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Prevalent Class I-Restricted T-Cell Response to the Theiler's Virus Epitope D^b:$VP2_{121–130}$ in the Absence of Endogenous CD4 Help, Tumor Necrosis Factor Alpha, Gamma Interferon, Perforin, or Costimulation through CD28

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C57BL/6 mice mount a cytotoxic T-lymphocyte (CTL) response against the Daniel's strain of Theiler's murine encephalomyelitis virus (TMEV) 7 days after infection and do not develop persistent infection or the demyelinating syndrome similar to multiple sclerosis seen in susceptible mice. The TMEV capsid peptide $VP2_{121–130}$ sensitizes *H-2D*$^{b+}$ target cells for killing by central-nervous-system-infiltrating lymphocytes (CNS-ILs) isolated from C57BL/6 mice infected intracranially. D^b:$VP2_{121–130}$ peptide tetramers were used to stain $CD8^+$ CNS-ILs, revealing that 50 to 63% of these cells bear receptors specific for $VP2_{121–130}$ presented in the context of D^b. No T cells bearing this specificity were found in the cervical lymph nodes or spleens of TMEV-infected mice. *H-2*^{b} mice lacking CD4, class II, gamma interferon, or CD28 expression are susceptible to persistent virus infection but surprisingly still generate high frequencies of $CD8^+$, D^b:$VP2_{121–130}$-specific T cells. However, CD4-negative mice generate a lower frequency of D^b:$VP2_{121–130}$-specific T cells than do class II negative or normal *H-2*^{b} animals. Resistant tumor necrosis factor alpha receptor I knockout mice also generate a high frequency of $CD8^+$ CNS-ILs specific for D^b:$VP2_{121–130}$. Furthermore, normally susceptible FVB mice that express a D^b transgene generate D^b:$VP2_{121–130}$-specific $CD8^+$ CNS-ILs at a frequency similar to that of C57BL/6 mice. These results demonstrate that $VP2_{121–130}$ presented in the context of D^b is an immunodominant epitope in TMEV infection and that the frequency of the $VP2_{121–130}$-specific CTLs appears to be independent of several key inflammatory mediators and genetic background but is regulated in part by the expression of CD4.

The Daniel's strain of Theiler's murine encephalomyelitis virus (TMEV) is a picornavirus which upon intracerebral injection causes a persistent infection of the white matter and demyelination in susceptible mouse strains (haplotypes *H-2*^{s}, *H-2*^{r}, *H-2*^{v}, *H-2*^{f}, *H-2*^{p}, or *H-2*^{q}) but not in resistant strains (haplotypes *H-2*^{b}, *H-2*^{d}, or *H-2*^{k}) of the C57BL lineage (35, 41, 44). The demyelinating disease observed in susceptible strains of mice is an excellent model of human multiple sclerosis (6, 12, 27). While the cause of pathology in susceptible strains remains unknown, the Th1 $CD4^+$ compartment has been demonstrated to mediate a delayed-type hypersensitivity inflammatory reaction with macrophages and cytokines leading to destruction of self tissue (26). However, depletion of the $CD8^+$ T-cell compartments has been shown to suppress demyelination, whereas depletion of $CD4^+$ T cells promotes demyelination (39). In addition, efforts to inhibit the effects of CD4 or CD8 T-cell involvement by disrupting major histocompatibility complex (MHC) class I and II expression or by knocking out CD4 and CD8 still results in demyelination and high virus titers (13, 14, 34, 37, 40). Therefore, the contribution of CD4 and CD8 T-cell subsets to demyelination in susceptible strains remains obscure.

In contrast to susceptible strains of mice, resistant mice clear TMEV from the central nervous system (CNS), and this clearance is associated with a virus-specific cytotoxic T-lymphocyte (CTL) response (43). Evidence supporting the importance of the CTL response in TMEV is indirect, but an essential role for a class I restricted response in viral clearance is demonstrated by several observations. First, the susceptible DBA/2 mice upon in vivo administration of interleukin-2 (IL-2)-secreting tumor readily clear TMEV. The viral clearance correlated with a three- to fourfold increase in virus-specific CTL activity (18). Second, perforin-deficient mice develop persistent TMEV infection (45). Perforin is an important effector molecule for both CTL and natural killer (NK) cells. Immunodepletion of NK cells in genetically resistant mice with anti-NK 1.1 antibody does not allow TMEV virus persistence, whereas a more pervasive depletion with anti-asialo GM1, which also targets some activated T cells, does (36). Third, the β_2 microglobulin (β_2m) knockout mice crossed onto the resistant C57BL/6 genetic background are no longer able to clear the virus (13, 37, 40). Finally, resistance has been genetically mapped to the *H-2D* class I alleles, since introduction of *H-2D*^{b} and *H-2D*^{d} transgenes into susceptible strains of mice allows TMEV clearance (2, 21, 38, 41, 42). The use of MHC recombinant mice suggests that *H-2D* class I alleles, more effectively than *H-2K* class I alleles, present viral peptides to CTLs which leads to TMEV clearance, either by direct killing of infected cells or by cytokine release (21, 42).

Support for the hypothesis that the *H-2D* class I molecules effectively present viral peptides to CTLs during viral clearance was demonstrated by two groups simultaneously. Borson et al. (5) subjected a VP2 viral capsid fusion protein to alkali hydro-

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lysis and used the resulting peptides to sensitize RMA-S target cells for killing by C57BL/6 CNS infiltrating lymphocytes (CNS-ILs). Viral protein 2 (VP2) amino acids 112 to 140 were identified as containing the relevant epitope. Upon synthesizing overlapping peptides from this region, $VP2_{121–130}$ (FHAGSLLV FM) was identified as the peptide that could sensitize target cells for CNS-IL killing. Independently, Dethlefs et al. (11) identified a 9-amino-acid peptide from VP2, amino acids 122 to 130, which is found in the VP2 capsid protein of both Mengo virus and TMEV. This peptide sensitized target cells for CTLs isolated from both Mengo virus- and TMEV-infected mice. While both of these studies found a relevant epitope for TMEV-specific CTL killing, the relative importance of $VP2_{121–130}$ for CTL mediated clearance of TMEV from the CNS in resistant C57BL/6 mice was not determined.

To better understand the class I-mediated T-cell response and the importance of $VP2_{121–130}$ presented in the context of D^b in a TMEV clearing inflammatory reaction, we constructed peptide-MHC tetramers. Altman et al. have described a method by which four class I heterodimers bound with a specific peptide can be assembled with fluorescent, conjugated streptavidin and used to stain T cells with T-cell receptors (TCRs) that have affinity for the peptide presented in the context of the class I molecule (1). Using this method, we assembled tetramers of D^b, $VP2_{121–130}$, and human β_2m to stain $CD8^+$ T cells with TCRs specific for this epitope and then assessed their frequency in the bulk population of brain-infiltrating $CD8^+$ lymphocytes. In addition, we addressed the importance of the CD4 and CD8 T-cell compartments, the *H-2D*^{b} allele, gamma interferon (IFN-γ), tumor necrosis factor alpha receptor I (TNF-αRI), perforin, MHC class II, and CD28 in $VP2_{121–130}$-specific T-cell infiltration by using knockout mice as hosts for intracerebral viral infection.

MATERIALS AND METHODS

Virus infection and mice. Male and female C57BL/6, C57BL/6-Pfp^{tm1sdz}, and IFN-γ$^{-/-}$ mice were obtained from Jackson Laboratory (Bar Harbor, Maine), and the Ab^o (class II$^{-/-}$) mice were a gift from Chris Benoist (Strasbourg, France). All other knockout mice (CD4$^{-/-}$, CD8$^{-/-}$, TNF-αRI$^{-/-}$, and CD28$^{-/-}$) were gifts from Tak Mak at Amgen (Thousand Oaks, Calif.). The FVB/D^b mice were generated with a D^b genomic transgene in Chella David's gene transfer lab at the Mayo Foundation (Rochester, Minn.). Mice ranged in age from 4 to 16 weeks. Mouse spleens, lymph nodes, and brains were harvested 7 days post-intracerebral injection with 2×10^6 PFU of Daniel's strain of TMEV. Lymphocytes were then extracted from the brain through homogenization and a homogeneous Percol gradient (24). An improved step gradient containing 35% Percol layered over 70% Percol was used to isolate cleaner preparations of lymphocytes from brains (see Fig. 4). The change in isolation procedures did not alter the ratio of tetramer. Stained $CD8^+$ T cells observed in C57BL/6 mice were infected with TMEV (data not shown). Erythrocytes were then removed from brain, spleen, and lymph node cell preparations through lysis with ammonium chloride, potassium carbonate, and EDTA (ACK). Handling of all animals conformed to the National Institutes of Health and Mayo Clinic institutional guidelines.

Preparation of *H-2D*^{b} tetramers. The expression vectors for *H-2D*^{b} and human β_2m were generous gifts from John Altman and Mark Davis, respectively. Proteins expressed from these reagents in bacteria were isolated and folded in gross excess of E7 or $VP2_{121–130}$ peptide as previously described (15, 25, 38). The folded monomer complexes were desalted with a PD-10 desalting column (Pharmacia, Upsala, Sweden), biotinylated with a BirA biotinylation kit (Avidity, Denver, Colo.), purified with a Mono Q cation-exchange column (Bio-Rad, Hercules, Calif.), and complexed with *R*-phycoerythrin–streptavidin (Molecular Probes, Eugene, Oreg.) at a 4.1:1 molar ratio. This tetramer complex was then purified by S-200 size exclusion gel filtration (Bio-Rad).

Flow cytometric analysis. Then, 10^6 cells isolated from brain, lymph nodes, and spleen were stained with R-phycoerythrin D^b:$VP2_{121–130}$ tetramer for 1 h, adding anti-CD8 fluorescein isothiocyanate during the final 20 minutes. Samples were then washed twice with fluorescence-activated cell sorter (FACS) buffer (1% bovine serum albumin and 2% sodium azide), resuspended in cold phosphate-buffered saline and fixed in 1% paraformaldehyde. Samples were run on a Becton Dickinson FACScan instrument (Mountain View, Calif.) and are reported on a logarithmic scale (250 channels/log intensity). Gates were drawn to include $CD8^+$ cells while excluding nonspecific binding of tetramer and/or anti-CD8. The data were analyzed in two ways. First, the quadrants were positioned to exclude the lymphocyte-negative signals present in normal, uninfected brain cell preparations (Fig. 1A) so that only $CD8^+$ cells would be scored. This procedure counts only cells expressing higher levels of CD8 and misses some positive cells that coincide in fluorescent intensity with the negative background. Although this approach has the disadvantage of not analyzing all the cells, it has the advantage of being objectively applied to all of the samples analyzed in a given experiment. In a second approach, we drew gates around populations of fluorescent cells not present in the normal brain preparation (Fig. 1A). We defined two $CD8^+$ populations in Fig. 1B: cells staining with the tetramer and cells not staining with the tetramer. The estimated ratios of tetramer-stained to nonstained cells by using either method was the same. These two strategies were applied to all the FACS data presented; both yielded comparable results.

In vitro CTL assay. A standard 4-h ^{51}Cr release assay was done to determine CTL activity in splenocyte and CNS-IL preparations, and 10^3 RMA-S target cells/well were used. RMA-S cells were labeled with ^{51}Cr for 2 h in the presence of $VP2_{121–130}$ peptide or irrelevant K^b-restricted dEV8 peptide (49). Both peptides were synthesized in the Mayo protein core facility. Freshly isolated effector cells were incubated with labeled target cells for 4 h. The percent specific lysis of target cells was calculated from released ^{51}Cr as follows: 100 × (experimental release − spontaneous release)/(total release in detergent − spontaneous release). The number of effector T cells added was estimated by counting the cells with lymphocyte morphology by microscopic inspection. Samples from the cell preparations were analyzed by FACS and found to contain fewer T cells than was estimated by microscopy. These effector/target ratios of brain cell preparations represent overestimates of the T cells added. This fact does not influence our conclusions, since populations containing fewer T cells exhibited cytolytic activity, while populations containing more T cells did not.

RESULTS AND DISCUSSION

Inflammatory mononuclear cells were isolated from the whole brains of noninfected and 7-day-post-TMEV-infected C57BL/6 mice. These CNS-ILs were stained with anti-CD8 antibody and D^b:$VP2_{121–130}$ tetramer or control tetramer specific for an irrelevant papillomavirus epitope as outlined in Materials and Methods. As expected, no CNS-ILs were detected in the brains from noninfected mice and no cells had demonstrable staining with anti-CD8 antibodies or D^b:$VP2_{121–130}$ tetramer (Fig. 1A). In contrast, as determined by four separate experiments, approximately 50 to 63% of CNS-ILs from TMEV-infected mice stained double positive for both of these reagents (Fig. 1B). BALB/c (*H-2*^{d}) mice infected with TMEV did not stain with the D^b:$VP2_{121–130}$ tetramer (Fig. 1C). These observations document the specificity of the tetramer staining and demonstrate that a majority of $CD8^+$ T cells in C57BL/6 mice harvested from the site of viral infection express TCR specific for the $VP2_{121–130}$ peptide presented in the context of D^b. Three-color staining revealed that the analyzed T-cell populations included approximately 30% $CD4^+/CD8^-$ cells that are presumably T helper cells (data not shown). None of these cells bear receptors stained by the tetramer probes.

To address whether there was a systemic clonal expansion of $CD8^+$ T cells specific for the D^b:$VP2_{121–130}$ epitope, lymphocytes were isolated from the brain, spleen, and cervical lymph nodes of infected C57BL/6 mice. No $VP2_{121–130}$-specific T cells were identified in the spleen or cervical lymph nodes, whereas 55% of the $CD8^+$ cells in the brain stained with the D^b:$VP2_{121–130}$ tetramer (Fig. 2A to C). A control tetramer, consisting of D^b loaded with a D^b binding peptide from the human papillomavirus E7 protein (25), did not stain this population (Fig. 2F). This control tetramer, however, stains greater than 99% of D^b:E7 epitope-specific $CD8^+$ T cells (data not shown). The observation that 55% of the CTLs are specific for one peptide is similar to frequencies previously reported in infections with lymphocytic choriomeningitis virus (LCMV). For example, Murali-Krishna et al., by using tetramers constructed with an immunodominant LCMV peptide presented in the context of L^d, stained up to 56% of splenic $CD8^+$ T cells in BALB/c mice (30).

We next addressed CTL activity in the lymphocytes isolated from the spleen and brain. In agreement with the staining

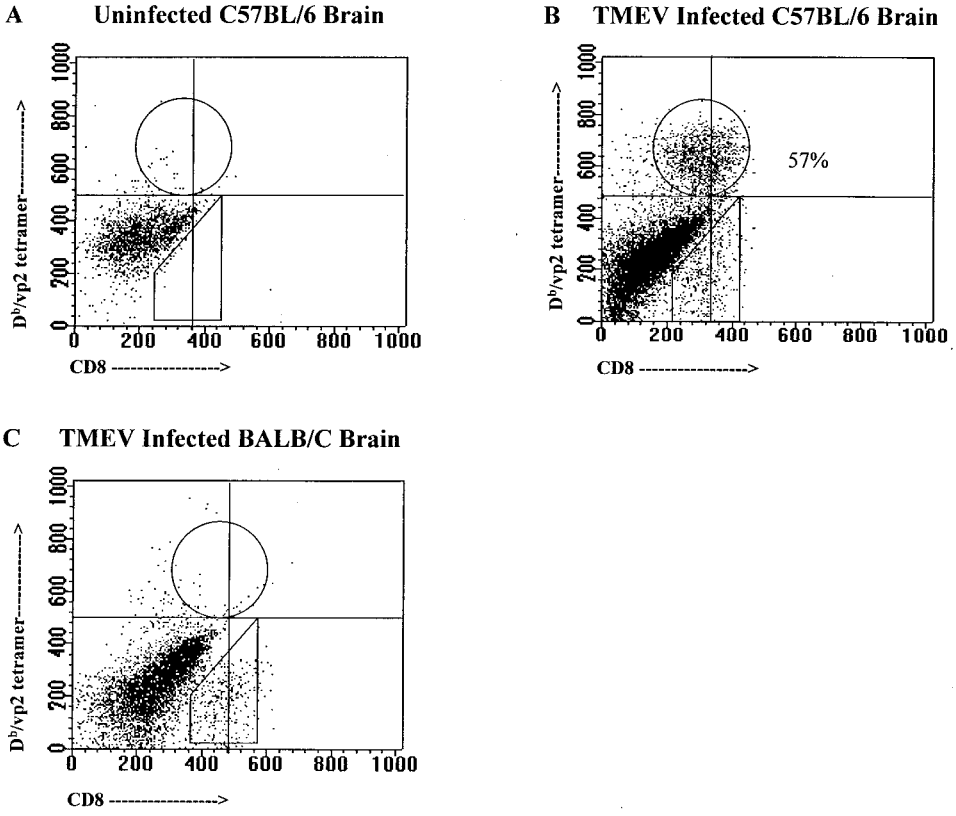

FIG. 1. Staining of inflammatory mononuclear cells isolated from the brains of noninfected C57BL/6 mice (A), TMEV-infected C57BL/6 mice (B), and TMEV-infected BALB/c mice (C). The circles population represents those lymphocytes that stain with both anti-CD8 and D^b:$VP2_{121–130}$ tetramer. The percentage given is the percentage of $CD8^+$ lymphocytes that stain with D^b:$VP2_{121–130}$ tetramer. This value is corrected by subtracting the nonspecific binding of irrelevant D^b:E7 tetramer. See Materials and Methods for details of the analysis.

shown by the D^b:$VP2_{121–130}$ tetramer, RMA-S cells loaded with $VP2_{121–130}$ were killed by freshly isolated CNS-ILs but not by lymphocytes isolated from the spleen (Fig. 3). In BALB/c mice infected with the JHM strain of mouse hepatitis virus (JHMV), JHMV-specific CTL activity was also localized only to the brain and not the cervical lymph node or spleen (48). It is possible that in the case of these virus infections, the brain acquires the responsibilities of a lymph node and is the site of CTL expansion.

The hierarchy of immunodominance among peptides in TMEV infection may be influenced by several factors. For example, peripheral immunization of C57BL/6 mice with TMEV can induce K^b-restricted splenocyte killing (21). In addition, subtle variations in TMEV itself may alter immunodominance. The TMEV protein L*, which is expressed by an alternative viral reading frame, can influence mouse susceptibility to TMEV infection and subsequent demyelinating disease (8). Recently, it has been shown that intracranial infection of C57BL/6 mice with the L*-deficient TMEV strain DAL* induces CNS-ILs capable of K^b-restricted antiviral killing (23). Whether these K^b-restricted epitopes are presented at low frequency during intracranial infection of C57BL/6 mice with Daniel strain TMEV remains to be established. Nevertheless, these results demonstrate that TMEV peptides other than $VP2_{121–130}$ are potential antigens for C57BL/6 $CD8^+$ T cells. Therefore, the observed dominance of $VP2_{121–130}$ as a T-cell epitope in *H-2D*^{b}-expressing mice must be regulated in some fashion.

To address how epitope dominance might be controlled, the influence of costimulation, inflammatory cytokines, perforin, or CD4 help on D^b:$VP2_{121–130}$ epitope dominance was investigated in TMEV-infected genetic knockout mice. Class II (34)-, CD4 (31)-, CD8 (31)-, IFN-γ (unpublished observation)-, perforin (32)-, and CD28 (unpublished observation)-deficient mice of the C57BL/6 lineage are all susceptible to TMEV persistent infection and demyelinating disease despite expression of *H-2D*^{b}. To address the effects of these inflammatory mediators which govern resistance to the virus, we assessed the frequency of $VP2_{121–130}$-specific T cells among CNS-ILs in the brains of infected mice. CNS-ILs were isolated 7 days post-TMEV infection from mice deficient for the indicated molecules and stained with D^b:$VP2_{121–130}$ tetramer and anti-CD8 antibodies. With the exception of the CD8-deficient strain (Fig. 4E), all of the other tested mouse strains developed a major population of T cells at the site of infection that recognize $VP2_{121–130}$ presented by D^b (Fig. 4A to I). Furthermore, the frequencies of these T cells in the class II, CD28, TNF-αRI, IFN-γ, and perforin knockout mice were not significantly different from that found in infected C57BL/6 animals. This indicates that the homing and clonal expansion of the population of $VP2_{121–130}$-specific $CD8^+$ lymphocytes is not affected by the absence of IFN-γ, TNF-αRI, class II molecules, perforin, or CD28. No staining by the D^b:$VP2_{121–130}$ tetramer was observed in TMEV-infected $CD8^{-/-}$ mice (Fig. 4E), demonstrating that CD8 molecules are required for the development of T cells capable of recognizing $VP2_{121–130}$ in the context of D^b. The expression of CD8 molecules could influence the development of the epitope-dominant T-cell response at the level of

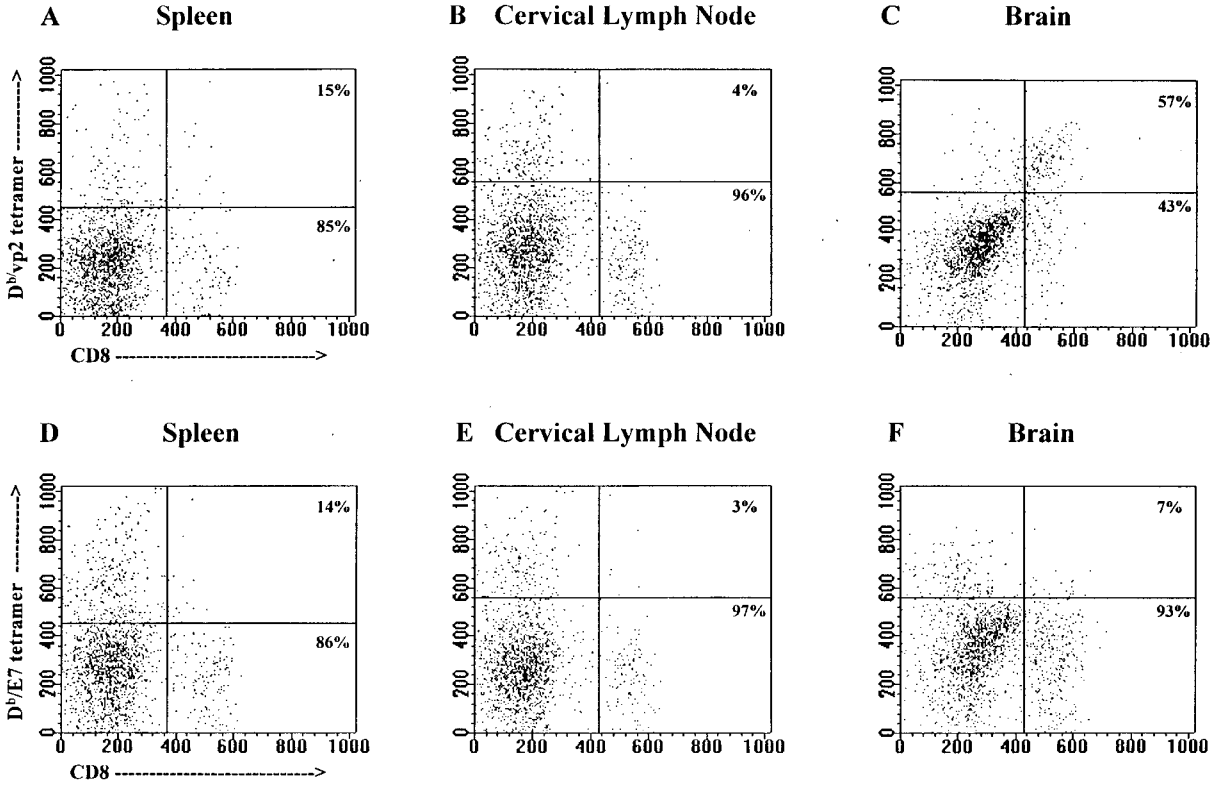

FIG. 2. Staining of lymphocytes isolated from mouse spleen, cervical lymph node, and brain at 7 days post-TMEV infection. Isolated lymphocytes were stained with either D^b:$VP2_{121–130}$ tetramer (A to C) or control irrelevant D^b:E7 tetramer (D to F). The percentage of $CD8^+$ T cells that stain positive or negative for D^b:$VP2_{121–130}$ or D^b:E7 tetramer is given.

repertoire selection, during T-cell activation, or in the process of recruitment of T cells to the brain.

The presence of significant populations of D^b:$VP2_{121–130}$-specific CTL at the site of TMEV infection in susceptible mice appears paradoxical at first glance. However, it is clear that the activation of CTL is not the only factor that can determine the outcome of viral infection. Lymphokines released by T cells can have both direct antiviral function and indirect influence on antiviral immunity by upregulating MHC encoded antigen-presenting molecules and by recruiting other effector cells, such as macrophages, to the site of infection. Cells harboring actively replicating TMEV could avoid the immune response in the absence of the timely upregulation of MHC gene expression or as a consequence of an inadequate cytokine response.

Of particular interest is the observation that $CD4^{-/-}$ mice consistently generate a lower but still appreciable (22 to 37%) frequency of D^b:$VP2_{121–130}$-specific $CD8^+$ T cells in the brain. This indicates that the focus of the $CD8^+$ T-cell response on the VP2 peptide is indeed regulated but the mechanism by which CD4 influences this process is not clear. If T helper cells were important in maintaining the dominant response to the VP2 epitope, then class II knockout mice would be predicted to also display diminished epitope dominance in their responses. As shown in Fig. 4C, this is not the case. The reason for this discrepancy is not known, but it may be an indication that the functions of CD4 molecules are not completely understood. Perhaps CD4 molecules perform additional functions not involving interactions with classically defined class II molecules. For example, another stage in the development of the immune response where CD4 expression could influence a CD8-restricted T-cell response is during the differentiation of immature T cells. Perhaps the absence of CD4 expression in the knockout mice at the CD4/CD8 double-positive stage in the thymus diminishes their subsequent dominance in the hierarchy of potential responding T cells to TMEV challenge. In

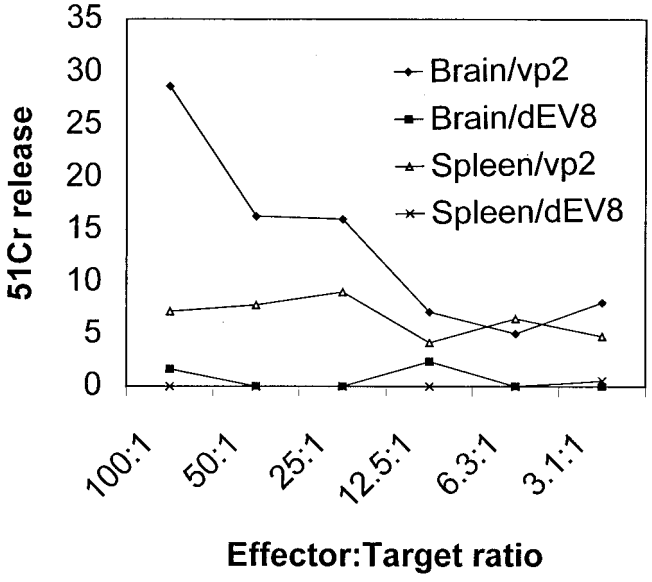

FIG. 3. D^b:$VP2_{121–130}$ epitope-specific cytolytic activity of lymphocytes isolated from the brain and spleen at 7 days post-TMEV infection. RMA-S cells were loaded with $VP2_{121–130}$ peptide or irrelevant dEV8 peptide before being subjected to killing by brain and spleen lymphocyte preparations.

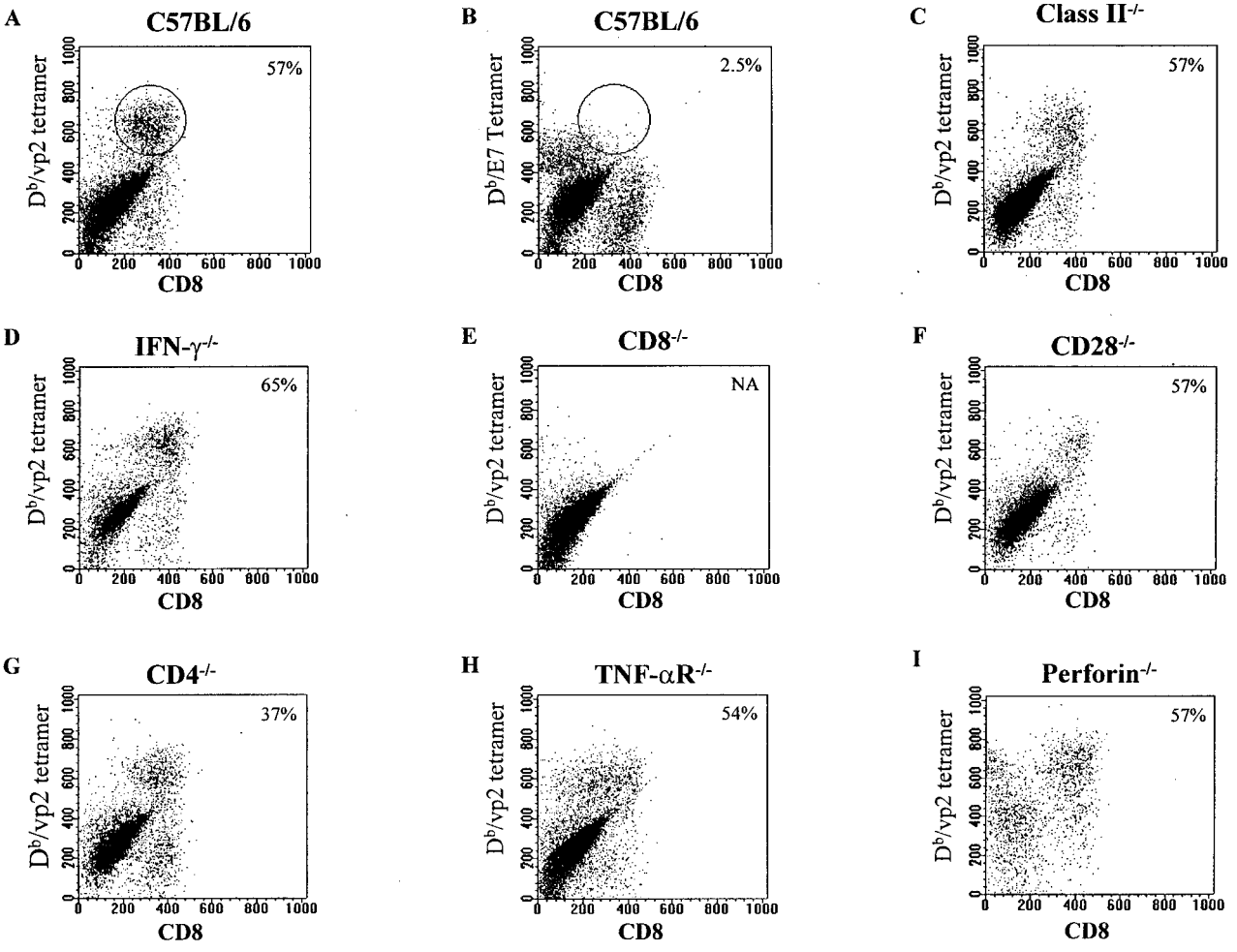

FIG. 4. Frequency of $CD8^+$ CNS-ILs that stain for both D^b:$VP2_{121–130}$ tetramer and CD8 in normal C57BL/6 mice (A), MHC-$II^{-/-}$ mice (C), IFN-$\gamma^{-/-}$ mice (D), $CD8^{-/-}$ mice (E), $CD28^{-/-}$ mice (F), $CD4^{-/-}$ mice (G), TNF-$\alpha RI^{-/-}$ mice (H), and perforin$^{-/-}$ mice (I). Typical background staining of CNS-ILs from TMEV-infected C57BL/6 mice with an irrelevant D^b:E7 tetramer is shown in panel B. Lymphocytes were isolated 7 days post-TMEV infection. The percentage of $CD8^+$ cells that stained with D^b:$VP2_{121–130}$ tetramer is given in the righthand corner. The $CD8^+$ population that recognizes the D^b:$VP2_{121–130}$ epitope is circles in panels A and B but not in panels C to I.

addition, the possibility that CD4 molecules could influence immune reactivity in ways not yet appreciated must be considered.

Why the frequency of $CD8^+$ T cells specific for a single epitope is so high and how this frequency resists change despite different environmental pressures remains unknown. The inflammatory mediators studied affect the kinetics of an immune response in several ways. For example, IFN-γ can upregulate the expression of many factors involved in antigen presentation, including class I, class II, β_2m, TAP1, TAP2, and tapasin (4). Likewise, TNF-α can modify inflammation by either working in synergy with IFN-γ or by activating NF-κB through degradation of the inhibitory subunit IκB (3, 47). Class II-restricted help could also modify an inflammatory response by providing the release of cytokines. Costimulation by B7-1 and B7-2 through CD28 is important for providing a second signal in T-cell activation, perhaps even dictating whether a Th1 or a Th2 response will occur (19). Perforin plays a central role in the effector process of T-cell-mediated cell killing. The fact that the frequency of $VP2_{121–130}$ peptide-specific T cells is essentially not different in perforin-deficient and normal C57BL/6 mice, indicates that the factors leading to epitope dominance precede the effector phase of the response. Remarkably, the mechanism responsible for focusing the immune response on the $VP2_{121–130}$ peptide is not influenced by costimulation, class II-mediated help, or the effects of IFN-γ or TNF-α.

As part of this study, we introduced a D^b transgene into the FVB strain to determine whether a population of $VP2_{121–130}$-specific CTLs could be generated in a TMEV-infected mouse with a completely different genetic background. Highly susceptible FVB mice are *H-2^q* and are not expected to generate D^b-restricted CTL responses. As predicted, no D^b:$VP2_{121–130}$-specific T cells were detected in the CNS-ILs of FVB mice (Fig. 5). Introduction of a D^b transgene into the FVB strain enables these highly susceptible mice to resist TMEV-induced demyelinating disease (2). A major population (55%) of CNS-ILs isolated from TMEV-infected FVB-D^b transgenic mice stained with anti-CD8 and D^b:$VP2_{121–130}$ tetramer (Fig. 5). This frequency is similar to that observed in C57BL/6 mice (50 to 63%). This finding stresses the importance of the D^b gene in the $VP2_{121–130}$-dominated T-cell response and the minimal effects contributed by other genes differing between these distantly related mouse strains.

The concept of an immunodominant peptide eliciting a strong CTL response is not new and has been described among both human and mouse viral infections (9, 17, 28, 51). The

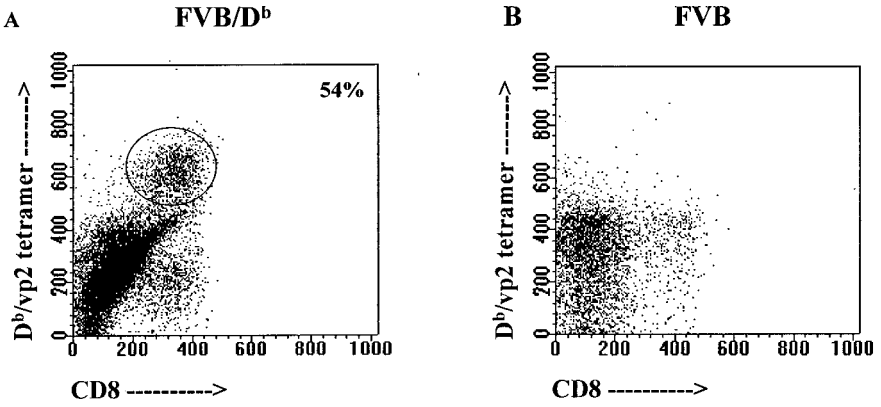

FIG. 5. Epitope dominance conferred by a D^b transgene. The staining patterns of CNS-ILs isolated from resistant FVB (*H-2D*^{b}) transgenic mice (A) and susceptible, parental FVB (*H-2*^{q}) mice (B) are shown. CNS-ILs were stained with D^b:$VP2_{121–130}$ tetramer and anti-CD8 antibodies and analyzed by FACS. The percentage of $CD8^+$ T cells that are double positive is given in the upper right corner of panel A.

mechanism by which this occurs is not well understood, but peptide affinity, peptide processing, and the available T-cell repertoire have all been implicated as factors in establishing immunodominance hierarchies among peptides (7, 9, 10, 16, 20, 29, 33, 46, 50, 52, 53). Here we have demonstrated that the D^b:$VP2_{121–130}$ epitope-restricted clonal dominance appears to persist in the absence of key inflammatory factors such as class II, CD4, CD28, IFN-γ, and TNF-αRI, indicating that the clonal dominance is independent of interaction with $CD8^+$ T cells and the help compartment.

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