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Contemporary Analysis of MHC-Related Immunodominance Hierarchies in the CD8\(^+\) T Cell Response to Influenza A Viruses

Gabrielle T. Belz, Philip G. Stevenson,\(^2\) and Peter C. Doherty\(^3\)

Early studies of influenza virus-specific CD8\(^+\) T cell-mediated immunity indicated that the level of CTL activity associated with H2D\(^b\) is greatly diminished in mice that also express H2K\(^k\). Such MHC-related immunodominance hierarchies are of some interest, as they could lead to variable outcomes for peptide-based vaccination protocols in human populations. The influence of H2K\(^k\) on the H2D\(^b\)-restricted response profile has thus been looked at again using a contemporary, quantitative, IFN-\(\gamma\)-based flow cytometric assay. The depressive effect of H2K\(^k\) was very apparent for the influenza D\(^b\) PA 224 epitope and was also reproduced when CTL activity was measured for H2D\(^b\)-expressing targets pulsed with the immunodominant NP 366 peptide. The secondary H2K\(^k\) D\(^b\) effectors, while comparable H2K\(^d\) D\(^b\) targets following exposure to in vivo stimulated H2\(^b\) and D\(^b\) NP 366 -specific response was much greater in parental H2\(^b\) than in H2\(^b\times1\timesF\(_4\) mice, but the sizes of the CD8\(^+\) sets specific for K\(^b\)NP\(_{50}\) and D\(^b\)NP\(_{366}\) were essentially equivalent in the F\(_4\) animals. Thus, although the immunodominance profile associated with D\(^b\)NP\(_{366}\) is lost when H2K\(^k\) is also present, the response is still substantial. A further, MHC-related effect was also identified for the K\(^b\)NS1\(_{152}\) epitope, which was consistently associated with a greater CD8\(^+\)IFN-\(\gamma\)-response in H2K\(^d\)D\(^b\) recombinant than in (H2K\(^d\)\times K\(^d\)\times K\(^d\))F\(_1\) mice. The diminished D\(^b\)PA\(_{224}\) response in H2\(^b\times1\timesF\(_4\) mice was characterized by loss of a prominent V\(\beta\)7 TCR responder phenotype, supporting the idea that TCR deletion during ontogeny shapes the available repertoire. The overall conclusion is that these MHC-related immunodominance hierarchies are more subtle than the early CTL assays suggested and, although inherently unpredictable, are unlikely to cause a problem for peptide-based vaccine strategies. *The Journal of Immunology*, 2000, 165: 2404–2409.

Experiments conducted >20 yr ago uncovered what seemed to be a profound MHC-related regulatory effect for the H2D\(^b\)-restricted responses to the influenza A viruses and vaccinia virus (1, 2). Potent influenza and vaccinia virus-specific CD8\(^+\) CTL activity was consistently apparent for H2K\(^d\) D\(^b\) or H2K\(^d\)D\(^b\) targets following exposure to in vivo stimulated H2\(^b\) (K\(^d\)D\(^b\)) effectors, while comparable H2K\(^d\)D\(^b\) recombinant and H2\(^b\times1\timesF\(_4\) (K\(^d\)\times K\(^d\)) spleen populations were only minimally lytic for H2K\(^d\)D\(^b\) cells infected with the homologous virus. Influenza virus is a small, negative strand RNA virus, while vaccinia virus is a large DNA virus, so this is not likely to reflect some inherent quality of the viral pathogens concerned. The overall conclusion was that we were seeing some sort of immunodominance hierarchy, where a concurrent H2K\(^d\)-restricted response functioned to minimize that associated with H2D\(^b\) (1, 2).

Several different types of experiments were performed in efforts to take this observation further. Naive B10.A(4R) T cells (H2K\(^d\)D\(^b\)) were depleted of alloreactive potential by in vivo filtration through lethally irradiated K\(^d\)D\(^b\) mice, then stimulated with vaccinia virus in an additional set of irradiated K\(^d\)D\(^b\) recipients (3). Under these conditions the vaccinia-specific K\(^d\)D\(^b\) T cells showed potent, virus-specific, H2D\(^b\)-restricted CTL activity. Later limiting dilution analysis (LDA)\(^4\) to determine influenza-specific CD8\(^+\) T cell frequencies showed that significant H2D\(^b\)-restricted memory populations were generated in mice that also expressed H2K\(^k\) (4). Both approaches thus indicated that the concurrent presence of H2K\(^k\) throughout ontogeny did not greatly compromise the development of H2D\(^b\)-restricted CTL precursors (CTLP) specific for vaccinia virus, although there was an effect on the generation of CTL effectors in normal mice. The opposite conclusion was drawn from other experiments that analyzed response patterns for H2K\(^d\)D\(^b\) T cells from mice that had been neonatally tolerized to H2K\(^k\) (5). In this case the vaccinia-specific, H2D\(^b\)-restricted CTL response was profoundly diminished. The favored interpretation was that the presence of H2K\(^k\) during development resulted in the deletion of CD8\(^+\) T cells that could recognize vaccinia virus associated with H2D\(^b\). These studies were all performed a very long time ago, before we understood that the primary function of MHC glycoproteins is to present viral peptides to the TCR (6, 7). The assays used (CTL and LDA) were either minimally quantitative (CTL) or at an early stage of development and far from optimized (LDA). We did not know that distinct CD8\(^+\) T cell clones could be specific for different peptides from the same virus bound to the same MHC class I glycoprotein (7, 8). Even so, it is clearly important as we move

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\(^4\)Abbreviations used in this paper: LDA, limiting dilution analysis; i.n., intranasally; BAL, bronchoalveolar lavage; ELISPOT, enzyme-linked immunospot; MLN, mediastinal lymph node; H, influenza hemagglutinin; M, matrix protein; N, neuraminidase; PA, polymerase 2 protein; PEL, peritoneal exudate lymphocytes; NP, nucleoprotein; NS1, nonstructural protein; NS2, nuclear export protein.
to the use of peptide-based vaccines (9) that we understand whether such MHC-related immunodominance hierarchies are real and, if so, how they operate. The experiment reported here used short term peptide stimulation followed by staining for cytoplasmic IFN-γ (8, 10, 11) to look at the relationship between H2Kk and H2Dd (1, 2) for the influenza-specific CD8+ T cell response. This approach gives very similar numbers (8, 10, 11) to those detected by staining with tetrameric complexes of MHC class I glycoprotein plus peptide (tetramers). Tetramer reagents were not available for most of the epitopes analyzed in this study.

Materials and Methods

Mice and tissue sampling

Female C57BL/6J (B6, H2b), C3H/HeJ (C3H, H2a), B6 × C3HFeJ (B6C3F1, H2b × H2a), B10.BR (H2b), and B10.A(2R) (H2k × Dd) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). They were anesthetized at 8 wk of age by i.p. injection with avertin (2,2,2-tribromoethanol) and were challenged intranasally (i.n.) with 10^6.8 EID_{50} of the HKx31 influenza A virus (12). Memory mice for secondary challenge experiments were infected i.p. at least 6 wk previously with 10^3 EID_{50} of the PR8 influenza virus (11). At the time of sampling, the mice were anesthetized and exsanguinated from the axillary artery. Lymphocytes were obtained from the pneumonic lung by bronchoalveolar lavage (BAL), and adherent cells were removed by incubating them on plastic for 1 h at 37°C (12). Peritoneal exudate lymphocytes (PEL) were processed in the same way. Spleen and mediastinal lymph node (MLN) samples were disrupted and enriched for CD8+ T cells by incubation with mAbs (PharMingen, San Diego, CA) to CD8 (53-67.14) and MHC class II glycoprotein (M5/114.15.2), followed by anti-rat and anti-mouse Ig-coated magnetic beads (Dynal, Oslo, Norway).

Peptides, epitopes, and the Pepy assay

The influenza virus nucleoproteins ASNSNEMT (NP_{56-37}) and SDYGERL1 (NP_{50-39}), nonstructural protein EEGAIVGEI (NS1_{152-160}), nuclear export protein RTFSQLI (NS2_{142-151}) polymerase 2 SSLENPRAYV (PA_{223-233}), and matrix protein MGLIVYRM (M_{123-133}) used this study have all been described previously (6, 8, 13, 14). They were synthesized at purified by HPLC. The H2K or H2D epitopes associated with these peptides are DHNP_{56-66}, DP_{32-42}, KN_{52-60}, KN_{51-60}, KN_{51-62}, and KN_{51-62}. The Pepy assay (8, 10, 11) used splen MLN and BAL populations that were enriched for CD8+ T cells and cultured for 5 h in 96-well round-bottom plates (Costar, Corning, NY) at a concentration of 5–8 × 10^5 cells/well in 200 μl of RPMI 1640 medium containing 10% FCS, 10 μg/ml human rIL-2, and 5 μg/ml Brefeldin A (Epicentre Technologies, Madison, WI) in the presence or the absence of 1 μM viral peptide. The T cells were then washed and stained with anti-mouse CD8α (GK1.5) and MHC class II glycoprotein (M5/114.15.2), followed by anti-rat and anti-mouse Ig-coated magnetic beads (Dynal, Oslo, Norway).

ELISPOT assay

The numbers of memory T cells in the spleens of mice primed with the PR8 virus were determined by the IFN-γ ELISPOT assay (15, 16). Nitrocellulose-bottom 96-well plates (Millipore, Bedford, MA) were coated overnight at 4°C with rat anti-mouse IFN-γ Ab (clone R4-6A2 from PharMingen). Dilutions of responder cells in complete medium were cultured for 48 h with 5 × 10^5 syngeneic feeder cells pulsed with (1 μM) or without peptide and 10 U/ml recombinant human IL-2. The plates were then washed and incubated with a biotinylated mAb to IFN-γ (clone XMG 1.2) followed by streptavidin-alkaline phosphatase and developed using 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium alkaline phosphatase substrate (Sigma, St. Louis, MO). Responses were considered positive when there were >10 ELISPOTs/well and the number of peptide-pulsed feeder ELISPOTs was more than two times the number of unpulsed feeder ELISPOTs. The frequency of peptide-specific CD8+ T cells present in the responding population was calculated by subtracting the mean number of spots for feeders with no peptide from the mean number of spots with peptide-pulsed feeders.

CTL assay

The target H2b- or H2Db-transfected L-929 cells (L cells) were labeled with Na^51 Cr for 1 h, pulsed with viral peptides or infected with the HKx31 influenza A virus for 60 min, washed, then plated at 5000 targets/well (8). The target cells were washed twice and incubated with the effector populations for 5 h before harvesting supernatants for gamma counting. Two-fold lymphocyte dilutions were assayed in triplicate, while untreated and irradiated L-929 cells were used as controls.

FIGURE 1. Levels of NP-specific CTL activity in the spleen, BAL, and MLN at 7 days after i.n. challenge of PR8-immune mice (10^8.5 EID_{50}, i.p. >6 wk previously) with 10^6.8 EID_{50} of the HKx31 influenza A virus. Lymphocyte populations from B6 (H2k × Dd) and B6C3F1 (H2k × Dd) mice were assayed against H2Dd-transfected L929 cells pulsed with 1 μM H2Dd-restricted NP_{56-66} peptide (A–D), while those from C3H (H2k × Dd) and B6C3F1 mice were assayed against L929 cells pulsed with 1 μM H2k-restricted NP_{56-66} peptide (E–H). The analysis (n = 5) used individual spleens (C), while the BAL and MLN samples were pooled from the B6 ( ), C3H ( ), or B6C3F1 ( ) mice.

FIGURE 2. A representative experiment showing the level of D^bNP_{56-66}-specific CTL activity following primary exposure to the HKx31 virus. The L929-D^b transgenic target cells were pulsed with titrated amounts of the H2D^b-restricted NP_{56-66} peptide. The pooled (n = 10) BAL populations (E:T cell ratio, 50:1) from naive H2b ( ) and H2k × H2b ( ) mice infected i.n. 10 days previously (see Fig. 1) were first adhered on plastic for 1 h at 37°C to remove monocytes/macrophages. These effectors were also assayed on targets infected with the HKx31 virus, with the level of specific 51Cr release being shown by the horizontal interrupted (H2b) and continuous (H2k × H2b) lines. Similar results were obtained following the ex vivo analysis of CTL from secondary stimulated spleen (see Fig. 1) and in vitro restimulation of primary and secondary spleen cultures (data not shown).
cell frequencies in the spleens of mice primed i.p. with the $10^{8.5}$ EID$_{50}$ of the PR8 virus 42 days previously. Unenriched spleen populations from individual mice (groups of five) were incubated on IFN-$\gamma$-coated ELISPOT plates with K$^{\text{NP}_{50}}$ (□) or D$^{\text{NP}_{566}}$-pulsed (○) syngeneic spleen cells. Peptide-specific IFN-$\gamma$ secretion was detected after 40–44 h with a second biotinylated anti-IFN-$\gamma$ mAb and streptavidin-alkaline phosphatase. Neither parent strain showed evidence of reactivity to the inappropriate peptide, with the limit of detection being 1/30,000 cells.

Triton-disrupted controls were measured in quadruplicate. The percent specific lysis was calculated as $100 \times (^{51}\text{Cr release from targets with effectors} - ^{51}\text{Cr release from targets alone})/( ^{51}\text{Cr release from targets with Triton})$. The level of $^{51}\text{Cr}$ release from targets incubated in the absence of T cells did not exceed 15% of the total Triton-mediated $^{51}\text{Cr}$ release. This background value was subtracted to give the values presented here.

Results

CTL activity following in vivo stimulation

The initial finding (1) that the influenza-specific, H2D$^b$-restricted response was substantially greater in the absence of H2K$^b$ was made using virus-infected L cell (K$^{\text{D}^b}$) and MC57G (K$^{\text{D}^b}$) targets and CTL populations taken directly from PR8-primed mice challenged i.n. with the HKx31 virus. This secondary challenge (HKx31→PR8) experiment (1, 11) was repeated, except that the CTL targets were L cells expressing D$^{\text{NP}_{566}}$ (Fig. 1, A–D) and K$^{\text{NP}_{50}}$ (Fig. 1, E–H). The level of D$^{\text{NP}_{566}}$-specific lysis was greater in every case for the parental B6 (K$^{\text{D}^b}$) than for B6C3F1 (K$^{\text{D}^b} \times $ K$^{\text{D}^b}$) effectors from the spleen, BAL and MLN (Fig. 1, A–D). An identical result was recorded for the BAL population recovered from mice after primary infection with the HKx31 virus (Fig. 2). The quality of CTL recognition did not, however, differ for the B6 and B6C3F1 responses to the NP$_{566}$ peptide, as the cut-off point for lysis of the peptide-pulsed L929-D$^b$ targets was $10^{-10}$ M in each case (Fig. 2).

These later experiments (Fig. 1) could, however, cause us to question the exclusivity of this F$_1$ effect to the H2D$^b$-restricted response (1). Cytotoxicity associated with the recognition of K$^{\text{NP}_{50}}$ by the secondarily stimulated B6C3F1 CTL was also diminished compared with the level of specific $^{51}\text{Cr}$ release caused by the C3H (K$^{\text{D}^b}$) T cells, with the effect being most obvious for the BAL population (Fig. 1G). We did not persist further with this, as we clearly now have much better assays (8, 11, 14, 16) for measuring virus-specific CD8$^+$ T cell responses (see subsequent sections). Also, our capacity to analyze the H2K$^b$-restricted response is limited by the fact that only two epitopes (13) have been identified (K$^{\text{NP}_{50}}$ and K$^{\text{NS1}_{52}}$), and it seems likely from the more detailed analysis that has been performed with the H2K$^b$ haplotype (6, 8, 14) that there are more to be found.

T cell memory

Other early LDA studies indicated that the numbers of H2D$^b$-restricted memory CTLp were not necessarily significantly different for mice expressing H2D$^b$ in the presence or the absence of H2K$^b$ (4). This was confirmed using the IFN-$\gamma$ ELISPOT assay (Fig. 3), which tends to give lower values than the Pepy protocol (11), but, because the lymphocytes are diluted, allows virus-specific memory T cells to be detected at lower frequencies. The prevalence of memory T cells specific for D$^{\text{NP}_{566}}$ was comparable for the B6 and B6C3F1 mice. This was also true for the set reactive to K$^{\text{NP}_{50}}$ in the C3H and B6C3F1 mice (Fig. 3). The diminished recall of CTL activity for the K$^{\text{NP}_{50}}$ and D$^{\text{NP}_{566}}$ epitopes in the F$_1$ group (Fig. 1) is thus not obviously explained by the relative availability of memory T cells (Fig. 3).
CD8+ IFN-γ+ T cells in parental and F₁ mice

The primary and secondary responses (HK×31→PR8) following i.n. exposure to the HK×31 virus were then analyzed using the Pep assay for parental (B6 and C3H) and F₁ mice. The results for the Dᵇ NP₃₆₆- and Kᵏ NP₅₀-specific sets are given as the percentage of cells staining in the BAL, MLN, and spleen for the H₂ᵇ, H₂ᵉ, and H₂ᵏ F₁ mice in Fig. 4, while the total cell counts for populations reactive to Dᵇ NP₃₆₆, Kᵏ NP₅₀, Kᵏ NS₁₁₅₂, and Kⁿ NS₂₁₁₄ are shown for the F₁ group only in Fig. 4. Cumulating the prevalence data for the different epitopes in the various sites sampled indicates that the magnitudes of the primary F₁ and parental responses are essentially equivalent up to 10 days after infection (Fig. 4, A, B, E, F, I, and J). The numbers of CD8⁺ Dᵇ NP₃₆₆ T cells in the F₁ BAL and spleen on day 13 were, however, 2- to 3-fold lower than the parental values (Fig. 4, A, E, and I). The HK×31 virus is generally cleared from the lung within 10 days of primary challenge, so the CD8⁺ Dᵇ NP₃₆₆⁺ response seems to resolve more rapidly in the F₁ than the B6 mice (17).

The secondary response to Dᵇ NP₃₆₆ was consistently lower in the H₂ᵏ F₁ than in the H₂ᵇ parent (Fig. 4, C, G, and K), an effect that was much less apparent for Kⁿ NP₅₀ encountered in the H₂ᵉ and H₂ᵏ F₁ situations (Fig. 4, D, H, and L). However, when we translated the percentages into cell numbers, the magnitudes of the Dⁿ NP₃₆₆⁺ and Kⁿ NP₅₀⁺reactive populations were essentially comparable for the B6C3F₁ response (Fig. 5). The divergence in relative prevalence of the Dⁿ NP₃₆₆⁺ and Kⁿ NP₅₀⁺reactive sets is thus seen between the parent and the F₁, not within the F₁ group. It is important in this regard to note that the scales in Fig. 4 are very different for the Dᵇ NP₃₆₆- and Kⁿ NP₅₀-reactive populations(Fig. 4, C, G, and K) and Kⁿ NP₅₀-reactive (Fig. 4, D, H, and L) populations, with the B6 results reflecting the dominance of Dⁿ NP₃₆₆ that we have recognized previously for the secondary influenza-specific response in B6 mice (8, 11, 17).

Responses to the Dᵇ PA₂₂₄ and Kⁿ NS₁₁₅₂ epitopes

We recently described (8) a new epitope (Dᵇ PA₂₂₄) that is at least as prominent as Dᵇ NP₃₆₆ in the primary, but not the secondary, response of B6 mice following i.n. challenge with the HKx31 virus. The same relationship was found for virus-specific CD8⁺ T cells in the spleen and PEL population from naive H₂ᵇ mice challenged i.p. with the PR8 virus (Fig. 6, A and C), the protocol used to prime for the secondary response (Figs. 1, 2, 4, and 5). However, this equivalence between the Dⁿ NP₃₆₆- and Dᵇ PA₂₂₄-reactive sets (Fig. 6, A and B) was not apparent for H₂ᵏ F₁ mice, in which the numbers of T cells specific for Dⁿ NP₃₆₆ were much higher (Fig. 6, B and D). The lower prevalence of Dᵇ PA₂₂₄-specific T cells in F₁ mice was also confirmed following primary i.n. challenge with the HK×31 virus (Fig. 7, A, C, and E). The profile for the Dᵇ PA₂₂₄-reactive set in the B10.A(2R) H₂Κᵇ Dᵇ recombinant (Fig. 7, B, D, and F) was intermediate between that for the H₂Κᵇ parent (Fig. 6, B and D) and the (H₂Κᵇ × H₂Κᵈᵇ) F₁ (Fig. 6, A and C, and Fig. 7, A, C, and E),

The response to Kⁿ NS₁₁₅₂ in B10.A (2R) mice was, if anything, greater than that to Kⁿ NP₅₀ (Fig. 7, B, D, and F). However, Kⁿ NS₁₁₅₂ was clearly less prominent following both primary (Fig. 5, A–C, and Fig. 7, A, C, and E) and secondary (Fig. 5, D–F) challenge of the B6C3F₁ mice. Comparison of primary BAL populations from congenic B10.BR (Kᵈᵇ) and B10.A (2R) mice also showed a relatively greater response to Kⁿ NS₁₁₅₂ in the recombinant (Table I) than in the parental strain, an impression that was confirmed by further analysis using C3H (H₂Κᵈᵇ) mice (Fig. 8). The response to Kⁿ NS₁₁₅₂ thus seems to be lower when H₂Κᵈ is also present.

Loss of a prominent TCR β phenotype in the H₂ᵇ×F₁ response to Dᵇ PA₂₂₄

The CD8⁺ Dⁿ NP₃₆₆⁺ response is characterized by prominent usage of a spectrum of Vᵇ8.3⁺ TCRs associated with a variety of TCR α-chains (18). Recent analysis indicates that Vβ7 dominates
the CD8\(^+\) D\(\beta\) PA 224-specific set generated in B6 mice (G.T.B. and P.C.D., unpublished observations). We thus asked whether the low CD8\(^+\) response in the H2\(k\)\(^3\)b F\(1\) mice might reflect some change in the pattern of TCR involvement. This was indeed found to be the case. When HK\(3\)\(^3\)1-immune H2\(b\) and H2\(k\)\(^3\)b F\(1\) spleen populations were stimulated separately in vitro with the PA 224 (Fig. 9\(A\)) or NP\(366\) (Fig. 9\(B\)) peptides, the diminished response in the F\(1\) animals was associated with a complete absence of V\(\beta\)7 in cultures established from all but one of five mice (Fig. 9\(A\)). There was, however, no difference in the V\(\beta\)8 staining profile for the B6 and B6C3F\(1\) mice (Fig. 9\(B\)). The effect for D\(\beta\) PA 224 could reflect clonal deletion of cross-reactive V\(\beta\)7 T cells specific for self peptide(s) presented in the context of H2K\(k\) or H2D\(k\) (5). The higher D\(\beta\) PA 224-specific response (spleen and MLN; Fig. 7) in the H2K\(k\)D\(\beta\) (compared with the H2K\(k\)D\(k\) F\(1\)) mice might indicate that the defect for D\(\beta\) PA 224 is more likely to be associated with H2D\(k\) than with the H2K\(k\) allele implicated in the early analysis of immunodominance hierarchies (1, 2). However, it is also possible that the Ag involved in the putative deletion of the V\(\beta\)7 D\(\beta\) PA 224-specific set is a peptide from the C3H background presented by H2K\(k\). This will be analyzed further.

**Discussion**

These experiments use a contemporary, quantitative approach (8, 11, 14) to confirm that there are indeed MHC-related immunodominance hierarchies in the CD8\(^+\) T cell responses to various

![Figure 7](http://example.com/figure7.png)

**FIGURE 7.** Comparison of the primary CD8\(^+\) T cell response in H2K\(\beta\)D\(\beta\) and H2K\(\beta\)D\(\beta\) \(\times\) H2K\(\beta\)D\(\beta\) F\(1\) mice following i.n. challenge with the HK\(3\)\(31\) virus. Naive B10.A(2R) and B6C3F\(1\) mice were infected i.n. with 10\(^{6.8}\) EID\(_{50}\) of the HK\(3\)\(31\) influenza A virus and sampled as described in Fig. 1. The responses to the D\(\beta\)NP\(366\) (C), D\(\beta\)PA\(224\) (D), K\(\beta\)NP\(50\) (E), and K\(\alpha\)NS1\(152\) (F) epitopes were measured by IFN-\(\gamma\) production using the PepCy assay (see Fig. 4). The total numbers of epitope-specific CD8\(^+\) T cells were calculated from the percentage of cells stained and the cell counts. The spleen results (A and B) are presented as the mean \(\pm\) SEM, while the MLN (C and D) and BAL (E and F) samples were pooled.

![Figure 8](http://example.com/figure8.png)

**FIGURE 8.** The primary response following i.n. challenge with the HK\(3\)\(31\) virus was measured for the CD8\(^+\) IFN-\(\gamma\)-set recovered from the BAL, MLN, and spleen of C3H (H2K\(k\)D\(\beta\)) mice.

![Figure 9](http://example.com/figure9.png)

**FIGURE 9.** Distribution of TCR V\(\beta\) phenotypes (18) in cultured D\(\beta\)PA\(224\)- and D\(\beta\)NP\(366\)-specific CD8\(^+\) T cells. Individual spleens (\(n = 5\)) from mice infected i.n. with the HK\(3\)\(31\) virus 8 days previously were divided, and aliquots were cultured separately with 1 \(\mu\)M PA\(224\) or NP\(366\) peptide for three cycles of in vitro stimulation (18). The lymphocytes were then stained (18) for CD8\(^+\) IFN-\(\gamma\)-set and analyzed in a FACScan using CellQuest software. The results are expressed as the mean \(\pm\) SEM. The V\(\beta\)7 CD8\(^+\) set represented 6 \(\pm\) 0.2 and 6.8 \(\pm\) 0.5% in naive B6+ B6C3F\(1\) mice (\(n = 5\)), respectively.

**Table I. Comparison of the CD8\(^+\) T cell response profile for congeneric mice**

<table>
<thead>
<tr>
<th>Source of Cells</th>
<th>Mouse Strain</th>
<th>Epitope</th>
<th>K(\beta)NP(50)</th>
<th>K(\alpha)NS1(152)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL</td>
<td>H2K(k)D(\beta)</td>
<td>10.0</td>
<td>7.2</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>H2K(\beta)D(\beta)</td>
<td>3.2</td>
<td>6.2</td>
<td>4.9</td>
</tr>
<tr>
<td>MLN</td>
<td>H2K(\beta)D(\beta)</td>
<td>1.3</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>H2K(\alpha)D(\beta)</td>
<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>H2K(\beta)D(\beta)</td>
<td>3.0</td>
<td>1.5</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>H2K(\beta)D(\beta)</td>
<td>1.5</td>
<td>0.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

\(^a\) The B6, B10.BR, and B10.A(2R) mice were infected i.n. with 10\(^{6.8}\) EID\(_{50}\) of the HK\(3\)\(31\) influenza A virus, and the percentage of CD8\(^+\) IFN\(\gamma\)-set T cells was determined 8 days later.
peptides derived from the influenza A viruses. The spectrum of MHC alleles expressed in a particular mouse strain can clearly modify the relative prevalence of CD8 T cells specific for an individual epitope. However, although we did confirm that the level of virus-specific and DNP366-specific 51Cr release caused by CD8 T cell effectors is lower when H2Kk is also present in the responding mouse strain, the effects are generally subtle and far from the all-or-none situation described in earlier studies (1, 2) in which the only in vitro measure of the CD8 T cell response was cytotoxicity.

We found that the magnitude of the primary DPA224-specific response is much lower in H2Kk × F1 mice than in H2Kk mice when measured quantitatively by the Pepy assay. However, the minimal CTL activity associated with DPA224 (8) indicates that this epitope is poorly presented on virus-infected cell lines, so the diminished numbers of DPA224-specific T cells in H2Kk × F1 mice fails to explain the H2Dβ-restricted difference between F1 and parent detected earlier by cytotoxicity (1). On the other hand, particular epitope-specific CD8 T cells were comparable for PR8-primed H2Kk × F1 and H2Kk mice immediately before i.n. challenge with the HK×31 virus, the extent of further BAL cell proliferation was lower in the F1 group. It thus seems that CD8 T cell frequency does not necessarily predict the magnitude of differentiated CTL function, at least in the primary response to the influenza A viruses. Unfortunately, we do not currently have an assay available that allows CTL activity to be measured at the single-cell level.

While the numbers of DNP366-specific CD8+ memory T cells were comparable for PR8-primed H2Kk × F1 and H2Kk mice immediately before i.n. challenge with the HK×31 virus, the extent of further clonal expansion (17) showed the hierarchy for the CD8-IFN-γ set that would have been predicted from the CTL assays. The dominance by the DNP366-specific population during the secondary influenza-specific CD8 T cell response in F1 mice had led to the impression that DNP366 is some sort of “superepitope” (8, 11). However, this is not the case in the H2Kk × F1, where the responses to DNP366 and KNP50 are essentially equivalent. The relative prevalence of a particular epitope-specific CD8+ population is clearly a function of the spectrum of MHC glycoproteins that are expressed in the responder environment. This effect is also seen for KNS1152. Which, compared with KNP50, shows the hierarchy H2KkD3β+H2KkD18+ (H2KkD3β × H2KkD18)+. The primary and recall responses to different epitopes were similar in magnitude for the H2Kk × F1, but not the H2Kk mice. The major difference is the prominence of the DNP366-specific set in the parental strain following secondary challenge. The situation for the H2Kk × F1 is much more comparable to that described previously for epitopes derived from Listeria monocytogenes (19). Immunodominance hierarchies apparently become unpredictable with the addition or removal of other MHC glycoproteins.

The idea that H2Dβ-restricted CD8+ T cells are deleted during thymic development as a consequence of exposure to self peptides presented by H2Kk glycoproteins throughout ontogeny could explain the much lower response to DPA224 in H2Kk × F1 than in H2Kk mice (5). This cross-tolerance concept was developed before it was known that MHC-restricted CD8+ T cells are specific for viral peptides. The observation that such effects are apparent for one (DPA224), but not another (DNP366), epitope makes sense in the context of established models of self tolerance (20–22). It is certainly the case that the absence of key TCRs in the mature repertoire can diminish the magnitude of a CD8+ T cell response (reviewed in Ref. 7).

The obvious question is whether we should be concerned about MHC-related immunodominance hierarchies as we move to develop vaccines that incorporate peptide epitopes by a spectrum of MHC molecules. A case in point is the polytopy approach that uses linked viral peptides that bind a range of HLA glycoproteins to protect, for instance, against EBV infection (9, 23). The results presented here indicate that any MHC-related hierarchies are generally much less absolute than suggested by the early CTL assays and are not likely to cause a problem for a vaccine incorporating multiple peptides. Even so, it is appropriate to assure that the peptides used are recognized widely by people that express the particular HLA glycoprotein.

References