Materials and methods

Animals, viral challenge studies and in vivo antibody treatment. C57BL/6 (B6, NCI, Frederick, MD) and B6.C-H-2^{bm8} (bm8, bred in our laboratory – at MSKCC or at OHSU, from the stock obtained from Dr. J. Sprent, The Scripps Research Institute, La Jolla, CA in 1990; originally from Jackson Labs, Bar Harbor, ME) mice, 6-12 wk of age, were maintained under SPF conditions and used throughout the experiments. Age-, sex- and weight-matched mice were challenged with indicated doses of HVH-1, strain 17. Mice were monitored daily and those exhibiting paralysis and inability to take food and water were euthanized, typically 7-10 days post-infection. All animals surviving this period without severe paralysis remained disease-free for at least 100 days. Results are shown as % survival and significance evaluated by Fischer's exact test.

Ablation of CD8α or β⁺ and Vβ8⁺ T cells was achieved by two i.p. injections of the anti-CD8α, anti-CD8β, anti-NK1.1 and anti-Vβ8 mAb 53.6.7, 53.5.8, PK136 and F23.1 (100-200μg/recipient, all produced in our laboratory in the form of bioreactor fluid), respectively, on days 7 and 3 prior to viral challenge (normal rat IgG or irrelevant mAb 19E12 was used as control). Control experiments showed that such treatment abrogated all CD8 functions (for anti-CD8γ⁻ or CD8β⁻-specific reagents), induced lack of toxicity against the K562 erythroleukemia cell line (for anti-NK1.1) and reduced proliferation of Vβ8⁺ cells by >95%, and abrogated generation of alloreactive Vβ8⁺ CTLs (for anti-Vβ8 treatment). For Vβ8 depletion, net corrected proliferation was: control mice, stimulation with anti-Vβ8 – B6 - \(26,349 ± 3,425\) c.p.m., bm8 – \(29,339± 4,288\); anti-CD3, B6 - 80,133±6,299, bm8 -89,643±8,239; in mice depleted of T-cells bearing Vβ8, anti-Vβ8 152±88 c.p.m., anti-CD3 76,842±5,246. Furthermore, the pattern of Vβ
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utilization in the 5-day MLR using splenocytes from depleted or non-depleted B6 and bm8 animals responding against irradiated BALB/c cells was comparable, except for the lack of Vβ8 response in depleted mice.

**CTL cultures, 51Cr-release assays, “cold” target inhibition assays and in vivo CTL transfers**. Virus immunization, restimulation, and ⁵¹Cr-release assay were performed as described (S1). For “cold” target inhibition, CTL derived against the whole virus were plated with virus-infected ⁵¹Cr-labelled targets (hot targets), in the presence or the absence of unlabeled control or HSV-8p peptide-coated targets, at a constant (36:1) effector/hot target ratio. As seen in Fig. 1C, lysis of HVH-1-infected targets by both types of HVH-1-specific CTLs was inhibited by >93% by the presence of the excess HSV-8p-coated, but not HSV-8p-negative, syngeneic “cold” targets. Unless indicated otherwise, LPS/ConA blasts were used as targets. These cells were used as is or were coated with indicated peptide concentrations (1 μM if not indicated otherwise) and incubated with syngeneic B6 and bm8 CTL populations in a standard ⁵¹Cr-release assay.

**Flow cytfluorometry and pMHC tetramer assays.** Antibodies were purchased from PharMingen (San Diego, CA). HSV-8p:H-2Kb tetramer encoding plasmids were provided by Dr. J. Altman (Emory University, Atlanta, GA) via Drs. S.C. Jameson (Univ. Of Minnesota, Minneapolis, MN) and tetramers prepared as described (S2). Day 7 poststimulation or day 5-6 post-infection CTLs were stained with antiVβ-FL (fluorescein) and αCD8-PE mAb, or with antiVβ-FL, Tetramer-PE and CD8-CyChrome. Staining with Kβ-tetramers bearing irrelevant peptides was always <0.2%. FACS analysis was performed as described (S1) and TCRVβ utilization normalized to the % of TCRqβ⁺CD8⁺ cells (>90% in all samples). Significance was determined by paired Student's T test.
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Tetramer association (Fig. S4) and tetramer decay assay (Fig. 4A) (TCR:pMHC “on-rate” and $t_{1/2}$ or "off-rate" determinations) were performed as described (S3,S4), respectively, with the exception that the off-rate assay was performed at 25°C and in the presence of saturating amounts of an anti-K$^b$ mAb, Y3. The above two studies (S3,S4) also showed that the results of these assays closely correspond to the K$_{on}$ and K$_{off}$ rates, respectively, of the interaction between the soluble TCR and immobilized pMHC molecules. In the course of both assays, B6 and bm8 CTLs displayed superimposable TCR and CD8 levels (<5% difference at any time point), indicating that the obtained results were due to differential TCR avidity and not levels of TCR or CD8 expression.

**CDR3 length analysis.** CDR3β length analysis was performed by a modified method of (S5), as described (S6).

**Supporting Text**

**Genotyping of F$_2$ animals.** F$_2$ mice were genotyped by PCR as follows. Genomic DNA was amplified in two separate PCR reactions using the following set of primers K$^b$ 5’: GAG CCC CGG TAC ATG GAA; K$^{bm8}$ 5’: GAG CCC CGG TTC ATC TCT; and K$^{b/bm8}$ 3’: CC CTT GCT CTG GTT GTA GTA GCC. Products were resolved on 1.5% agarose gel and visualized using a BioRad Fluor-S Multimager (BioRad, ). Animals segregated into the three genotypes at the expected mendelian 1:2:1 ratios.

**On depletion of CD8 T-cells using different mAbs.** Experiments shown in Fig. S1A document that both B6 and bm8 mice are rendered similarly susceptible to a low dose of HVH-1 after anti-CD8α mAb treatment. However, virtually identical results were obtained when an anti-CD8β mAb (53.5.8) was used for depletion, demonstrating that differential susceptibility to HVH-1 segregates with the CD8∀∃+ T-cells (and not, perhaps, with the CD8∀∀+ dendritic cells).
Ruling out negative selection/tolerance by K\textsuperscript{b} as a possible reason for higher susceptibility of B6 mice. It was possible that protective T-cells with broad TCR repertoire were present in both strains, but were eliminated or functionally silenced via negative selection by K\textsuperscript{b}, thus rendering B6 mice more susceptible to infection. However, deletion/silencing of T-cells by tolerizing MHC is a dominant trait (S7). Therefore, if negative selection (deletion or nondeletional tolerance) of the protective TCR repertoire by K\textsuperscript{b} were the cause for lower resistance of B6 mice, then in F\textsubscript{1} and F\textsubscript{2} hybrids and in F\textsubscript{1}→P bone marrow irradiation chimeric animals even a single copy of K\textsuperscript{b} should render the animal susceptible to HVH-1. By contrast, in F\textsubscript{1} hybrids and the F\textsubscript{1}→P bone marrow irradiation chimera (S8) as well as in F\textsubscript{2} animals (Fig. 1B of the main paper), the presence of K\textsuperscript{b}m8 dominantly conferred protection regardless of the presence of K\textsuperscript{b}. This rules out negative selection and, is consistent with positive selection effects (see S8 for additional data on that topic).

Supporting evidence for the extrapolation of the frequencies of HSV-8p:MHC-specific CD8 T-cells in bm8 mice. Three independent lines of evidence indicate that amongst the HSV-8p-specific bm8 CTL there are about 2/3 crossreactive, HSV-8p:K\textsuperscript{b}–reactive and about 1/3 of HSV-8p:K\textsuperscript{b}m8-monospecific cells: (i) analysis of over 80 CTL clones isolated from these animals (18); (ii) cold-target inhibition data using bulk CTL lines (9); and tetramer staining from Fig. S2. While in each case measurements were made on in vitro expanded cells, the fact that all these cells were generated using the whole virus and syngeneic (bm8) cells, and that those from (ii) and (iii) were minimally manipulated, makes it unlikely that the repertoire of cells during infection ex vivo would behave much differently. We thus feel justified in our claim that there are no major differences in frequencies of CTL mobilized in the face of lethal infection.
Supporting evidence against differential K\textsuperscript{b} and K\textsuperscript{bm8} trafficking/synthesis. To rule out the possibility that the two MHC molecules are synthesized at different rates, we measured re-expression of K\textsuperscript{b} and K\textsuperscript{bm8} molecules at the cell surface after peptide stripping using brief mild acid treatment (pH 4.5). Such treatment reduced surface MHC expression by ~ 60-70% in both cases. Moreover, under these conditions, we saw no difference in the kinetics of their re-expression at the cell surface.

Note on the issue of increased avidity during primary responses. Several groups have shown that CTL avidity increases in the secondary immune response (S3,S4). It may be of interest to test whether the avidity differences between B6 and bm8 CTL would change in the secondary response. This notwithstanding, our data highlight the importance of avidity in the primary infection.
SUPPLEMENTAL FIGURES:

A. Effects of CD8 T cell depletion on HVH-1 susceptibility of B6 and bm8 mice. B6 and bm8 mice were left untreated (open histograms) or were depleted of CD8 T cells using mAb 53.6.7. Both B6 and bm8 mice exhibited 100% mortality at 5 x 10⁶ pfu (9/9 and 11/11 mice, respectively). Note that untreated CD8⁻/⁻ F₁ animals used in Fig. 1D of the main paper were more resistant to HVH-1 than CD8-depleted B6 animals used in this figure, likely due to the adaptive mechanisms compensating for the genetic defect in CD8 expression.

B. Effects of NK cell depletion on HVH-1 susceptibility of B6 and bm8 mice. B6 (left panels) and bm8 mice (right panels) were left untreated (solid histograms) or were depleted of NK cells (striped histograms) and were infected with 5 and 30 x 10⁶ p.f.u. HVH-1. % Animal survival is shown as bar graphs for 15 animals/group pooled from three experiments. Note that at 30 x 10⁶ p.f.u. occasional bm8 mice may succumb to infection regardless of treatment. There were no significant differences in survival between treated and untreated groups.
Figure S2. HSV-8p:Kb tetramers detect ~2/3 bm8 CTLs specific for HSV-8p. B6 and bm8 CTLs were generated against HSV-8p from lethally infected animals by one in vitro restimulation with peptide-coated syngeneic splenocytes and were analyzed on day 5 after restimulation. Cells were stained with CD8-specific mAb and the HSV-8p:Kb tetramer. A typical example of one set of comparable cell lines (out of five) is shown.
Figure S3. TCR Vβ repertoire usage in response to Influenza (control) and HVH-1 infection.  
A. B6 and bm8 TCR repertoires against a H-2D-restricted antigen are comparable.  
B6 and bm8 mice were immunized with the Influenza virus A/PR8/34 and CTLs expanded using  
the immunodominant Db-restricted peptide ASNENMETM. Vβ repertoire was analyzed as in ref.  
9, and revealed no statistically significant difference (by Student’s paired t test) in Vβ utilization  
between B6 and bm8 CTLs.  Results shown are from 5 mice/group (± S.D.), representative  
of two experiments.  
B. B6 and bm8 TCR repertoires against HSV-8p are significantly
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different. The samples from experiment shown in Fig. 2. were analyzed for TCRVβ expression
among the CD8^+HSV-8p:K^b^+ cells, gated as in Fig. 2B. and are representative of three experiments.
Figure S4. On-rates of TCR:pMHC association of B6 and bm8 CTL. Ex vivo staining of B6 (circles) and bm8 (triangles) CTLs with increasing concentration of HSV-8p:Kb tetramers. Counter-staining for the expression of CD8 was used to gate on CD8+ cells. Staining was performed on ice for 45' following extensive washing and FCM analysis.
SUPPORTING REFERENCES


