

The Cytotoxic T-Cell Response to Herpes Simplex Virus Type 1 Infection of C57BL/6 Mice Is Almost Entirely Directed against a Single Immunodominant Determinant

MORGAN E. WALLACE,¹ RACHAEL KEATING,¹ WILLIAM R. HEATH,²
AND FRANCIS R. CARBONE^{1*}

*Department of Pathology and Immunology, Monash Medical School, Prahran, Victoria 3181,¹ and
Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville,
Victoria 3050,² Australia*

Received 9 April 1999/Accepted 10 June 1999

Many virus infections give rise to surprisingly limited T-cell responses directed to very few immunodominant determinants. We have been examining the cytotoxic T-lymphocyte (CTL) response to herpes simplex virus type 1 (HSV-1) infection. Previous studies have identified the glycoprotein B-derived peptide from residues 498 to 505 (gB_{498–505}) as one of at least three determinants recognized by HSV-1-specific CTLs isolated from C57BL/6 mice. We had previously found that in vitro-derived CTLs directed to gB_{498–505} show a characteristic pattern of T-cell receptor (TCR) usage, with 60% of gB_{498–505}-specific CD8⁺ T cells expressing BV10⁺ TCR β chains and a further 20% expressing BV8S1. In this report, we confirm that this TCR V-region bias is also reflected in the ex vivo response to HSV-1 infection. A high proportion of activated CD8⁺ draining lymph node cells were found to express these dominant V regions, suggesting that a substantial number of in vivo responding T cells were directed to this one viral determinant. The use of an HSV-1 deletion mutant lacking the gB_{498–505} determinant in combination with accurate intracellular gamma interferon staining allowed us to quantify the extent of gB-specific T-cell dominance. Together, these results suggested that between 70 and 90% of all CD8⁺ HSV-1-specific T cells target gB_{498–505}. While deletion of this determinant resulted in an attenuated CD8⁺ T-cell response, it also permitted the emergence of one or more previously unidentified cryptic specificities. Overall, HSV-1 infection of C57BL/6 mice results in an extremely focused pattern of CD8⁺ T-cell selection in terms of target specificity and TCR expression.

While some virus-specific cytotoxic T lymphocyte (CTL) responses show considerable diversity at the level of determinant recognition, many are confined to an extremely restricted number of antigenic targets. Both the helper T-cell and CTL populations activated by even the most complex of pathogenic agents are frequently directed to less than a handful of major or immunodominant determinants (49, 58). Such focusing on just a few key determinants provides a simple means for viruses to escape immune surveillance. Even the most complex virus can potentially evade T-cell recognition by limited mutations within these few dominant determinants (2, 18, 42). These types of CTL escape variants are found within human immunodeficiency virus-infected patients, where they are thought to contribute to progressive immune evasion with a corresponding gradual worsening of disease (6, 35, 43). Alternatively, this immunodominance can potentially provide advantages to the infected individual, such as the ability to mount a rapid and high-affinity response while minimizing the possibility of cross-reactivity with self components (20).

Immunodominance is likely to arise as a consequence of a combination of factors which span the complete class I-restricted presentation and T-cell recognition process. Firstly, the strength of peptide-class I interaction has been shown to be a key parameter in determining immunodominance (13, 50, 54, 55), while antigen processing can also play an important role in controlling the hierarchy of presented peptides (41). In addition,

there is emerging evidence that competition at the level of T-cell–antigen-presenting cell interaction could potentially influence the composition of the responding CTL populations (11). Finally, T-cell receptor (TCR) expression and selection appear to make critical contributions toward the extent of determinant dominance in what would otherwise be a complex immune response (58).

Of all these parameters, it has proven most difficult to assess the TCR contribution to immunodominant T-cell responsiveness. Firstly, the extensive and detailed characterization of a T-cell repertoire specific for any given peptide represents a major undertaking, especially at the clonal level. Combined with this, antiviral CTLs of a single specificity can be quite diverse in terms of their TCR expression (8, 14, 26, 31). Most importantly, it is often difficult to determine the relationship between antigen-specific TCR selection and the preexisting repertoire found within the naive T-cell pool, especially when the specificity is associated with a highly diverse TCR expression.

Given this, antigens that select restricted patterns of TCR expression are providing important insights into how the TCR repertoire can potentially influence immune responsiveness to complex pathogens (8, 9, 51). We have characterized CTLs selected by a murine *H-2^b*-restricted determinant from the herpes simplex virus (HSV) glycoprotein B (gB) encompassing residues 498 to 505 (gB_{498–505}) (15, 16). These CTLs are known to protect mice against lethal infection with this virus (4, 5, 25, 34). Extensive analysis of TCR expression at both the population and the clonal level has revealed an unusually consistent pattern of TCR V-region utilization combined with a high degree of diversity in terms of clonally distributed TCR se-

* Corresponding author. Mailing address: Department of Pathology and Immunology, Monash Medical School, Prahran, Victoria 3181, Australia. Phone: 61-3-9903-0744. Fax: 61-3-9903-0731. E-mail: carbone@cobra.path.monash.edu.au.

quence expression (15, 16, 27, 53). These repeating patterns of TCR element usage within the gB response provide an opportunity to examine how the naive T-cell repertoire composition can affect the CTL response directed to this virus. An important element in this analysis is an accurate assessment of the gB determinant's contribution to the overall HSV type 1 (HSV-1)-specific immune response. Limiting dilution analyses have previously suggested that gB₄₉₈₋₅₀₅-specific cells comprise less than a quarter of the HSV-specific CTL population (45, 57). In this report, we have reexamined this issue by quantifying the relative contribution of gB₄₉₈₋₅₀₅ with preferential TCR V-region usage and gamma interferon (IFN- γ) expression within the lymph nodes draining the site of primary cutaneous HSV-1 infection. In contrast to previous reports, we have found that the gB₄₉₈₋₅₀₅ determinant is truly immunodominant, with up to 90% of all HSV-1-specific CD8⁺ T cells isolated from infected C57BL/6 mice being specific for this single viral determinant.

MATERIALS AND METHODS

Mice. Female C57BL/6 mice at 6 to 8 weeks of age were purchased from the Monash University Central Animal Facility. Mice were immunized intradermally in the hind feet with 4×10^5 PFU of each virus, diluted to a total volume of 20 μ l per footpad in phosphate-buffered saline or gB₄₉₈₋₅₀₅ peptide (1 mg/ml in Hanks balanced salt solution) emulsified in complete Freund's adjuvant (CFA).

Peptide and cells. Primary mouse embryonic fibroblasts (PMEF) from day 13 C57BL/6 embryos were generated in the laboratory by Claerwen Jones and propagated in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum (FCS). The *H-2^b*-expressing thymoma cell line EL4 was grown in Dulbecco modified Eagle medium supplemented with 10% FCS. Vero cells and D6 transfectants expressing HSV-1 gB (21) were grown in minimum essential medium supplemented with 5% FCS. The gB₄₉₈₋₅₀₅ (5), RR₈₂₂₋₈₂₉ (48), and ICP27₄₄₅₋₄₅₂ (44) peptides were synthesized with an Applied Biosystems model 431A synthesizer (ABI, Foster City, Calif.) and kindly provided by J. Fecondo (Swinburne University, Melbourne, Australia).

Viruses. The KOS strain of HSV-1 was propagated and titrated in Vero cells. The KOS-derived gB-deletion mutant strains K Δ 318 and K Δ 5C (21) were grown and titrated by using the gB-producing D6 cell line, which complements the gene inactivation. Both mutant viruses were also checked for the absence of plaque formation on Vero cells to exclude any possibility of reversion to wild type.

Ex vivo analysis of BV element expression. Draining popliteal lymph nodes were removed from mice immunized 5 days previously with either the KOS, K Δ 318, or K Δ 5C virus or gB₄₉₈₋₅₀₅ peptide emulsified in CFA. Lymph node cells were triple labelled with anti-CD25-phycoerythrin (PE) (Pharmingen, San Diego, Calif.); anti-CD8-allophycocyanin (Pharmingen); and a panel of biotinylated antibodies specific for TCR BV elements, BV2, BV4, BV9, BV10, BV11, and BV12 from Pharmingen and BV7 from Caltag, followed by streptavidin-fluorescein isothiocyanate (FITC) (Molecular Probes, Eugene, Oreg.). The BV8 and BV5 subfamily member staining was carried out with hybridoma supernatants from antibodies MR9-4 (BV5S1 specific) (3), MR9-4 (BV5S1 and BV5S2 specific) (3), MR5-2 (BV8S1 and BV8S2 specific) (28), F23.2 (BV8S2 specific) (29), and F23.1 (BV8S1, BV8S2, and BV8S3 specific) (52) followed by the FITC-labelled isotype-specific secondary antibodies (Pharmingen). After staining, cells were analyzed by flow cytometry with a FACScalibur (Becton Dickinson, San Jose, Calif.) cell sorter. Data analysis was performed with CELLQuest software (Becton Dickinson).

Assessment of gB₄₉₈₋₅₀₅ and RR₈₂₂₋₈₂₉-specific CTL activity. Lymph node cells harvested from mice 5 days postinfection were cultured in 24-well tissue culture plates at a density of 5×10^6 cells/well in 2 ml of RPMI medium containing 10% FCS (RP10) without exogenous antigen for 3 days. The resulting level of cytotoxicity was assayed with EL4 cells alone or pulsed for 1 h with 1 μ g of either gB₄₉₈₋₅₀₅ or RR₈₂₂₋₈₂₉ per ml in a 4-h ⁵¹Cr-release assay.

Assay for IFN- γ production. Lymph node suspensions were prepared from pooled lymph nodes of two to four mice that were immunized 5 days previously with KOS, K Δ 318, or K Δ 5C. Cells were cultured at 37°C for 6 h in 96-well round-bottomed tissue culture plates at a density of 0.8×10^6 cells per well in 200 μ l of RP10 supplemented with 10 U of interleukin-2 per ml. Stimulator cells were added at a density of 0.2×10^6 cells per well. Stimulator cells were PMEF alone, infected for 12 h with virus at a multiplicity of infection (MOI) of 1, or pulsed with 1 μ g of peptide per ml for 1 h. Staining was carried out with a Cytofix/Cytoperm kit (Pharmingen). Following the 6-h stimulation, cells were washed twice with phosphate-buffered saline–0.5% bovine serum albumin–0.1% azide and then stained for the expression of cell surface markers with anti-CD8-APC (Pharmingen) and anti-CD25-biotin (Caltag). Following this, the cells were washed, incubated with an FITC-streptavidin conjugate (Molecular Probes), and then fixed and permeabilized according to the protocol supplied with the Cytofix/Cytoperm kit. Finally, the cells were stained with either a PE-conjugated anti-IFN- γ or a PE-labelled isotype control antibody. Samples were analyzed by flow

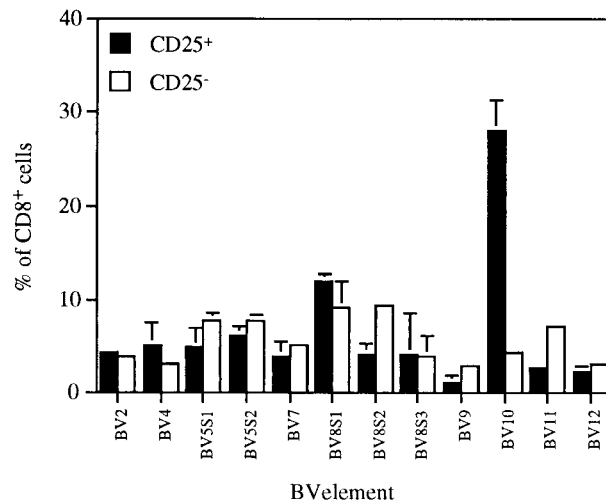


FIG. 1. TCR BV expression by CD25⁺ CD8⁺ T cells following HSV-1 infection. Draining popliteal lymph nodes were harvested at the peak of infection from mice immunized in the hind feet 5 days previously with the KOS strain of HSV-1. Lymph node cells were stained for CD8 and CD25 expression in combination with a panel of BV-element-specific antibodies and then subjected to flow cytometric analysis. BV-element expression in the CD25⁻ subset, shown by the white bars, corresponds to the expression level seen in unimmunized controls (data not shown).

cytometry with a FACScalibur (Becton Dickinson) cell sorter, and data analysis was performed with CELLQuest software (Becton Dickinson). Viable cell counts were determined for all samples on the basis of trypan blue exclusion prior to stimulation. The number of CD8⁺ cells producing IFN- γ was calculated by multiplying the percentage of IFN- γ ⁺ cells by the total cell number per mouse determined by the cell counts.

RESULTS

The accumulation of CD8⁺ T cells expressing TCR BV10 and BV8S1 in the lymph nodes draining the site of HSV-1 infection is a direct consequence of gB specificity. We have previously shown that cultured CTL lines specific for the gB₄₉₈₋₅₀₅ determinant consistently show a biased expression of two major TCR β -chain elements (16). Nearly 60% of CD8⁺ gB-specific T cells derived from mice infected with HSV-1 were found to express TCR BV10⁺ β chains while a further 20% contained the BV8S1 element. This in vitro-derived V-element bias was also evident in T cells isolated directly ex vivo after footpad infection with HSV-1. Figure 1 shows the relative expression of each of 12 TCR BV elements on activated CD8⁺ T cells isolated from the popliteal lymph nodes from mice immunized in the hind feet with the KOS strain of HSV-1. In this case, interleukin-2 receptor (CD25) expression was used as a marker of T-cell activation. These results reveal a preferential accumulation of BV10 and, to a lesser extent, of BV8S1 cells within the CD25⁺ CTL subset. This is largely consistent with an earlier finding of a BV10⁺ TCR accumulation in the CD8⁺ T-cell blast subset (15) and emphasizes that this bias represents a preferential activation of the gB-specific T cells within the lymph nodes draining the site of HSV-1 footpad infection.

To further examine the association between gB specificity and BV10 accumulation, we examined the CTL response to KOS-derived gB-deletion mutant strains of HSV-1, K Δ 5C and K Δ 318 (21) (Fig. 2). The K Δ 5C deletion encompasses residues 43 to 595, which include the immunodominant epitope gB₄₉₈₋₅₀₅ peptide, while gB residues 616 to 711 are deleted in the K Δ 318 mutant, so that the truncated gene product still

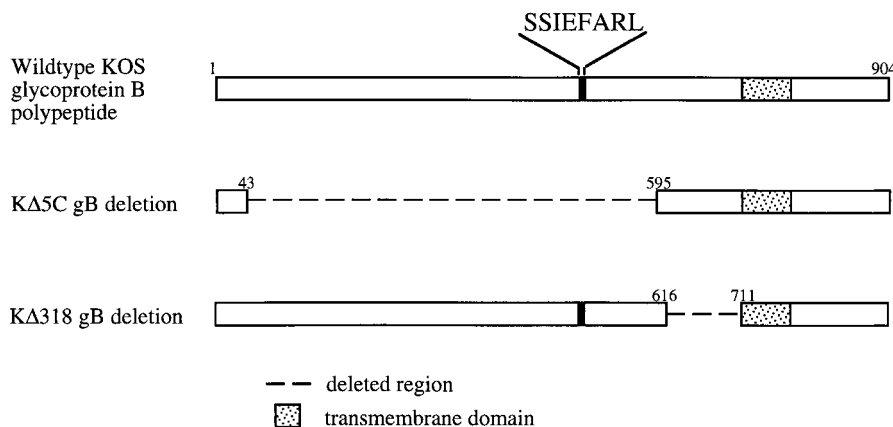


FIG. 2. Schematic diagram of the gB-deletion mutant strains KΔ5C and KΔ318. The KΔ5C mutant lacks amino acids 43 to 595, which encompass the gB₄₉₈₋₅₀₅ determinant, while the truncated polypeptide produced by the KΔ318 mutant lacks only amino acids 616 to 711 and thus retains the determinant.

contains the *K^b*-restricted CTL determinant. It should be noted that neither the KΔ318 nor the KΔ5C strain expresses a functional gB, and consequently, both can undergo only a single round of replication following infection. Mice were immunized with either the wild-type KOS strain of HSV-1 or the gB-deletion mutant strains KΔ318 and KΔ5C. Figure 3 shows that mice immunized with the KΔ5C virus generate no gB-specific cytotoxicity, whereas the KΔ318 virus gives a strong gB-specific CTL response equivalent to that of immunization with wild-type KOS virus. The level of cytotoxicity directed against a subdominant determinant from the viral ribonucleotide reductase RR₈₂₂₋₈₂₉ (48) remains the same across all three virus strains and serves as a control to demonstrate effective infection and some CTL activation by the KΔ5C mutant. Analysis of BV-element expression on cells isolated from mice immunized with KΔ5C showed no evidence for any other V-element bias occurring in the absence of a gB-specific response (data not shown). In contrast, lymph node cells isolated from KΔ318-infected animals showed preferential TCR BV10 accumulation approaching the level seen after KOS immunization (Fig. 4). It

is also interesting to note that the level of BV10 accumulation following immunization with the gB₄₉₈₋₅₀₅ peptide is equivalent to that achieved following immunization with the whole virus, arguing that CD8⁺ T cells directed to the gB₄₉₈₋₅₀₅ determinant must represent a considerable proportion of all C57BL/6-derived CTLs directed to the whole HSV-1.

The gB-derived determinant (gB₄₉₈₋₅₀₅) is the major target recognized by HSV-1-specific CD8⁺ T cells. The preceding data suggesting a significant gB dominance was in conflict with previous estimates of its contribution to the overall HSV-1-specific CTL response. Early limiting dilution analyses showed that only 5 to 25% of virus-specific CTLs derived from HSV-1-infected C57BL/6 mice were specific for the gB₄₉₈₋₅₀₅ determinant (45, 57). However, we have consistently found that, on average, more than 40% of activated CD8⁺ T cells from the draining lymph nodes preferentially express BV10⁺ β chains (Fig. 4 and data not shown) with highly conserved junctional sequences indicative of gB-specific CTLs (15). Given that approximately 60% of in vitro-derived CTL lines directed to gB₄₉₈₋₅₀₅ express these same TCR β-chains (16), our results

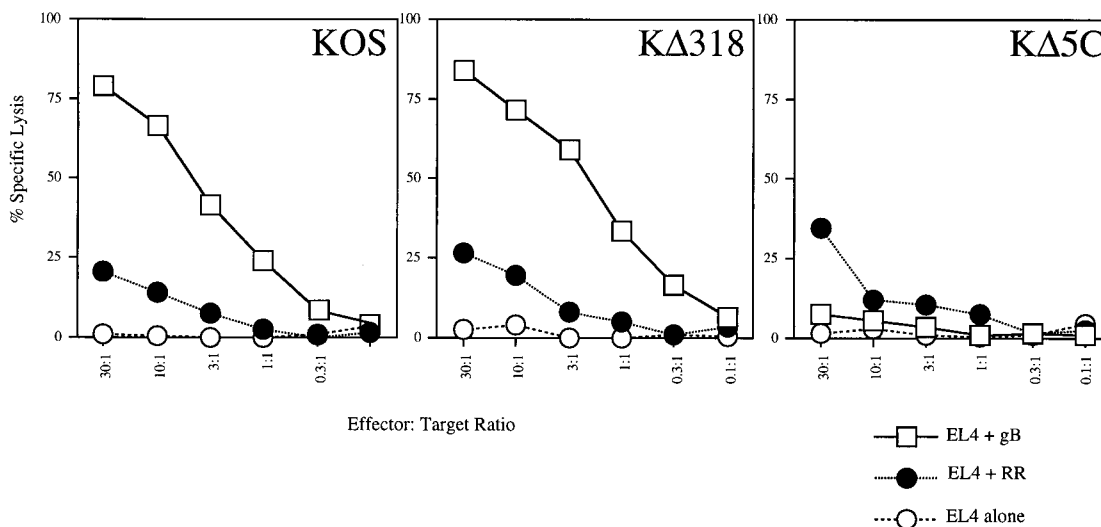


FIG. 3. CTL target specificities following cutaneous infection with KOS, KΔ318, or KΔ5C virus. Mice were immunized in the hind feet with wild-type KOS or gB-deletion mutant viruses, and the draining popliteal lymph nodes were removed 5 days postinfection. Lymph node cells were cultured for 3 days in the absence of exogenous antigen and then assayed in a 4-h ⁵¹Cr-release assay for reactivity against EL4 cells pulsed with 1 μg of either the gB₄₉₈₋₅₀₅ or RR₈₂₂₋₈₂₉ peptide per ml.

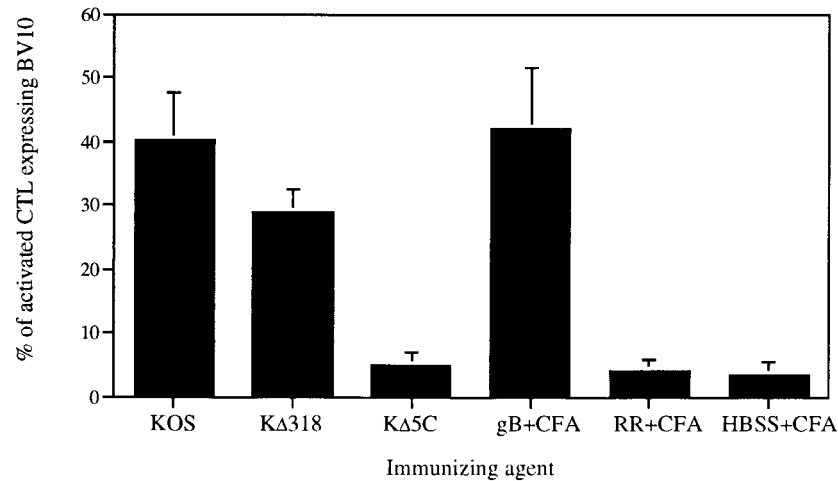


FIG. 4. Accumulation of BV10⁺ cells in the lymph node CD8⁺ CD25⁺ T-cell subset. Lymph node cells derived from mice immunized 5 days previously with either virus (KOS, KA318, or KA5C) or peptide (gB₄₉₈₋₅₀₅ or RR₈₂₂₋₈₂₉) in CFA were triple labelled with anti-CD8, anti-CD25, and anti-BV10 antibodies and then subjected to flow cytometric analysis. The graph shows the percentages of CD25⁺ CTLs expressing TCR BV10. The level of BV10 expression seen in unimmunized controls is 5% (data not shown). HBSS, Hanks balanced salt solution.

suggest that CTLs targeting this one peptide must represent at least two-thirds of the total HSV-specific response. This is an underestimate since it excludes nonspecific lymph node T-cell activation. Even at its lowest, this value is a significantly higher proportion of the overall HSV-1 response than that estimated from the earlier limiting dilution analyses.

To clarify this issue, we quantified the relative contribution of gB-specific T cells to the overall virus-specific CD8⁺ T-cell response following immunization with each of the mutant virus strains by measuring IFN- γ production within lymph node cells draining the site of infection. Popliteal lymph node cells taken at day 5 postinfection, which is the peak of the CTL response (26a), were stimulated for 6 h with PMEF loaded with the gB₄₉₈₋₅₀₅ or RR₈₂₂₋₈₂₉ peptide or PMEF infected with the KOS virus to induce IFN- γ production. Intracellular IFN- γ staining in CD8⁺ T cells could be detected only following stimulation with appropriately presented PMEF, for example, those infected with HSV-1 (compare Fig. 5A with 5B). Moreover, all IFN- γ -expressing CD8⁺ T cells were found to coexpress CD25 (Fig. 5C), consistent with our previous report that the gB-specific cells are found within the activated T-cell subset (15).

The results presented in Fig. 6 show that the viruses containing the gB determinant, KOS and KA318, induce nearly equal proportions of IFN- γ -producing CD8⁺ T cells when stimulated with either the isolated gB₄₉₈₋₅₀₅ peptide or cells infected with the complete HSV-1 virus. From the results presented in Fig. 6, we estimate that about 92 and 95% of HSV-1-specific CD8⁺ T cells induced by KOS and KA318 infection, respectively, are directed to the gB₄₉₈₋₅₀₅ determinant. T cells specific for the RR₈₂₂₋₈₂₉ determinant make up a relatively minor proportion of responding lymph node cells, with less than 0.5% of all lymph node CD8⁺ T cells being specific for this determinant. A third *H-2^b*-restricted determinant derived from HSV-1 ICP27 (ICP27₄₄₅₋₄₅₂) has also been identified (44), although we could find no IFN- γ ⁺ T cells directed to this determinant (data not shown). Immunization with the gB₄₉₈₋₅₀₅ deletion mutant KA5C induced a lower, but still respectable, level of intracellular IFN- γ staining when the CD8⁺ lymph node cells were stimulated with HSV-1-infected PMEF, but gB activity was absent from this response (Fig. 6). Surprisingly, while the KA5C mutation virtually abolished the response to the dominant gB₄₉₈₋₅₀₅ determinant, the emerging specificities did not appear to be directed to the previously identified sub-

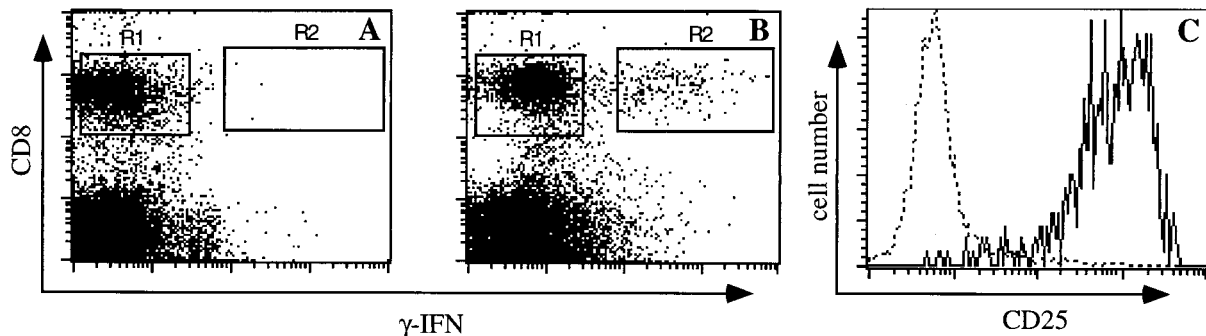


FIG. 5. Intracellular CD25 and IFN- γ expression in CD8⁺ lymph node cells after HSV-1 infection. Lymph node cells harvested 5 days postinfection with the KOS strain of HSV-1 were stimulated *in vitro* for 6 h with either uninfected PMEF (A) or PMEF infected with KOS (MOI = 1) (B). Following stimulation, cells were triple labelled for CD8 and CD25 expression, and intracellular IFN- γ production was measured by flow cytometric analysis. Also shown is CD25 expression on CD8⁺ IFN- γ ⁺ cells (located in region R2 in panel B and represented by a solid line) and CD8⁺ IFN- γ ⁻ cells (located in region R1 in panel B and represented by a dotted line) (C).

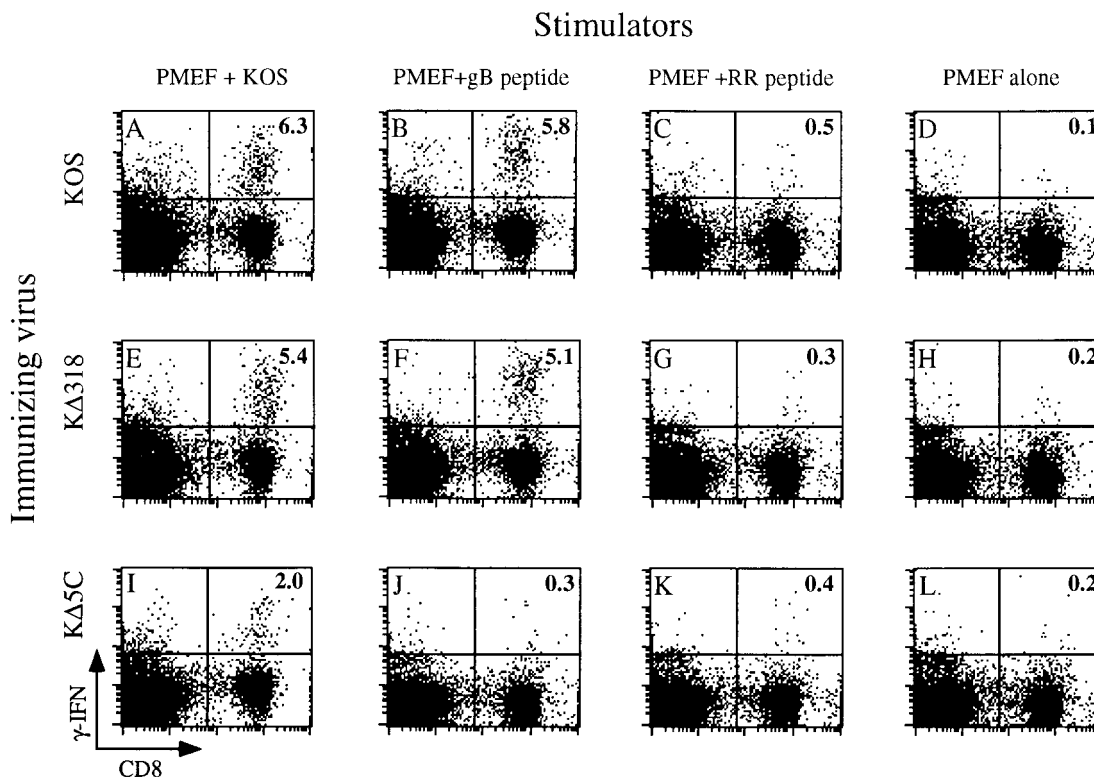


FIG. 6. Intracellular IFN- γ staining in lymph node cells from KOS- and gB-deletion mutant-immunized mice. Lymph node cells from mice immunized with either KOS HSV-1 or the KOS-derived gB-deletion mutant strain K Δ 318 or K Δ 5C were stimulated in vitro with either KOS-infected PMEF, PMEF loaded with 10 μ M gB₄₉₈₋₅₀₅ peptide, or uninfected PMEF. Following the 6-h culture period, cells were stained with anti-CD8-APC, fixed and permeabilized, and then stained with anti-IFN- γ -PE prior to flow cytometric analysis. The percentage of CD8⁺ cells producing IFN- γ is indicated in the top right-hand corner of each panel. (A to D) CTLs from KOS-immunized mice; (E to H) K Δ 318-immunized mice; (I to L) K Δ 5C-immunized mice.

dominant determinant RR₈₂₂₋₈₂₉ (Fig. 6) or ICP27₄₄₅₋₄₅₂ (data not shown). Consequently, deletion of the immunodominant gB determinant from the HSV-1 genome reveals the existence of one or more hidden or cryptic class I-restricted determinants expressed by this virus.

Finally, the gB dominance of the HSV-1 CD8⁺ T-cell response was confirmed by determining the proportion of lymph node cells expressing IFN- γ after in vitro stimulations with either the gB₄₉₈₋₅₀₅-containing viruses (KOS and K Δ 318) or the K Δ 5C variant, which lacks this determinant (Fig. 7). Lymph node cells from mice infected with the replication-defective mutant strains were used for this experiment. The results clearly showed that stimulation with K Δ 5C-infected PMEF caused a significant reduction in the K Δ 318-specific IFN- γ response, confirming that gB₄₉₈₋₅₀₅ dominates the CD8⁺ T-cell response when it is present within the immunizing virus. It should also be noted that, while gB accounts for the bulk of the response, 20 to 30% of K Δ 318-specific CTLs appear to be responding to non-gB determinants, although this may be due in part to an increased representation of non-gB determinants on K Δ 5C-infected stimulator cells.

Deletion of the immunodominant gB₄₉₈₋₅₀₅ determinant attenuates the CD8⁺ T-cell response to HSV-1 in C57BL/6 mice and results in expansion of T cells specific for one or more cryptic determinants. The data presented in Fig. 6 suggests that removal of the immunodominant gB₄₉₈₋₅₀₅ determinant results in expansion of CTLs directed to a cryptic determinant within HSV-1. However, the actual level of expansion cannot be assessed from the proportion of cells expressing IFN- γ since

the deletion viruses induce a lower level of T-cell activation and lymph node cell recruitment compared with the wild-type KOS virus (data not shown). Therefore, rather than presenting the results as proportions, we examined the total number of HSV-1-specific, IFN- γ -producing CD8⁺ T cells within lymph nodes draining tissues infected with the KOS virus and two mutant strains of virus (Fig. 8). While Fig. 6 shows that the majority of HSV-1-specific CD8⁺ T cells from K Δ 5C-immunized animals were now specific for an otherwise cryptic determinant, Fig. 8 shows that, in terms of actual IFN- γ ⁺ CD8⁺ T-cell numbers recovered from responding lymph nodes, this response was less than half that of K Δ 318 virus-immunized mice. Comparison of results for KOS and K Δ 318 shows the attenuation associated with differences in virus replication, since both give responses that are largely gB₄₉₈₋₅₀₅ specific (Fig. 6) while only KOS is able to undergo multiple rounds of replication. In contrast, the decrease in IFN- γ ⁺ CD8⁺ T-cell numbers shown by comparing K Δ 318 and K Δ 5C comes about from the deletion of gB residues 43 to 495, which include the gB₄₉₈₋₅₀₅ CTL determinant. Regardless of the attenuation, these results still represent an overall increase in otherwise silent T cells specific for determinants other than the usually dominant gB₄₉₈₋₅₀₅.

DISCUSSION

Our results show that the primary HSV-1-specific CD8⁺ T-cell response to cutaneous HSV-1 infection is largely directed against a single immunodominant gB-derived determi-

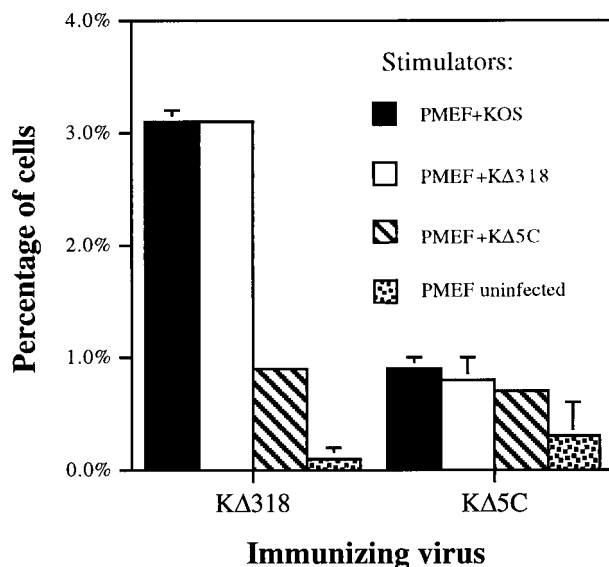


FIG. 7. Production of IFN- γ following stimulation with gB-deletion virus-infected PMEF. Lymph node cells isolated from mice immunized 5 days previously with either the K Δ 318 or K Δ 5C gB-deletion mutant strain of HSV-1 were stimulated in vitro for 6 h with PMEF infected with KOS, K Δ 318, or K Δ 5C at an MOI of 1 or with uninfected PMEF. Following stimulation, cells were stained for CD8 and then fixed and stained with a PE-conjugated anti-IFN- γ antibody prior to flow cytometric analysis.

nant. Even our lowest estimation, based on preferential V-region usage, necessitates that more than two-thirds of HSV-1-specific T cells are specific for the gB₄₉₈₋₅₀₅ determinant, while the IFN- γ results suggest that this peptide is likely to be targeted by 70–90% of all virus-specific CD8⁺ T cells. This range is at odds with previous limiting dilution analyses which predicted that 5 to 25% of HSV-1-specific CTLs isolated from lymph nodes draining the site of infection were directed to this determinant (45, 57). One possible explanation for the differences between our results and those published previously is that only a small proportion of gB₄₉₈₋₅₀₅-specific responders detected by TCR or IFN- γ analyses are actually cytotoxic, which was the parameter used in the limiting dilution assays. Alternatively, it is now recognized that the limiting dilution analyses, as distinct from direct determination with TCR expression or IFN- γ production, grossly underestimate the actual proportion of specific precursors within a responding cell population (10, 11, 22, 39).

There are two other H-2^b-restricted HSV-1-derived determinants that have so far been identified, RR₈₂₂₋₈₂₉ and ICP27₄₄₅₋₄₅₂ (44, 48), and these were expected to make some contribution to the T-cell response to virus infection. Here we show that the RR₈₂₂₋₈₂₉-specific T cells are found at relatively low, but detectable, frequencies. In contrast, we could not detect any ICP27₄₄₅₋₄₅₂-specific T cells, either by IFN- γ production or by cytotoxicity assays (data not shown). Differences in virus substrain usage or subtle host variations may explain the observed lack of ICP27 response in our hands. Regardless, our results provide direct quantitation of the frequency of T cells responding to the dominant gB₄₉₈₋₅₀₅ determinant and suggest that they make up the bulk of HSV-1-specific T cells as well as a major proportion of the total activated CD8⁺ subset found within lymph nodes draining the site of primary infection. While the estimates of absolute responding cell numbers are greater than those calculated from previous limiting dilution analyses (45, 57), they are largely in line with our earlier

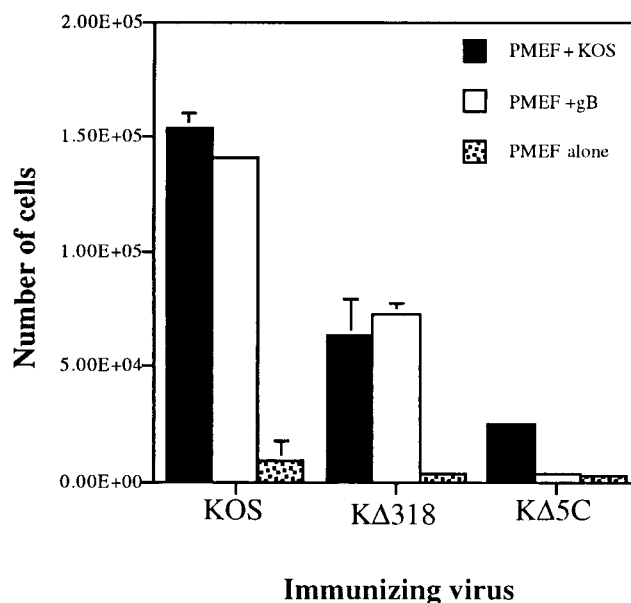


FIG. 8. Deletion of the immunodominant gB₄₉₈₋₅₀₅ determinant attenuates the CD8⁺ T-cell response to HSV-1. Lymph node cells isolated from mice immunized 5 days previously with either KOS, K Δ 318, or K Δ 5C were stimulated in vitro for 6 h with PMEF pulsed with 1 μ g of gB₄₉₈₋₅₀₅ peptide per ml for 1 h, infected with the KOS strain of HSV-1 (MOI = 1), or left uninfected. Following stimulation, cells were stained for CD8 and IFN- γ expression and subjected to flow cytometric analysis. The number of IFN- γ ⁺ cells per lymph node was calculated from viable cell counts performed prior to in vitro stimulation, and the proportion of lymph node cells expressing IFN- γ was determined by flow cytometry.

predictions based solely on direct TCR sequence analysis of the cutaneous HSV-1 response (15). This study suggested that specific T cells make up a larger proportion of responding cell populations than previously appreciated, subsequently documented in more detail for CTL responses involving systemic infections (10, 11, 39).

Given the complexity of HSV-1, which encodes over 100 viral products (47), it may appear unexpected that virtually the entire HSV-1-specific CTL response is directed against a single class I-restricted determinant. However, dominance by one or a very few key determinants is often observed in T-cell responses to even the most complex antigen (49, 58). Even so, the gB₄₉₈₋₅₀₅-specific immunodominance highlights a number of interesting features. For example, antiviral responses tend to be directed to proteins synthesized during the earliest phases of the viral replication cycle (17), and yet the gB protein is produced relatively late in infection (47). There was a formal possibility that the immunogenic components might have come from the virion itself rather than from de novo protein synthesis, as noted in the CTL response to the betaherpesvirus human cytomegalovirus (33). This would have explained why gB could so efficiently generate a CTL response. However, immunization experiments involving the mutant viruses K Δ 318 and K Δ 5C clearly showed that at least for these variants the gB-specific response is reliant on de novo protein synthesis. Both these replication-deficient gB-deletion mutants acquire the functional gB molecules necessary for infection from the D6 virus-packaging cell line (21), but only the determinant-expressing K Δ 318 was able to prime gB₄₉₈₋₅₀₅-specific T cells (Fig. 3). Significantly, not only was the anti-gB response maintained with the replication-defective K Δ 318 virus but it was surprisingly potent compared to that of a fully competent par-

ent virus. Efficient CTL induction has been reported previously for another replication-defective HSV-1 strain (36). In that case, it was suggested that prolonged infection by the input variant was the major contributor to the efficiency of this immune response (37).

While the results clearly emphasize the importance of the gB₄₉₈₋₅₀₅ determinant, its elimination in the KΔ5C mutant revealed the existence of an otherwise silent CTL determinant. Interestingly, there was little increase in the response to the already-defined subdominant determinant RR₈₂₂₋₈₂₉. Lewicki et al. (30) showed that mutation of all three codominant determinants from lymphocytic choriomeningitis virus (LCMV) resulted in the emergence of an otherwise hidden response to "cryptic" epitopes already present in the virus. A similar emergence of undefined cryptic determinants is also noted here, although the deletion of the dominant gB₄₉₈₋₅₀₅ nonetheless results in some level of immune response attenuation. For example, the total IFN-γ response by CD8⁺ T cells from animals immunized with the gB₄₉₈₋₅₀₅-deletion virus KΔ5C was about half that found for the similarly defective mutant, KΔ318, that contains the immunodominant determinant. While this decrease is modest, it nonetheless provides evidence that elimination of a dominant determinant does make the resultant variant "less fit" at generating an effective immune response. This type of response attenuation has been noted in other systems involving mutations of immunodominant, but not subdominant, target peptides (38, 40).

Probably the most striking feature of the dominant gB₄₉₈₋₅₀₅-specific response is the highly restricted pattern of TCR usage within the responding population. CTL responses to immunodominant determinants are characterized by both restricted (1, 19, 32, 46, 51) and unconstrained (8, 12, 14, 26) patterns of TCR usage. We previously found that nearly 80% of all gB-specific CTLs use the two preferred BV elements, BV8S1 and BV10 (16). Combined with the data presented here showing up to 90% of all HSV-specific CTLs being directed to this one determinant, these two V regions define over 80% of all CD8⁺ T cells that respond to this virus in C57BL/6 mice. A similar level of V-region bias is also found in one of the few other antiviral CTL responses showing similar levels of overwhelming single-determinant dominance. Over 90% of all LCMV-specific CTLs derived from BALB/c mice are directed to a single nucleoprotein-derived peptide (39, 56), and three BV elements encompass 70% of the overall antiviral response (51). Moreover, both the LCMV (51) and HSV (16) TCR BV biases are associated with highly restricted junctional sequences encoding the TCR β chain CDR3, which is involved in direct contact with the presented peptide (23, 24, 53). Despite this, there is considerable diversity within the remainder of the β-chain sequence and little conservation in TCR α-chain pairing within the gB₄₉₈₋₅₀₅-specific T cells (16, 53). We found that different individuals responded with a highly diverse and unique set of TCR sequences (16), although all had the consistent pattern of TCR BV usage shown here.

The extent of the determinant immunodominance described in this report, coupled with the highly consistent pattern of TCR gene expression, argues that the gB₄₉₈₋₅₀₅-specific T cells, and therefore their specific TCRs, are relatively abundant within the naive lymphocyte population. Indeed, while peptide affinity can control immunodominance (13), there is increasing evidence that T-cell frequencies and TCR availability also play important roles in this selection (20, 54, 55). The conserved pattern of TCR BV usage and the restricted junctional sequence in the gB₄₉₈₋₅₀₅-specific response permit comparison between these antigen-selected T-cell populations and the naive receptor repertoire, as recently reported for a simple tumor

model which also expanded a CTL population with a very restricted TCR BV10-junctional sequence combination (7). However, in our case, the responding cell population represents nearly all the CD8⁺ T cells specific for a much more complex antigenic system, in the form of HSV-1, providing a unique opportunity to examine how the naive receptor repertoire can control the overall immune response to an infectious agent.

ACKNOWLEDGMENTS

We thank Stanley Person for the kind gift of the HSV-1 gB-deletion mutant virus strains and the D6 packaging cell line.

This work was supported by grants from the Australian National Health and Medical Research Council and the Australian Research Council.

REFERENCES

- Aebischer, T., S. Oehen, and H. Hengartner. 1990. Preferential usage of Vα4 and Vβ10 T cell receptor genes by lymphocytic choriomeningitis virus glycoprotein-specific H-2D^b-restricted cytotoxic T cells. *Eur. J. Immunol.* **20**: 523-531.
- Bertoletti, A., A. Sette, F. V. Chisari, A. Penna, M. Levrero, M. Decarli, F. Fiaccadori, and C. Ferrari. 1994. Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells. *Nature* **369**:407-410.
- Bill, J., O. Kanagawa, J. Linten, Y. Utsunomiya, and E. Palmer. 1990. Class I and class II MHC gene products differentially affect the fate of Vβ5 bearing thymocytes. *J. Mol. Cell. Immunol.* **4**:269-279.
- Bonneau, R. H., and S. R. Jennings. 1989. Modulation of acute and latent herpes simplex virus infection in C57BL/6 mice by adoptive transfer of immune lymphocytes with cytolytic activity. *J. Virol.* **63**:1480-1484.
- Bonneau, R. H., L. A. Salvucci, D. C. Johnson, and S. S. Tevethia. 1993. Epitope specificity of H-2K^b-restricted, HSV-1-, and HSV-2-cross-reactive cytotoxic T lymphocyte clones. *Virology* **195**:62-70.
- Borrow, P., H. Lewicki, X. Wei, M. S. Horwitz, N. Peffer, H. Meyers, J. A. Nelson, J. E. Gairin, B. H. Hahn, M. B. Oldstone, and G. M. Shaw. 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat. Med.* **3**:205-211.
- Bouso, P., A. Casrouge, J. D. Altman, M. Hauray, J. Kanellopoulos, J. P. Abastado, and P. Kourilsky. 1998. Individual variations in the murine T cell response to a specific peptide reflect variability in naive repertoires. *Immunity* **9**:169-178.
- Busch, D. H., and E. G. Pamer. 1998. MHC class I peptide stability: implications for immunodominance, in vitro proliferation, and diversity of responding CTL. *J. Immunol.* **160**:4441-4448.
- Busch, D. H., I. Pilip, and E. G. Pamer. 1998. Evolution of a complex T cell receptor repertoire during primary and recall bacterial infection. *J. Exp. Med.* **188**:61-70.
- Busch, D. H., I. M. Pilip, S. Vihj, and E. G. Pamer. 1998. Coordinate regulation of complex T cell populations responding to bacterial infection. *Immunity* **8**:353-362.
- Butz, E. A., and M. J. Bevan. 1998. Massive expansion of antigen-specific CD8⁺ T cells during an acute virus infection. *Immunity* **8**:167-175.
- Casanova, J. L., P. Romero, C. Widmann, P. Kourilsky, and J. L. Maryanski. 1991. T-cell receptor genes in a series of class-I major histocompatibility complex-restricted cytotoxic lymphocyte-T clones specific for a Plasmodium berghei nonapeptide—Implications for T-cell allelic exclusion and antigen-specific repertoire. *J. Exp. Med.* **174**:1371-1383.
- Chen, W. S., S. Khilko, J. Fecondo, D. H. Margulies, and J. McCluskey. 1994. Determinant selection of major histocompatibility complex class I-restricted antigenic peptides is explained by class I-peptide affinity and is strongly influenced by nondominant anchor residues. *J. Exp. Med.* **180**:1471-1483.
- Cole, G. A., T. L. Hogg, and D. L. Woodland. 1994. The MHC class I-restricted T cell response to Sendai virus infection in C57BL/6 mice: a single immunodominant epitope elicits an extremely diverse repertoire of T cells. *Int. Immunol.* **6**:1767-1775.
- Cose, S. C., C. M. Jones, M. E. Wallace, W. R. Heath, and F. R. Carbone. 1997. Antigen-specific CD8⁺ T cell subset distribution in lymph nodes draining the site of herpes simplex virus infection. *Eur. J. Immunol.* **27**:2310-2316.
- Cose, S. C., J. M. Kelly, and F. R. Carbone. 1995. Characterization of a diverse primary herpes simplex virus type 1 gB-specific cytotoxic T-cell response showing a preferential Vβ bias. *J. Virol.* **69**:5849-5852.
- Coupar, B. E., M. E. Andrew, G. W. Both, and D. B. Boyle. 1986. Temporal regulation of influenza hemagglutinin expression in vaccinia virus recombinants and effects on the immune response. *Eur. J. Immunol.* **16**:1479-1487.
- de Campos-Lima, P. O., R. Gavioli, Q. J. Zhang, L. E. Wallace, R. Dolcetti,

- M. Rowe, A. B. Rickinson, and M. G. Masucci. 1993. HLA-A11 epitope loss isolates of Epstein-Barr virus from a highly A11+ population. *Science* **260**:98–100.
19. Deckhut, A. M., W. Allan, A. McMickle, M. Eichelberger, M. A. Blackman, P. C. Doherty, and D. L. Woodland. 1993. Prominent usage of V β 8.3 T-cells in the H-2D^b-restricted response to an influenza-A virus nucleoprotein epitope. *J. Immunol.* **151**:2658–2666.
 20. Deng, Y. P., J. W. Yewdell, L. C. Eisenlohr, and J. R. Bennink. 1997. MHC affinity, peptide liberation, T cell repertoire, and immunodominance all contribute to the paucity of MHC class I-restricted peptides recognized by antiviral CTL. *J. Immunol.* **158**:1507–1515.
 21. Desai, P., F. L. Homa, S. Person, and J. C. Glorioso. 1994. A genetic selection method for the transfer of HSV-1 glycoprotein B mutations from plasmid to the viral genome: preliminary characterization of transdominance and entry kinetics of mutant viruses. *Virology* **204**:312–322.
 22. Gallimore, A., A. Glithero, A. Godkin, A. C. Tissot, A. Pluckthun, T. Elliott, H. Hengartner, and R. Zinkernagel. 1998. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I peptide complexes. *J. Exp. Med.* **187**:1383–1393.
 23. Garboczi, D. N., P. Ghosh, U. Utz, Q. R. Fan, W. E. Biddison, and D. C. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* **384**:134–141.
 24. Garcia, K. C., M. Degano, R. L. Stanfield, A. Brunmark, M. R. Jackson, P. A. Peterson, L. Teyton, and I. A. Wilson. 1996. An $\alpha\beta$ T cell receptor structure at 2.5 angstrom and its orientation in the TCR-MHC complex. *Science* **274**:209–219.
 25. Hanke, T., F. L. Graham, K. L. Rosenthal, and D. C. Johnson. 1991. Identification of an immunodominant cytotoxic T-lymphocyte recognition site in glycoprotein B of herpes simplex virus by using recombinant adenovirus vectors and synthetic peptides. *J. Virol.* **65**:1177–1186.
 26. Horwitz, M. S., Y. Yanagi, and M. B. Oldstone. 1994. T-cell receptors from virus-specific cytotoxic T lymphocytes recognizing a single immunodominant nine-amino-acid viral epitope show marked diversity. *J. Virol.* **68**:352–357.
 - 26a. Jones, C., S. Cose, and F. Carbone. Submitted for publication.
 27. Jones, C. M., S. C. Cose, and F. R. Carbone. 1997. Evidence for cooperation between TCR V region and junctional sequences in determining a dominant cytotoxic T lymphocyte response to herpes simplex virus glycoprotein B. *Int. Immunol.* **9**:1319–1328.
 28. Kanagawa, O. 1988. Antibody-mediated activation of T cell clones as a method for screening hybridomas producing antibodies to the T cell receptor. *J. Immunol. Methods* **110**:169–178.
 29. Kappler, J. W., U. Staerz, J. White, and P. C. Marrack. 1988. Self-tolerance eliminates T cells specific for MIs-modified products of the major histocompatibility complex. *Nature* **332**:35–40.
 30. Lewicki, H. A., M. G. vonHerrath, C. F. Evans, J. L. Whitton, and M. B. A. Oldstone. 1995. CTL escape viral variants. II. Biologic activity in vivo. *Virology* **211**:443–450.
 31. Malissen, M., J. Trucy, E. Jouvin-Marche, P. A. Cazenave, R. Scollay, and B. Malissen. 1992. Regulation of TCR α and β gene allelic exclusion during T-cell development. *Immunol. Today* **13**:315–322.
 32. Maryanski, J. L., C. V. Jongeneel, P. Bucher, J. L. Casanova, and P. R. Walker. 1996. Single-cell PCR analysis of TCR repertoires selected by antigen in vivo: a high magnitude CD8 response is comprised of very few clones. *Immunity* **4**:47–55.
 33. McLaughlin-Taylor, E., H. Pande, S. J. Forman, B. Tanamachi, C. R. Li, J. A. Zaia, P. D. Greenberg, and S. R. Riddell. 1994. Identification of the major late human cytomegalovirus matrix protein pp65 as a target antigen for CD8⁺ virus-specific cytotoxic T lymphocytes. *J. Med. Virol.* **43**:103–110.
 34. McLaughlin-Taylor, E., D. E. Willey, E. M. Cantin, R. Eberle, B. Moss, and H. A. Openshaw. 1988. A recombinant vaccinia virus expressing herpes simplex virus type 1 glycoprotein B induces cytotoxic T lymphocytes in mice. *J. Gen. Virol.* **69**:1731–1734.
 35. McMichael, A. J., and R. E. Phillips. 1997. Escape of human immunodeficiency virus from immune control. *Annu. Rev. Immunol.* **15**:271–296.
 36. Morrison, L. A., and D. M. Knipe. 1994. Immunization with replication-defective mutants of herpes simplex virus type 1—sites of immune intervention in pathogenesis of challenge virus infection. *J. Virol.* **68**:689–696.
 37. Morrison, L. A., and D. M. Knipe. 1996. Mechanisms of immunization with a replication-defective mutant of herpes simplex virus 1. *Virology* **220**:402–413.
 38. Moskophidis, D., and R. M. Zinkernagel. 1995. Immunobiology of cytotoxic T-cell escape mutants of lymphocytic choriomeningitis virus. *J. Virol.* **69**:2187–2193.
 39. Murali-Krishna, K., J. D. Altman, M. Suresh, D. J. Sourdive, A. J. Zajac, J. D. Miller, J. Slansky, and R. Ahmed. 1998. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunology* **8**:177–187.
 40. Mylin, L. M., R. H. Bonneau, J. D. Lippolis, and S. S. Tevethia. 1995. Hierarchy among multiple H-2^d-restricted cytotoxic T-lymphocyte epitopes within simian virus 40 T antigen. *J. Virol.* **69**:6665–6677.
 41. Niedermann, G., S. Butz, H. G. Ihlenfeldt, R. Grimm, M. Lucchiari, H. Hoshutzky, G. Jung, B. Maier, and K. Eichmann. 1995. Contribution of proteasome-mediated proteolysis to the hierarchy of epitopes presented by major histocompatibility complex class I molecules. *Immunity* **2**:289–299.
 42. Niewiesk, S., S. Daenke, C. E. Parker, G. Taylor, J. Weber, S. Nightingale, and C. R. Bangham. 1995. Naturally occurring variants of human T-cell leukemia virus type I Tax protein impair its recognition by cytotoxic T lymphocytes and the transactivation function of Tax. *J. Virol.* **69**:2649–2653.
 43. Nowak, M. A., R. M. May, R. E. Phillips, S. Rowland-Jones, D. G. Lalloo, S. McAdam, P. Klenerman, B. Koppe, K. Sigmund, C. R. M. Bangham, and A. J. McMichael. 1995. Antigenic oscillations and shifting immunodominance in HIV-1 infections. *Nature* **375**:606–611.
 44. Nugent, C. T., J. M. McNally, R. Chervenak, R. M. Wolcott, and S. R. Jennings. 1995. Differences in the recognition of CTL epitopes during primary and secondary responses to herpes simplex virus infection in vivo. *Cell. Immunol.* **165**:55–64.
 45. Nugent, C. T., R. M. Wolcott, R. Chervenak, and S. R. Jennings. 1994. Analysis of the cytolytic T-lymphocyte response to herpes simplex virus type 1 glycoprotein B during primary and secondary infection. *J. Virol.* **68**:7644–7648.
 46. Rodewald, H. R., U. H. Koszinowski, K. Eichmann, and I. Melchers. 1989. Predominant utilization of V β 8⁺ T cell receptor genes in the H-2L^d-restricted cytotoxic T cell response against the immediate-early protein pp89 of the murine cytomegalovirus. *J. Immunol.* **143**:4238–4243.
 47. Roizman, B., and A. E. Sears. 1993. Herpes simplex viruses and their replication, p. 11–68. *In* B. Roizman et al. (ed.), *Human herpesviruses: biology, pathogenesis, and treatment*. Raven Press, New York, N.Y.
 48. Salvucci, L. A., R. H. Bonneau, and S. S. Tevethia. 1995. Polymorphism within the herpes simplex virus (HSV) ribonucleotide reductase large subunit (ICP6) confers type specificity for recognition by HSV type 1-specific cytotoxic T lymphocytes. *J. Virol.* **69**:1122–1131.
 49. Sercarz, E. E., P. V. Lehmann, A. Ametani, G. Benichou, A. Miller, and K. Moudgil. 1993. Dominance and crypticity of T-cell antigenic determinants. *Annu. Rev. Immunol.* **11**:729–766.
 50. Sette, A., A. Vitiello, B. Reherman, P. Fowler, R. Nayarsina, W. M. Kast, C. J. Melief, C. Oseroff, L. Yuan, J. Ruppert, et al. 1994. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. *J. Immunol.* **153**:5586–5592.
 51. Sourdive, D. J. D., K. Murali-Krishna, J. D. Altman, A. J. Zajac, J. K. Whitmire, C. Pannettier, P. Kourilsky, B. Evavold, A. Sette, and R. Ahmed. 1998. Conserved T cell receptor repertoire in primary and memory CD8 T cell responses to an acute viral infection. *J. Exp. Med.* **188**:71–82.
 52. Staerz, U. D., H.-G. Rammensee, J. D. Benedetto, and M. J. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* **134**:3994–4000.
 53. Turner, S. J., S. C. Cose, and F. R. Carbone. 1996. TCR α -chain usage can determine antigen-selected TCR β -chain repertoire diversity. *J. Immunol.* **157**:4979–4985.
 54. van der Most, R. G., A. Sette, C. Oseroff, J. Alexander, K. Murali-Krishna, L. L. Lau, S. Southwood, J. Sidney, R. W. Chesnut, M. Matloubian, and R. Ahmed. 1996. Analysis of cytotoxic T cell responses to dominant and subdominant epitopes during acute and chronic lymphocytic choriomeningitis virus infection. *J. Immunol.* **157**:5543–5554.
 55. Vitiello, A., L. L. Yuan, R. W. Chesnut, J. Sidney, S. Southwood, P. Farness, M. R. Jackson, P. A. Peterson, and A. Sette. 1996. Immunodominance analysis of CTL responses to influenza PR8 virus reveals two new dominant and subdominant K^b-restricted epitopes. *J. Immunol.* **157**:5555–5562.
 56. Whitton, J. L., A. Tishon, H. Lewicki, J. Gebhard, T. Cook, M. Salvato, E. Joly, and M. B. Oldstone. 1989. Molecular analyses of a five-amino-acid cytotoxic T-lymphocyte (CTL) epitope: an immunodominant region which induces nonreciprocal CTL cross-reactivity. *J. Virol.* **63**:4303–4310.
 57. Witmer, L. A., K. L. Rosenthal, F. L. Graham, H. M. Friedman, A. Yee, and D. C. Johnson. 1990. Cytotoxic T lymphocytes specific for herpes simplex virus (HSV) studied using adenovirus vectors expressing HSV glycoproteins. *J. Gen. Virol.* **71**:387–396.
 58. Yewdell, J. W., and J. R. Bennink. 1999. Immunodominance in MHC class I restricted T lymphocyte responses. *Annu. Rev. Immunol.* **17**:1–30.