

# Direct Link Between *mhc* Polymorphism, T Cell Avidity, and Diversity in Immune Defense

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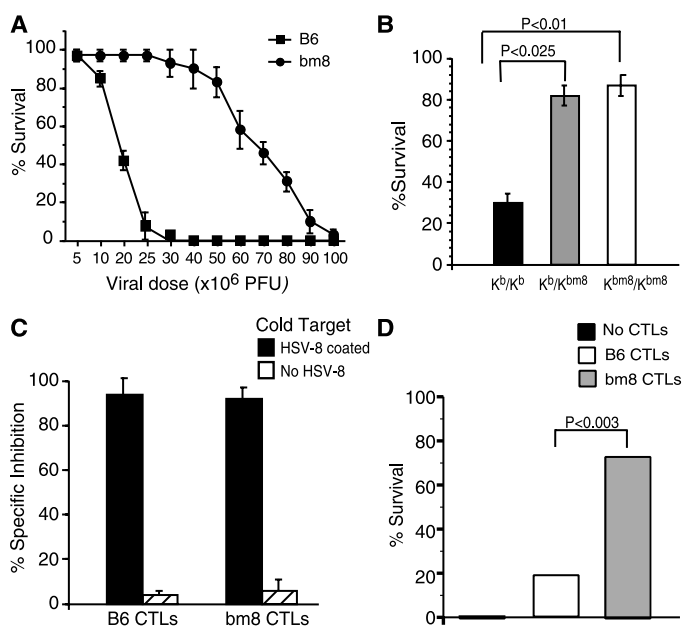
Major histocompatibility complex (*mhc*)-encoded molecules govern immune responses by presenting antigenic peptides to T cells. The extensive polymorphism of genes encoding these molecules is believed to enhance immune defense by broadening the array of antigenic peptides available for T cell recognition, but direct evidence supporting the importance of this mechanism in combating pathogens is limited. Here we link *mhc* polymorphism-driven diversification of the cytotoxic T lymphocyte (CTL) repertoire to the generation of high-avidity, protective antiviral T cells and to superior antiviral defense. Thus, much of the beneficial effect of the *mhc* polymorphism in immune defense may be due to its critical influence on the properties of the selected CTL repertoire.

Major histocompatibility complex (*mhc*)-encoded class I molecules present cytosolically processed peptides to CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs) (1). The same class I molecules shape the repertoire of CD8<sup>+</sup> CTLs through positive and negative selection of immature precursors in the thymus (2). Mammalian *mhc* class I genes are very highly polymorphic (3), and this polymorphism is believed to have arisen in response to the evolutionary pressures generated by encounters with pathogens such as intracellular bacteria and viruses (4). At the population level, a polymorphism at the *mhc* locus theoretically would ensure that, even if a proportion of individuals succumbed to a new pathogen, others, bearing distinct MHC molecules, would be potentially resistant by virtue of efficacious antipathogen CTL responses. The prevalent mechanism by which *mhc* polymorphism is considered to contribute to immune defense is by binding a broad array of antigenic determinants that are presented to T cells (1, 3–6). However, mammalian susceptibility or resistance to infectious diseases has rarely been directly and unambiguously linked to the *mhc* haplotype (5, 6). Similarly, T cell receptor (TCR) diversity, which is a direct result of MHC selection, has not been linked specifically to infectious disease susceptibility.

We sought to determine how a specific *mhc* polymorphism might directly influence

class I-restricted T cell-mediated immune defense. A gene conversion-mediated *mhc* class I polymorphism separates the coisogenic mouse strains C57BL/6 (B6; *mhc* haplotype H-2<sup>b</sup>) and B6.C-H-2<sup>bm8</sup> (bm8; *mhc* H-2<sup>bm8</sup>) (7), which differ from each other by only four amino acid substitutions (Y22→F, M23→I, E24→S, and D30→N) (7, 8) located on the floor of the H-2K peptide binding

**Fig. 1.** Relationship between HVH-1 resistance, *mhc* haplotype, and CTL immunity in B6 and bm8 mice. (A) HVH-1 resistance in B6 and bm8 mice. Mice were infected intraperitoneally by the indicated doses of live HVH-1, strain 17 (13). Percent survival after 100 days is shown as mean ± SD, pooled from >10 experiments (10 to 60 mice per point; three experiments per point). (B) Segregation of HVH-1 resistance with the H-2K locus. (B6 × bm8)<sub>F2</sub> mice were divided according to H-2K genotype and were challenged with 25 × 10<sup>6</sup> PFU of HVH-1. Survival and statistical significance (13) are shown (*n* = 15 mice per haplotype), pooled from two experiments. (C) HSV-8p immunodominance in B6 and bm8 mice. B6 and bm8 HVH-1-specific CTL lines, derived by restimulation on virally infected cells (13), were used to test HSV-8p immunodominance in a cold target inhibition assay (13). Lysis of HVH-1-infected targets by both types of HVH-1-specific CTL was specifically inhibited (>93%) by the excess HSV-8p-coated syngeneic cold targets, indicating similar HSV-8p dominance in both strains. (D) Protection by adoptively transferred B6 and bm8 CTLs. CD8<sup>-/-</sup> (B6 × bm8)<sub>F1</sub> recipients received no cells (black bar) or 1 × 10<sup>6</sup> HSV-8p-specific CD8<sup>+</sup> CTLs of B6 (open bar) or bm8 (gray bar) origin intravenously and were infected with a HVH-1 dose lethal for unmanipulated animals (15 × 10<sup>6</sup> PFU). Percent survival (*n* = 22 per group; three experiments) is shown (*P* value is for B6 versus bm8 CTL transfer) (13).



site (9). The resulting H-2K<sup>b</sup> and H-2K<sup>bm8</sup> molecules are identical at the TCR-contacting residues (7, 9) but differ in their ability to present some peptides and/or to enable certain T cell responses. One of these peptides, HSV-8p, is the immunodominant determinant derived from the glycoprotein B (gB) of the *Herpesvirus hominis* type 1 (HVH-1; herpes simplex virus type 1) (10).

To examine the influence of the H-2K<sup>b</sup>/K<sup>bm8</sup> polymorphism on the defense against HVH-1, we challenged B6 and bm8 mice by increasing doses of HVH-1. The 50% lethal doses for B6 and bm8 mice were 15 × 10<sup>6</sup> and 65 × 10<sup>6</sup> plaque-forming units (PFU), respectively (Fig. 1A). Thus, bm8 mice were four to five times more resistant than were B6 mice to HVH-1. Immunity to HVH-1 is known to be mediated by lymphocytes and by the components of the innate immune system, including cytokines and natural killer (NK) cells (11, 12). However, because B6 and bm8 mice are coisogenic at the H-2K locus, differences would be expected to relate to class I-dependent adaptive immune response rather than to innate immunity by the animals. This was confirmed by the fact that the difference in resistance between bm8 and B6 mice was abrogated upon depletion of CD8 T cells with monoclonal antibodies (mAb) directed against CD8α or CD8β chains (fig. S1) (13). Depletion of NK cells did not affect

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## REPORTS

differences between B6 and bm8 mice (fig. S1), further emphasizing the critical role of CD8 T cells in mediating the observed differences. Finally, we generated cohorts of (B6 × bm8) $F_2$  mice that were divided into H-2K<sup>b/b</sup>, H-2K<sup>b/bm8</sup>, and H-2K<sup>bm8/bm8</sup> genotype groups (13) and challenged with the HVH-1 dose known to kill about 80% of B6 and about 10% of bm8 mice (Fig. 1A). We reasoned that if the non-*mhc* genes played a role in bm8 resistance, one would expect similar survival rates in each of the groups, regardless of the *mhc* haplotype, because of random segregation of non-*mhc* genes. In contrast, a single copy of H-2K<sup>bm8</sup> conferred higher HVH-1 resistance to  $F_2$  hybrids, which was indistinguishable from that in bm8 mice (Fig. 1B).

Up to 90% of the B6 CD8<sup>+</sup> CTL response against HVH-1 is directed against the immunodominant H-2K<sup>b</sup>-restricted gB<sub>498–505</sub> peptide SSIEFARL (HSV-8p) (8, 14–17). This peptide is also overwhelmingly immunodominant in bm8 mice, because lysis of HVH-1-infected targets by HVH-1-specific bm8 CTLs was nearly completely (>93%) inhibited by the presence of HSV-8p-coated syngeneic “cold” (unlabeled) targets, as is also observed for B6 CTLs (Fig. 1C). This finding ruled out the possibility that H-2K<sup>bm8</sup> may present alternative HVH-1 determinants, not presented by H-2K<sup>b</sup>, and allowed us to focus our analysis on this immunodominant *in vivo*-protective peptide (14, 15).

We first performed CTL transfer experiments to directly determine whether the origin of HSV-8p-specific CTLs dictates improved resistance of bm8 mice. We trans-

ferred HSV-8p-specific CTLs from B6 and bm8 mice (13) intravenously into CD8-deficient (B6 × bm8) $F_1$  mice that were simultaneously infected with a supralethal dose of HVH-1. Transfer of B6 HSV-8p-specific CTLs provided little additional protection against HVH-1 (~18%), compared with bm8 CTLs, which conferred a very high degree of protection (~73%) (Fig. 1D). Thus, a strong monoallelic association could be detected between H-2K<sup>bm8</sup>, CD8<sup>+</sup> CTL-mediated immunity against HSV-8p and increased protection against HVH-1.

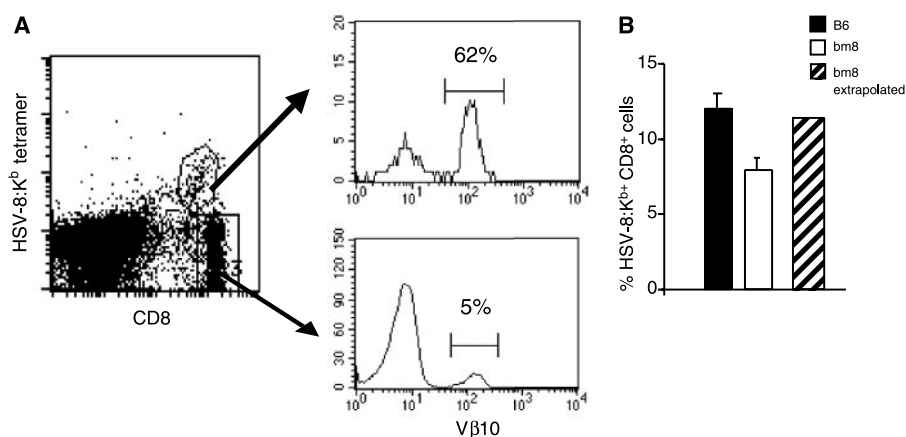
The above *mhc*-linked differences in CTL-mediated HVH-1 resistance could be explained by differential binding of the viral peptide to MHC proteins or by differential TCR recognition of pMHC complexes generated by positive or negative intrathymic selection of a distinct TCR repertoire by H-2K<sup>bm8</sup> and H-2K<sup>b</sup>. We ruled out differential binding of HSV-8p to H-2K<sup>b</sup> and H-2K<sup>bm8</sup>, because the kinetics of HSV-8p binding to the two molecules was indistinguishable (18). Moreover, we discarded the possibility that H-2K<sup>b</sup>-mediated negative selection of the TCR repertoire interferes with resistance, because negative selection should confer dominant nonresponsiveness on  $F_1$ ,  $F_2$ , and  $F_1 \rightarrow P$  bone marrow irradiation chimeric animals, which was not experimentally observed (10, 13).

We recently demonstrated that during intrathymic development, H-2K<sup>bm8</sup> positively selects a CD8 T cell population exhibiting a broader HSV-8p-specific TCR repertoire than H-2K<sup>b</sup> (10). This prompted us to test the impact of repertoire selection on antiviral

defense through the quality and/or quantity of HSV-8p-specific CTL responses. Five days after lethal infection with HVH-1, *ex vivo* enumeration of HSV-8p:H-2K<sup>b</sup>-specific CD8<sup>+</sup> T cells with pMHC tetramers revealed that B6 mice mobilized one-third more HSV-8p-specific CD8<sup>+</sup> T cells than were found in bm8 animals (Fig. 2). Clonal analysis (19), cold target inhibition studies (10), and tetramer staining of CD8 cells (fig. S2) had already suggested that at least two-thirds of all HSV-8p-specific bm8 CTLs cross-recognize HSV-8p:H-2K<sup>b</sup> (and are therefore detected by HSV-8p:H-2K<sup>b</sup> tetramers). The maximal total numbers of HSV-8p-specific CD8<sup>+</sup> cells in bm8 mice that recognize the HSV-8p:H-2K<sup>bm8</sup> complex, therefore, can be extrapolated to 11.5%, which is comparable to the values found in B6 mice (Fig. 2B). Thus, quantitative differences in numbers of mobilized CTL precursors between these coisogenic strains cannot be responsible for the stronger bm8 resistance found in our experiments.

To investigate the nature of putative qualitative differences in CTL responses, we examined the TCR repertoires of HSV-8p-specific B6 and bm8 CTLs, which differ from one another. The B6 response is relatively narrow, dominated by usage of TCRs bearing only two of the possible 20 TCR V $\beta$  chains—V $\beta$ 10 (60 to 70%) and V $\beta$ 8 (15 to 25%)—whereas bm8 CTLs use these two as well as five other V $\beta$  families (fig. S3) (10, 20, 21). Moreover, even within the shared V $\beta$  families, bm8 HSV-8p-specific CTLs exhibited greater TCR diversity at the molecular level, as measured by CDR3 length distribution (13) (Fig. 3A). CDR3 segments are the most polymorphic parts of TCR chains, differing by length and sequence between TCRs, and their diversity reflects the complexity of T cell populations. Naïve mice exhibit Gaussian distribution of CDR3 length frequencies within each TCR V $\beta$  family, with an average of eight CDR3 $\beta$  length peaks spaced by single codons (13) (Fig. 3A). In polyclonal immune responses, the diverse pattern is maintained, as seen in V $\beta$ 8 CDR3 profiles of the HSV-8p-specific CTLs from B6 and bm8 mice (Fig. 3A). But if the response is oligoclonal, the Gaussian distribution is lost and individual peaks dominate. This type of restricted CDR3 pattern was always present among the HSV-8p-specific V $\beta$ 10<sup>+</sup> B6, but not bm8, CTLs (Fig. 3A). Thus, V $\beta$ 8<sup>+</sup> CD8 cells provide significant molecular TCR diversity to B6 mice (Fig. 3A) because their V $\beta$ 10 CTLs are oligoclonal.

This allowed us to directly examine the role of TCR diversity in HVH-1 resistance. Elimination of the V $\beta$ 8<sup>+</sup> T cells should reduce the numbers of HSV-8p-specific T cells comparably in B6 and bm8 mice (in both strains, these cells make up 15 to 25% of the



**Fig. 2.** *Ex vivo* frequencies of B6 and bm8 CD8 T cells responding to lethal HVH-1 infection. B6 and bm8 mice were lethally infected ( $25 \times 10^6$  and  $100 \times 10^6$  PFU per mouse, respectively) with HVH-1; splenocytes were stained 6 days later with antibody to CD8, HSV-8p:H-2K<sup>b</sup>-tetramer, and V $\beta$ -specific mAbs and analyzed by flow cytometry (FCM). (A) Three-color FCM shows the CD8/tetramer profile of a B6 mouse (representative of five analyzed) used to analyze precursor frequencies [see (B)] and V $\beta$  expression (fig. S3) among the CD8<sup>+</sup>HSV-8p:H-2K<sup>b</sup><sup>+</sup> or the CD8<sup>+</sup>HSV-8p:H-2K<sup>b</sup><sup>-</sup> cells (specific enrichment of percent TCR V $\beta$ 10 expression among tetramer-positive cells verifies the specificity of tetramers). (B) Percent splenic HSV-8p:H-2K<sup>b</sup>-specific CD8 cells mobilized by B6 and bm8 mice in response to lethal HVH-1 infection. Staining was as in (A), with percent Tet<sup>+</sup>CD8<sup>+</sup> cells shown (eight mice per group; mean  $\pm$  SD; two experiments). Extrapolation for the bm8 mice (hatched bar) is explained in the text and in (13).

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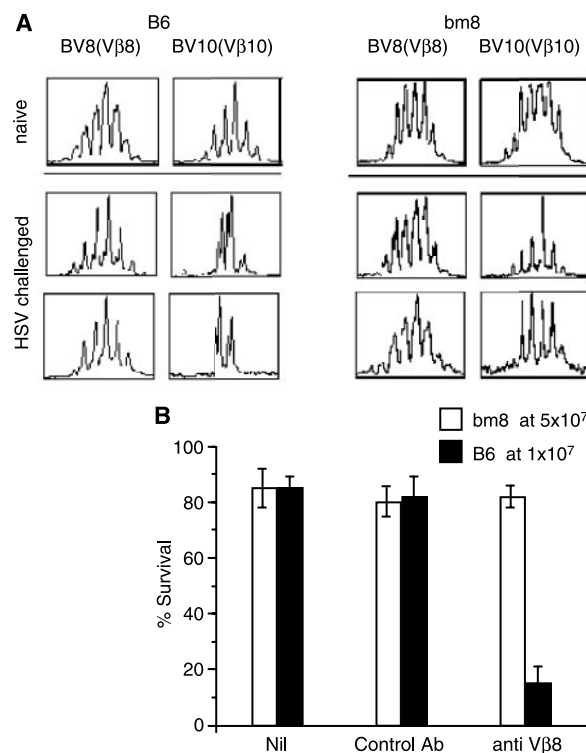
total response against HSV-8p) (fig. S3) (10) but should reduce the already more limited HSV-8p-specific TCR diversity severely in B6 mice and less so in bm8 animals. If the numbers of HSV-8p-specific CTLs are the key to an efficient defense, then the resistance of both strains should be comparably compromised by this depletion. If, by contrast, TCR diversity plays a role, B6, but not bm8, mice should become less resistant. Mice of both strains were depleted of V $\beta$ 8 cells (13). Upon challenge with HVH-1 doses sublethal for both strains, V $\beta$ 8-depleted B6 mice exhibited a striking 85% drop in survival, whereas V $\beta$ 8-depleted bm8 mice showed no difference in mortality (Fig. 3B), causally linking high HVH-1 resistance to a diverse (bm8) CD8 T cell repertoire. These data offer strong evidence for CTL diversity as an important parameter of resistance.

We next examined the qualitative characteristics of B6 and bm8 HSV-8p-specific TCRs by determining TCR:pMHC interaction kinetics with pMHC tetramer staining assays (13). We discovered that, although both CTL types expressed equivalent levels of CD8 and TCR expression (13), the bm8 CTLs exhibited at least three times better tetramer association (fig. S4) and about 20 times slower dissociation (Fig. 4A) rates than B6 CTLs, which suggests considerably higher bm8 TCR avidity for pMHC. Moreover, bm8 CTLs exhibited strikingly higher peptide sensitivity, requiring a  $10^4$ -fold lower HSV-8p concentration than B6 CTLs to achieve comparable levels of target cell lysis (Fig. 4B). bm8 CTLs also required much lower HSV-8p concentrations than did B6 CTLs to kill B6 targets (Fig. 4B). Thus, the difference in sensitivity is caused by the properties intrinsic to the CTL and not the target cell (such as differential peptide-MHC stability or resistance to lysis). We also found that bm8 CTLs were capable of killing infected EL-4 cells as early as 3 to 4 hours after infection, whereas B6 CTLs required 16 hours of infection to effect EL-4 lysis (Fig. 4C). This experiment further supported the previous observation that the differential TCR avidity, rather than differential HSV-8p processing and presentation by H-2K<sup>b</sup> and H-2K<sup>bm8</sup>, is responsible for differential lysis. HVH-1 is known to efficiently reduce class I antigen presentation via ICP47, a protein that disrupts TAP (transporter associated with peptide processing) function (22, 23). Therefore, the presence of high-avidity CTLs that efficiently recognize infected cells bearing very low viral peptide-MHC copy numbers is likely crucial for controlling HVH-1 infection by allowing prompt containment of viral spread early in primary infection via lytic (24) or nonlytic (25) mechanisms.

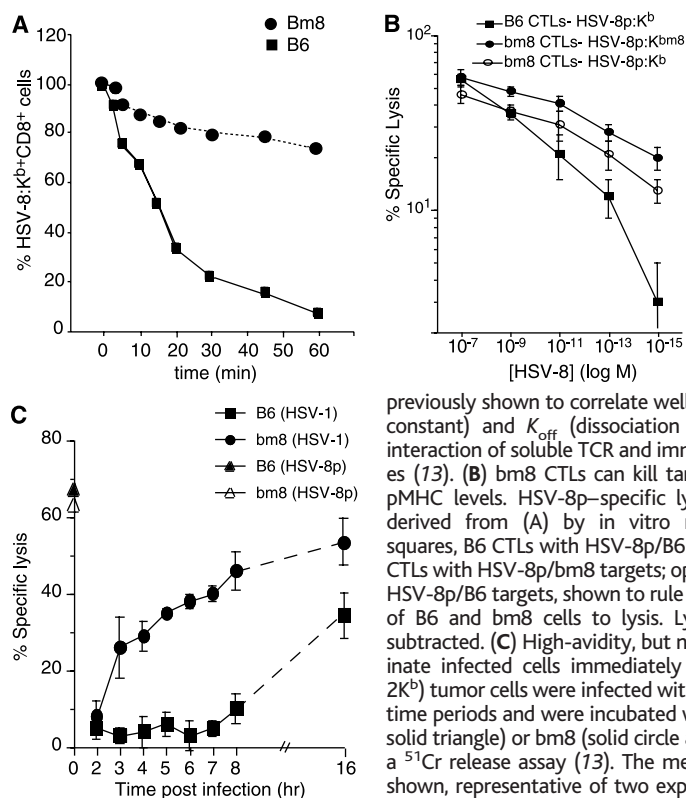
Our results demonstrate that bm8 mice with a diverse antiviral TCR repertoire, but

not B6 mice with a restricted antiviral TCR repertoire, can mobilize high-avidity, peptide-sensitive antiviral CTLs that lyse infected cells immediately after infection. It is believed that a diverse TCR repertoire enhances pathogen control by preventing microbial escape (26, 27), but this is unlikely to explain

our observations: HVH-1 mutates infrequently and is very unlikely to accumulate mutations within structural proteins (the HSV-8p-containing gB is essential for virus packaging) over the short time frame (7 to 10 days) that decides the outcome of infection. Rather, we suggest that the broad TCR repertoire



**Fig. 3.** Role of repertoire diversity in immune defense against HVH-1. (A) Restricted V $\beta$ 10 CDR3 utilization by B6 mice. TCR V $\beta$  CDR3 length analysis of HSV-8p-specific CTL lines derived from individual B6 and bm8 mice (20). Splenocytes of naive (top row) or virus-infected (bottom two rows) B6 or bm8 mice were analyzed, representative of 20 mice per strain over six experiments (see also fig. S3). BV refers to DNA and RNA products, and V $\beta$  refers to proteins. (B) Experimental reduction of HSV-8p-specific T cell diversity drastically reduces HVH-1 resistance. Forty mice per group (two experiments) were treated with experimental (anti-V $\beta$ 8) or control (13) mAbs and infected with an otherwise nonlethal dose of HSV-1 ( $1 \times 10^7$  and  $5 \times 10^7$  PFU per mouse for B6 and bm8 mice, respectively); survival was assessed as in Fig. 1.



**Fig. 4.** Diverse, but not restricted, TCR repertoire enables recruitment of high-avidity T cells. (A) TCR:pMHC off-rate (tetramer decay) determination was performed on B6 and bm8 splenic CD8 cells obtained from pools of five mice per group ex vivo 5 days after HVH-1 infection (13), representative of three experiments. Measurements shown in (A) and in fig. S4 were

previously shown to correlate well with the  $K_{on}$  (association constant) and  $K_{off}$  (dissociation constant) values of the interaction of soluble TCR and immobilized pMHC complexes (13). (B) bm8 CTLs can kill targets expressing very low pMHC levels. HSV-8p-specific lytic activity of CTL lines derived from (A) by in vitro restimulation (13). Solid squares, B6 CTLs with HSV-8p/B6 targets; solid circles, bm8 CTLs with HSV-8p/bm8 targets; open circles, bm8 CTLs with HSV-8p/B6 targets, shown to rule out differential sensitivity of B6 and bm8 cells to lysis. Lysis of control targets is subtracted. (C) High-avidity, but not low-avidity, CTLs eliminate infected cells immediately after infection. EL-4 (H-2K<sup>b</sup>) tumor cells were infected with HVH-1 for the indicated time periods and were incubated with B6 (solid squares and solid triangle) or bm8 (solid circle and open triangle) CTLs in a  $^{51}Cr$  release assay (13). The mean  $\pm$  SD ( $n = 3$  to 5) is shown, representative of two experiments.

## REPORTS

against a single epitope in bm8 mice provides a pool of precursors from which high-avidity CTLs can be selected. It is possible that diversity is not causally linked to recruitment of high-avidity CTLs and that it is simply easier for bm8 mice to generate high-affinity CTLs. However, we believe it more likely that high-avidity ("better fit") CTLs can be generated more readily from a diverse, broad repertoire produced by positive selection by H-2K<sup>bm8</sup> in the thymus (10).

Both CTL avidity and the speed of target cell elimination (28–31) are known to play a role in immune defense. To date, however, neither has been linked to *mhc* polymorphism or to CTL precursor diversity. A recent study suggested a role for high-avidity CD4 T cells in resistance to *Leishmania donovani* (32); however, deployment of high-avidity T cells was linked not to *mhc* polymorphism or TCR repertoire but rather to factors of innate immunity. To our knowledge, our data provide the first clear link between the *mhc* polymorphism and defense against pathogens and implicate both the increased T cell repertoire diversity and T cell avidity in this process. Contrasting the widely held view that binding of a broad array of a pathogen's peptides is the main mechanism by which *mhc* polymorphism contributes to immune defense, we show that variant MHC molecules significantly shape the immune defense by allowing selection of the best and most efficient precursors from a diverse TCR repertoire. Moreover, together with our previous work (10), these data highlight the role of intrathymic positive selection in generating a diverse T

cell repertoire that leads to protective immunity. This mechanism at least partly explains the advantage of MHC heterozygosity verified in experimental settings (10, 33) and could play a role in the recently observed advantage of HLA heterozygosity among long-term HIV-1 survivors (34).

### References and Notes

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### Supporting Online Material

[www.sciencemag.org/cgi/content/full/298/5599/1797/DC1](http://www.sciencemag.org/cgi/content/full/298/5599/1797/DC1)

Materials and Methods

Figs. S1 to S4

References

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