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Characterization of a Diverse Primary Herpes Simplex Virus Type 1 gB-Specific Cytotoxic T-Cell Response Showing a Preferential Vβ Bias

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The glycoprotein B (gB) from herpes simplex virus type 1 is a major target of cytotoxic T lymphocytes (CTL) in C57BL/6 mice. The majority of these T cells are directed to a single K^b-restricted determinant, $gB_{498–505}$. We have analyzed the T-cell receptor (TCR) usage in gB-specific CTL lines derived shortly after virus infection. The CTL populations preferentially used two Vβ regions, a dominant Vβ10 element and a subdominant Vβ8 element. Detailed sequence analysis revealed considerable TCR β-chain heterogeneity despite a striking level of predicted amino acid conservation at the Vβ-Dβ junction. This junction forms part of the third hypervariable loop of the TCR thought to directly contact the major histocompatibility complex-bound antigenic peptide. The results reveal considerable diversity within the primary T cells responding to a single viral determinant while still maintaining a high degree of TCR Vβ bias and sequence conservation at the V-D-J junction.

Cytotoxic T lymphocytes (CTL) constitute a major component of the adaptive immune response and accelerate viral clearance by directly lysing infected cells (19). CTL expressing the CD8 cell surface marker recognize viral antigens as peptide fragments presented in combination with the products encoded by the class I locus of the major histocompatibility complex. This recognition is mediated by an immunoglobulin-like α- and β-chain T-cell receptor (TCR) heterodimer consisting of variable (V), diversity (D), joining (J), and constant (C) elements (16, 24). In further analogy with the immunoglobulins, the TCR polypeptides have three areas of hypervariability corresponding to the antibody complimentarity-determining regions (CDR) (9, 26). T cells exhibit a combined specificity for both the foreign peptide antigen and the self major histocompatibility complex which is determined by the sequence of the respective components within the TCR. Recent studies have suggested that the third hypervariable regions (the CDR3) found at the V-J and V-D-J junctions preferentially interact with the peptide antigen while the V-encoded CDR1 and CDR2 elements contact the helical regions of the major histocompatibility complex molecule (11, 12, 18). Various examples of TCR junctional region conservation consistent with this arrangement for TCR-peptide interaction have been described (17, 18, 21).

In an effort to assess the extent of TCR diversity present in virus-specific responses, we have characterized the TCR expressed by CTL specific for the herpes simplex virus type 1 (HSV-1) envelope glycoprotein B (gB) isolated shortly after virus infection. T cells directed to this protein play an important role in the primary immune response to HSV-1 infection in C57BL/6 ($H\text{-}2^b$) mice and limit the extent of viral infection on adoptive transfer (6, 7, 15, 25, 27). The animals generate a predominantly K^b-restricted response to HSV-1 (15), and gB represents one of only two known HSV-derived CTL targets in these particular mice (28). Furthermore, virtually all CTL specific for the HSV-1 gB glycoprotein appear to be directed to a single K^b-presented peptide fragment mapped to the $gB_{498–505}$ minimal determinant (i.e., the gB fragment from amino acid 498 to 505) (6, 27, 28).

Many T-cell responses appear to be dominated by a restricted number of Vα and Vβ elements, often in conjunction with preferred junctional sequences (1, 29, 33, 35). We set out to determine whether this was also true for the gB-specific response. Polyclonal CTL lines were derived from mice infected in the footpad with 4×10^5 PFU of the McIntyre strain of HSV-1 (ATCC VR-539). Draining popliteal lymph node cells were isolated after 5 days and stimulated once in vitro with 3×10^6 irradiated (12,000 rads) MC57gB cell transfectants expressing HSV-1 $gB_{1–613}$ (34) in the absence of interleukin 2 and two more times with the addition of 15 U of interleukin 2 per ml and 20×10^6 irradiated (3,000 rads) syngeneic splenocytes in addition to the MC57gB cells. All cultures were grown in upright T25 tissue culture flasks (Greiner, Frickenhausen, Germany) in 10 ml of complete RPMI 1640 medium containing 10% fetal calf serum. At the end of this culture period CTL lines were shown to be specific for the MC57gB transfectant and the $gB_{498–505}$ peptide by a CTL lysis assay (20). The resultant CTL were then double stained with the Vβ-specific antibodies and a fluorescein isothiocyanate-labelled anti-CD8 antibody (Caltag, South San Francisco, Calif.) with a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.). This analysis revealed that the gB-specific responses showed preferential expression of just two Vβ elements in six lines (B63 to B68) derived from separate mice (Table 1). On average, 17% of the predominantly $CD8^+$ population was $V\beta8^+$ (identified by the F23.1 antibody [30]) while 61% were $V\beta10^+$ (identified by the KT10 antibody [31]). Consequently, $V\beta8^+$ and $V\beta10^+$ CTL make up the overwhelming majority of $gB_{498–505}$-specific CTL, and no other V region is consistently expressed on more than 10% of T cells (data not shown).

The extent of TCR diversity within each responding population was determined by sequencing of the TCR transcripts derived from the cell lines shown in Table 1. The mRNA was isolated at the time of flow cytometric analysis and subjected to a combination of reverse transcriptase cDNA synthesis and

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TABLE 1. Biased Vβ usage in HSV gB-specific CTL lines[a]

Cell line	% of cells showing Vβ region usage		% $CD8^+$ cells
	Vβ8	Vβ10	
B63	19	63	100
B64	11	57	100
B65	19	61	100
B66	16	68	99
B67	12	68	100
B68	17	51	100
Avg	17	61	99
$CD8^+$ LNTC[b]	15	4	

[a] CTL lines B63 through B68 were derived from different C57BL/6 mice by immunization with the McIntyre strain of HSV-1 and stimulation in vitro three times with the gB transfectant MC57gB (34). Bulk CTL lines were double stained with an anti-CD8 antibody and anti-Vβ8 (F23.1 [30]) or anti-Vβ10 (KT10 [31]) antibody. Values are the percentages of cells which stained positive.

[b] Lymph node T cells (LNTC) from nonimmune C57BL/6 mice were double stained with the antibodies described in footnote *a*.

PCR amplification with Vβ8- or Vβ10-specific and Cβ-specific oligonucleotides as described previously (20). The PCR products were cloned into the pGEM-T plasmid for sequence analysis as described in the manufacturer's instructions (Promega, Madison, Wis.). A number of transformed bacterial colonies containing $V\beta10^+$ or $V\beta8^+$ plasmid inserts were isolated from each T-cell population. We have focused predominantly on the dominant $V\beta10^+$ subset (Fig. 1) and carried out only a limited analysis of the $V\beta8^+$ transcripts from four T-cell lines (Fig. 2).

Sequencing was performed on these inserts by double-stranded cycle sequencing as described previously (20). The predicted amino acid sequences of the in-frame transcripts and the frequency with which each appeared in a given response are shown in Fig. 1 and 2. T cells express only one productively rearranged β-chain gene as a direct consequence of allelic exclusion between the respective genetic loci (23, 32). Therefore, the TCR β-chain transcripts provide us with an accurate assessment of T-cell heterogeneity within each responding population, assuming that the sequence frequencies reflect the relative abundance of individual transcripts found in the original starting material. On the basis of this assumption it is clear that each CTL population contained a variety of T-cell clones. The populations were not dominated by any single clone, since each contained between four and eight distinct $V\beta10^+$ sequences (Fig. 1) which make up the major T-cell subset (Table 1). Interestingly, every individual responded with a unique array of clones, since no two populations in Fig. 1 contained the same TCR β-chain sequence. The Vβ8-specific amplification showed that the sequence variation within and between animals extended to this minor T-cell subset (Fig. 2).

Figures 1 and 2 also provide an assessment of the overall variation in $gB_{498–505}$-specific TCR. In total we were able to isolate 13 different $V\beta8^+$ sequences from four different CTL populations and 39 different $V\beta10^+$ sequences from a total of six independent T-cell lines. The sequence heterogeneity results largely from a promiscuity in Jβ usage. It is this diversity in Jβ usage and the resultant Dβ-Jβ junction that directly contributes to the diverse nature of TCR usage despite the presence of only two dominant Vβ elements. In contrast to the heterogeneous Dβ-Jβ junction, the sequences in the vicinity of the Vβ-Dβ junction appears relatively conserved even among different Vβ groups. The majority of the gB-specific TCRs have a junctional glycine located exactly six residues from the V-region-encoded cysteine. Furthermore, 8 of the 13 $V\beta8^+$ sequences and 14 of the 39 $V\beta10^+$ β chains encode a junctionally encoded arginine at the first position of the CDR3 according to the definition of Chothia et al. (9). It should be noted that both the arginine and glycine residues are found at the Vβ-Dβ junction, which is usually one of the most variable regions of the TCR β chain (12). Thus, about half of the TCR β chains specific for the HSV-1 $gB_{498–505}$ determinant contain an RXXG junctional motif spanning the first four residues of the CDR3 despite variations in Vβ and Jβ usage.

Our data show that the $gB_{498–505}$-specific CTL are relatively diverse within and between different C57BL/6 mice. Therefore,

Vβ10	N-Dβ2-N	Jβ	Frequency
Cell line B63			
L C A S	R N W G	N Y A E Q F F G	Jβ2.1 3/10
L C A S S	L W G	N T G Q L Y F G	Jβ2.2 1/10
L C A S S	Y W G A	S A E T L Y F G	Jβ2.3 1/10
L C A S S	S W G G A	A E T L Y F G	1/10
L C A S	G T W G G A	D T Q Y F G	Jβ2.5 1/10
L C A S	H W G G G	D T Q Y F G	1/10
L C A S S	S W G G A	Y E Q Y F G	Jβ2.6 1/10
L C A S	R S G G S	S Y E Q Y F G	1/10
Cell line B64			
L C A S S	D W G	N Y A E Q F F G	Jβ2.1 1/10
L C A S	R D W G V	Y A E Q F F G	1/10
L C A S S	F W G R	A E Q F F G	1/10
L C A S	R D W G	A E T L Y F G	Jβ2.3 2/10
L C A S S	Y W G G	Q N T L Y F G	Jβ2.4 2/10
L C A S S	Y W G	S Q N T L Y F G	1/10
L C A S S A	W G G Q H	T Q Y F G	Jβ2.5 2/10
Cell line B65			
L C A S S	L W G T	N Y A E Q F F G	Jβ2.1 3/9
L C A S	R D W G I V S	E Q F F G	1/9
L C A S	R D W G	N Y A E Q F F G	1/9
L C A S	K D W G T	S Q N T L Y F G	Jβ2.4 1/9
L C A S S	H W G G E G	T Q Y F G	Jβ2.5 1/9
L C A S	R N W G S	S Y E Q Y F G	Jβ2.6 2/9
Cell line B66			
L C A S S	Y W G A N	A E Q F F G	Jβ2.1 1/9
L C A S S	S W G T	T G Q L Y F G	Jβ2.2 3/9
L C A S S	Y W G S	N T G Q L Y F G	1/9
L C A S	R P W G G A	Y E Q Y F G	Jβ2.6 1/9
L C A S	R D W G D	S Y E Q Y F G	2/9
L C A S	R H W G G A F	E Q Y F G	1/9
Cell line B67			
L C A S	R P W G	N Y A E Q F F G	Jβ2.1 4/9
L C A S S	H W G G G	D T Q Y F G	Jβ2.5 2/9
L C A S S	F W G A	Q D T Q Y F G	1/9
L C A S S	S W G V	Y E Q Y F G	Jβ2.6 2/9
Cell line B68			
L C A S	R P W G A	N T E V F F G	Jβ1.1 1/9
L C A S S	Y W G	S A E T L Y F G	Jβ2.1 1/9
L C A S S	Y W G S	S A E T L Y F G	1/9
L C A S	R V W G T	S Q N T L Y F G	Jβ2.4 1/9
L C A S S	S W G	S Q N T L Y F G	2/9
L C A S	S W G A L	N T L Y F G	1/9
L C A S S	S W G T	S Y E Q Y F G	Jβ2.6 1/9
L C A S	R S W G S	S Y E Q Y F G	1/9

FIG. 1. $V\beta10^+$ TCR β-chain sequences derived from gB-specific T-cell populations. HSV gB-specific Vβ10 sequences were isolated from 3-week-stimulated bulk T-cell cultures by extracting the total RNA and treating it with reverse transcriptase and then subjecting it to PCR amplification with Cβ- and Vβ10-specific primers (20). PCR products were cloned into plasmid pGEM-T and then used to transform *Escherichia coli*. Plasmid inserts were amplified with Cβ- and Vβ10-specific primers and directly sequenced by double-stranded cycle sequencing. Only the predicted amino acid sequences are shown. The Vβ and Jβ regions were deduced from Vβ10 (4, 5) and Jβ (14, 22) genomic sequences. The remaining sequences were assigned to the intervening N-Dβ-N region.

Vβ8	N-Dβ-N	Jβ	Frequency
Cell line B64			
F C A S	R G Q G	N T E V F F G	Jβ1.1 1/8
F C A S	R G Q G I	S D Y T F G	Jβ1.2 1/8
F C A S S D	A L G L G G R	Y A E Q F F G	Jβ2.1 4/8
F C A S	R G Q G T	E T L Y F G	Jβ2.3 1/8
F C A S	R G Q G	Q N T L Y F G	Jβ2.4 1/8
Cell line B65			
F C A S	R G Q G Y	T E V F F G	Jβ1.1 2/4
F C A S	R G Q G	N T E V F F G	1/4
F C A S S D	R H P S	Q N T L Y F G	Jβ2.4 1/4
Cell line B67			
F C A S	R G Q G D	T E V F F G	Jβ1.1 1/5
F C A S S	E R T G G S	Q N T L Y F G	Jβ2.4 1/5
F C A S S	D A G T G R	N T L Y F G	3/5
Cell line B68			
F C A C	R G Q G D	T E V F F G	Jβ1.1 1/4
F C A S	R G Q G	N T E V F F G	1/4
F C A S	G G Q G E	N T L Y F G	Jβ1.3 1/4
F C A S	R G Q G V	Y E Q Y F G	Jβ2.6 1/4

FIG. 2. Vβ8$^+$ TCR β-chain sequences derived from gB-specific T-cell populations. HSV gB-specific Vβ8 sequences were isolated from 3-week-stimulated bulk T-cell cultures by extracting the total RNA and treating it with reverse transcriptase and then subjecting it to PCR amplification with a Cβ-specific primer and a Vβ8-specific primer (20). PCR products were cloned into plasmid pGEM-T and then used to transform *E. coli*. Plasmid inserts were amplified by PCR and directly sequenced by double-stranded cycle sequencing. Only the predicted amino acid sequences are shown. The Vβ and Jβ regions were deduced from Vβ8 (10) and Jβ (14, 22) genomic sequences. The remaining sequences were assigned to the intervening N-Dβ-N region.

the observed V-region bias is a consequence not of the repeated selection of dominant Vβ8- or Vβ10-expressing clones but rather of the activation of diverse populations with a conserved junctional motif. It should be noted that we have isolated a number of Vβ8$^+$ and Vβ10$^+$ CTL clones specific for $gB_{498–505}$ which contain the same junctional sequences as that found in the bulk populations (data not shown). Together with the striking level of junctional conservation, this suggests that the bulk T-cell lines examined in this study largely consist of a single specificity. In addition, the RXXG sequence is not found in unrelated K^b-restricted CTL (20) or in other responses with preferential Vβ8 or Vβ10 biases (2, 8, 13), consistent with the notion that this junctional motif is selected by the K^b presentation of the $gB_{498–505}$ determinant.

Finally, TCR analysis of CTL specific for a different member of the herpes virus family, human Epstein-Barr virus (EBV), has found an unprecedented level of TCR conservation. Argaet et al. (3) isolated EBV-specific HLA-B8 restricted T-cell clones expressing identical TCR sequences from a number of unrelated individuals. These investigators suggested that the EBV-specific repertoire conservation reflected the existence of long-term latency within their donor population. The HSV gB-specific CTL examined here were isolated from newly infected animals. Consequently, the observed heterogeneity could simply reflect the selection of a broad low-affinity primary response. CTL isolation when little free virus persists in the latently infected animals may reveal a far less heterogeneous memory T-cell pool expressing only a selected subset of the TCR found at the initiation of viral replication.

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