived from C57BL/6 and C57/L mice could lead to differences in the fine specificity of gB peptide recognition, CTL clones were derived from two of the gB-specific C57/L CTL lines (lines 2 and 4). These clones were compared with TCRBV10- and TCRBV8S1-expressing, gB-specific CTL clones previously derived from C57BL/6 CTL lines (20) for the recognition of the gB<sub>498-505</sub> peptide and 22 peptide analogues containing single amino acid substitutions
TCRBV availability and antigen-dependent TCR selection

Fig. 1. TCR β chain sequences of HSV-1 gB-specific T cell populations derived from four C57L mice. RNA was extracted from 3-week-stimulated bulk T cell cultures and amplified by 5' RACE using a nested set of anchor (Gibco/BRL) and TCRBC-specific primers. PCR products were ligated into the plasmid pGEM-T and used to transform E. coli (DH5α). Plasmid inserts were amplified using a further nested set of anchor (Gibco/BRL) and TCRBC primers, and directly sequenced by double-stranded cycle sequencing. The predicted amino acid sequences of the in-frame transcripts are shown together with the frequency with which each transcript appeared in each of the CTL lines. The TCRBV and TCRBJ regions were deduced from published genomic sequences (31,33,34). The remaining sequences were assigned to the intervening N–D–N region. Nomenclature of the TCRBV region genes is according to Arden et al. (31).
at the major TCR contact sites at determinant positions 4, 6 and 7 (37–40). These peptide analogues involved both conservative and non-conservative amino acid substitutions of the respective parent residue at each of these determinant positions (Fig. 2). The majority of the gB-specific CTL clones derived from C57BL/6 mice strongly recognized six of the seven position 6 peptide analogues. The CTL clones derived from C57/L mice, on the other hand, showed very little cross-reactivity in their recognition of the TCR contact residue peptide analogues. The only peptide analogues recognized strongly by these CTL clones had conservative substitutions at position 6: Ala to Gli, Ala to Ser. Only one of the C57/L-derived clones, L4/3, contained the junctional residues at positions 3 and 4 of the CDR3 typical of the gB-specific C57BL/6-derived CTL clones (Fig. 3). Interestingly, it was clone L4/3 which showed considerable strong cross-reactivity with the position 7 peptide analogues (Fig. 2). These data show that the C57BL/6 clones are extremely cross-reactive at position 6 while the TCR β chain sequence differences in the C57/L-derived clones correlate with a significant restriction in the level of antigen cross-reactivity.

Structural difference in TCR specific for a particular peptide–MHC complex can lead to differences not only in antigenic cross-reactivity but also in affinity for the peptide–MHC complex. We therefore addressed the question of whether the loss of the dominant TCRBV10- and TCRBV8S1-expressing CTL in the C57/L mice led to the selection of T cells with a lower affinity for the K0–gB498–505 complex. A titration of gB498–505 peptide showed that the gB-specific CTL clones derived from C57BL/6 and C57/L mice responded equally well to subpicomolar levels of peptide in a CTL lysis assay (Fig. 4). Furthermore, a similar quantity of anti-CD8 antibody was required for 50% inhibition of specific lysis of H-2K0 target cells pulsed with the gB498–505 peptide in an anti-CD8 inhibition assay (data not shown). Thus it appears that while the TCRBV gene polymorphism resulted in changes in sequence selection with a concomitant change in antigen cross-reactivity, it did not affect TCR–ligand affinity.

**Discussion**

Allelic polymorphisms in the TCRBV genes have been shown to lead to the loss of specific immune responses to antigenic peptides which are dominated by T cells bearing particular TCRBV genes. Nanda et al. (17) showed that the deletion of the TCRBV8S2 and TCRBV13 gene segments from the germline of mice strains with the TCRBV4 genotype resulted in the inability of these mice to respond to two antigenic determinants, sperm whale myoglobin 111–121/I-Ed and myelin basic protein 1–11/I-Ak. Here we find that the removal from the available TCR repertoire of the preferred TCRBV10 and BV8S1 gene elements used by CTL specific for the dominant gB498–505 determinant of HSV-1 does not lead to the ablation of this response in C57/L mice following primary infection. Rather, CTL expressing other TCRBV gene elements appear to replace the previously dominant subset. Moreover, TCR isolated from a number of independent C57/L responses to this determinant showed no preferential TCRBV usage or biased CDR3 sequence composition. The diversification of the CTL response to this viral determinant mirrors that previously observed for the HLA-B8-restricted EBV response (16). Briefly, these individuals mount a strikingly biased CTL response, consisting of an invariant TCR sequence, to the immunodominant EBNA-3 determinant of EBV. Deletion of the receptor from the available repertoire results in the complete diversification of the responding population.

The TCR repertoire diversification associated with the C57/L response to gB appears to reflect expansion of minor subpopulations present in the C57BL/6 response. The subdominance of such CTL may arise as a consequence of their overall lower affinity for the K0–gB498–505 complex compared with the TCRBV10- and BV8S1-expressing T cells as suggested for the HLA-B8-restricted EBNA-3 response. However, this appears
TCRBV availability and antigen-dependent TCR selection

Fig. 3. TCR β chain sequences of HSV-1 gB-specific T cell clones derived from C57L and C57BL/6 CTL lines. CTL clones were isolated by limiting dilution from two C57L-derived CTL lines (lines 2 and 4 from Fig. 1) and from four C57BL/6-derived CTL lines (20). RNA was extracted and reverse transcribed into cDNA followed by PCR amplification using a TCRBC-specific primer and a panel of TCRBV family-specific primers (32). Specific PCR products were ligated into the plasmid pGEM-T and used to transform E. coli (DH5α). Plasmid inserts were amplified using a nested TCRBC-specific primer and the appropriate TCRBV-specific primer, and directly sequenced by double-stranded cycle sequencing. Only the predicted amino acid sequences of each CTL clone are shown. The TCRBV and TCRBJ regions were deduced from published genomic sequences (31,33,34). The remaining sequences were assigned to the intervening N–D–N region. Nomenclature of the TCRBV region genes is according to Arden et al. (31). The conserved junctional residues at positions 3 and 4 of the CDR3 typical of gB-specific C57BL/6-derived CTL are highlighted in bold typeface.

unlikely since gB-specific CTL clones derived from C57L and C57BL/6 mice were found to give similar dose responses to the gB498–505 determinant (Fig. 4) suggesting that both populations had similar overall affinities. More likely, the emerging C57L clones may simply represent a minor population of precursors in the starting naive T cell pool. To some extent this is consistent with the reduction in precursor frequencies found in the responding lymph node populations, although the CTLp reduction does not appear to account for the complete deletion of the otherwise dominant CTL subset which makes up to 80% of the C57BL/6 response to the gB determinant. Nonetheless, the loss of the TCRBV10- and BV8S1-expressing CTL in the C57L mice appears to permit the emergence of an otherwise subdominant population of gB-specific CTL to expand and fill the void left by the elimination of the dominant T cell subset.

The recently published crystal structure of the αβTCR location. Interestingly, this position also appears to be involved in direct peptide contact in the high resolution confirmed previous proposals (9–11) that the CDR3 region is principally involved in peptide interaction. However, the crystal structure further revealed that the CDR1 and CDR2 regions also play a role in peptide interaction by contact with the terminal residues of the peptide in the MHC binding groove (7,8). Therefore class I-bound peptide can determine both V region selection as well as CDR3 sequence expression by the responding T cell subset. Indeed, the preferential TCRBV10 and BV8S1 usage in the response to the Kβ–gB498–505 complex is clearly peptide dependent since this V region bias is not evident in other Kβ-restricted peptides (41–43). Thus, one of the V region-encoded CDR1 and CDR2 elements is likely to be involved in either direct peptide contact or interaction with a region of the MHC which undergoes some form of peptide-dependent conformational alteration. A likely candidate for the TCR residue involved is position 28 found within the CDR1 (nomenclature according to 31). The two dominant V regions, TCRBV10 and BV8S1, share a common aspartic acid at this position while the other two closely related members of the TCRBV8 family, TCRBV8S2 and BV8S3, which never appear in the gB-specific response, contain the complete deletion of the otherwise dominant T cell subset.

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Interestingly, this position also appears to be involved in direct peptide contact in the high resolution TCR–MHC–peptide structure of Garboczi et al. (7).

These results also highlight the functional importance of CDR combinations in determining antigen-dependent TCR selection. Not only do the TCRBV allelic changes abolish the V region bias in the responding CTL populations, but they also eliminate the striking CDR3 conservation found in these dominant subsets. Nearly all TCRBV10- and >55% of TCRBV8S1-expressing gB-specific CTL derived from C57BL/6 mice showed an absolute conservation of positions 3 and

<table>
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<tr>
<th>Clone</th>
<th>TCR V Gene</th>
<th>V</th>
<th>N-D-N</th>
<th>J</th>
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<tr>
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<td>F C A S</td>
<td>R G Q G</td>
<td>Q N T L Y F G</td>
<td>BD1B72S4</td>
</tr>
<tr>
<td>HSV-2.3</td>
<td>BV8S1</td>
<td>F C A S</td>
<td>R G Q G V</td>
<td>S D Y T F G</td>
<td>BD1B71S2</td>
</tr>
</tbody>
</table>

| L2/1 | BV4S1 | F C A S S | Q E D W G | S A E T L Y F G | BD2B72S3 |
| L2/2 | BV16S1A2 | F C A S S | Q D S | S Y E Q Y F G | BD1B72S6 |
| L2/3 | BV7S1 | F C A S S | P L G G | N Y A E Q F F G | BD2B72S1 |
| L4/3 | BV6S1A2 | L C A S S | Q W G V | Q N T L Y F G | BD2B72S4 |
| L4/6 | BV2S1 | Y C T C S | G D W G S | S Q N T L Y F G | BD2B72S4 |
TCRBV availability and antigen-dependent TCR selection

Fig. 4. Response to gB peptide titration by gB-specific CTL clones derived from C57BL/6 (A) and C57/L (B) mice. Titrations of gB\(_{498-505}\) peptide (10\(^{-6}\) to 10\(^{-16}\) M) added to \(^{51}\)Cr-labeled H-2\(^b\) target cells were incubated with the gB-specific CTL clones at an E:T of 3:1 in a standard CTL lysis assay. Spontaneous lysis was 11%.

4 within the CDR3 (19) which was minimal in other T cell populations, such as those that emerge in the C57/L response to this determinant. Thus, alterations within the CDR1 and CDR2 regions also effect the selection of those TCR regions most involved in direct peptide interactions, specifically the CDR3. This altered CDR3 selection would explain the altered peptide specificity manifested as a diminished promiscuity for variants at the exposed position 6 in the target peptide. Such focusing of an otherwise promiscuous pattern of peptide cross-reactivity may reflect an increased CDR3 contribution to peptide recognition that compensates for the loss of preferential V region interaction with other parts of the MHC–peptide ligand complex. While the changes in peptide specificity could also reflect alterations in TCR \(\alpha\) chain usage, the lack of restriction in gB-specific selection with this chain in C57BL/6 mice (20) suggests that the \(\alpha\) chain has a lesser role in the response to the K\(^b\)-gB\(_{498-505}\) complex.

In conclusion, this study shows that changes in TCRBV availability results in the loss of a dominant gB-specific CTL pool expressing TCRBV10 and BV8S1 gene elements, and the emergence of gB-specific CTL that otherwise represent a minor subset in the responding T cell population. In addition to the obvious differences in V region usage, the major and minor populations appear to contain altered patterns of CDR3 sequence inclusion reflecting an alteration in their contribution to target specificity. It would therefore appear that preferential
interactions with CDR1 at the expense of CDR3 residues allow the TCRBV10- and BV8S1-expressing CTL to dominate the response to HSV-1 gB, possibly by selecting a broader pool of CTL precursors.

Acknowledgements

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Abbreviations

CDR complementarily determining region
CTL cytotoxic T lymphocyte
CTLP CTL precursors
EBV Epstein–Barr virus
gB glycoprotein B
HSV-1 herpes simplex virus type 1
TCRBV TCR β chain variable region

References


