Generation of Antiviral Major Histocompatibility Complex Class I-Restricted T Cells in the Absence of CD8 Coreceptors

Nicolas P. Andrews, Christopher D. Pack, and Aron E. Lukacher*

Department of Pathology, Emory University School of Medicine, Atlanta, Georgia 30322

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The CD8 coreceptor is important for positive selection of major histocompatibility complex I (MHC-I)-restricted thymocytes and in the generation of pathogen-specific T cells. However, the requirement for CD8 in these processes may not be essential. We previously showed that mice lacking β2-microglobulin are highly susceptible to tumors induced by mouse polyoma virus (PyV), but CD8-deficient mice are resistant to these tumors. In this study, we show that CD8-deficient mice also control persistent PyV infection as efficiently as wild-type mice and generate a substantial virus-specific, MHC-I-restricted, T-cell response. Infection with vesicular stomatitis virus (VSV), which is acutely cleared, also recruited antigen-specific, MHC-I-restricted T cells in CD8-deficient mice. Yet, unlike in VSV infection, the antiviral MHC-I-restricted T-cell response to PyV has a prolonged expansion phase, indicating a requirement for persistent infection in driving T-cell inflation in CD8-deficient mice. Finally, we show that the PyV-specific, MHC-I-restricted T cells in CD8-deficient mice, while maintained long term at near-wild-type levels, are short lived in vivo and have extremely narrow T-cell receptor repertoires. These findings provide a possible explanation for the resistance of CD8-deficient mice to PyV-induced tumors and have implications for the maintenance of virus-specific MHC-I-restricted T cells during persistent infection.

CD8 T cells are critical mediators of immune surveillance for viral infections. Antiviral CD8 T cells express T-cell receptors (TCRs) that recognize virus-derived oligopeptides fitted into a solvent-accessible groove formed by the α1 and α2 domains of major histocompatibility complex I (MHC-I) molecules. The topology defined by a particular peptide-MHC-I (pMHC-I) complex creates a ligand for a select number of clonally distributed TCRs. The α chain of CD8 molecules, which are expressed at the cell surface as αα homodimers or αβ heterodimers, contacts a nonpolymorphic site in the juxtamembrane α3 domain of MHC-I heavy chains. CD8 operates as a TCR coreceptor by stabilizing T-cell binding to pMHC-I ligands and by amplifying proximal TCR signal transduction via colocalizing the tyrosine kinase p56\(\text{Lck}\), which is associated with the cytoplasmic domain of the CD8α chain. By these mechanisms, engagement of the CD8 coreceptor can dramatically enhance sensitivity for antigenic peptides and thereby compensate for low-affinity TCRs (20, 23). Centrally, CD8 molecules promote efficient positive selection of MHC-I-restricted thymocytes. In the T-cell periphery, once CD8-amplified TCR signaling reaches a sufficient activation threshold, CD8 T cells mobilize antiviral effector functions, including cytokine production and cytotoxic activity.

The requirement for CD8 coreceptors in the selection and generation of MHC-I-restricted T-cell responses, however, may not be absolute. Mice genetically disrupted for CD8α gene expression (CD8KO mice) exhibit a profound defect in mounting MHC-I-restricted T-cell responses against viruses that are acutely cleared in wild-type mice (3, 14). However, cytotoxic CD4\(^{-}\) T cells in CD8KO mice have previously been shown to be capable of rejecting MHC-I-disparate skin grafts (8). In a recent report of a familial CD8α gene missense mutation, three CD8-deficient siblings, of which two were asymptomatic and one suffered recurrent non-life-threatening bacterial infections, were shown to have high frequencies of circulating CD4\(^{-}\)CD8\(^{-}\) TCRαβ\(^{+}\) T cells that phenotypically resembled effector CD8 cytotoxic T lymphocytes; whether these double-negative T cells were MHC-I-restricted was not addressed (10).

Using the oncogenic mouse polyoma virus (PyV) infection model, we came across the unexpected finding that CD8KO mice were largely resistant to PyV-induced tumors, although β2-microglobulin-deficient mice were highly susceptible to PyV tumorigenesis (13). This observation led us to hypothesize that PyV might elicit an MHC-I-restricted T-cell response in CD8KO mice. In this study, we demonstrate that CD8KO mice infected by PyV indeed generate an MHC-I-restricted virus-specific T-cell response. This antiviral T-cell response, though, was found to differ from that elicited in wild-type mice in terms of the time course of expansion, functional competence, and TCR repertoire diversity. Thus, CD8 coreceptors are dispensable for generating antiviral MHC-I-restricted T-cell responses and provide a possible explanation for the PyV-induced tumor resistance of CD8KO mice.

MATERIALS AND METHODS

Mice. C57BL/6 (B6) mice were purchased from the National Cancer Institute. CD8\(^{\text{αβ/αβ}}\) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred at Emory University Department of Animal Resources. All animal protocols were conducted according to the guidelines established by the Institutional Animal Care and Use Committee and the Department of Animal Resources of Emory University. All mice were between 6 and 12 weeks of age at the time of infection.

Viruses. Stocks of PyV (strain A2) and PyV.OVA-I were prepared in baby mouse kidney cells, as described previously by Lukacher and Wilson (28).
were injected subcutaneously (s.c.) in hind footpads with 2 × 10^6 PFU of virus. Recombinant PyV-OVA-A1 was generated through the insertion of the SINEFEK encoding sequence in frame at a unique BspI restriction site in the coding region of middle T antigen (Ag) (4). SINFEK-encoding fragments were generated with high-fidelity Taq polymerase (Invitrogen) using a forward overlapping primer also encoding the BspI restriction site (5’-GGTTTCTGAGACATAAATGCTGAAAACCTGACGCGATGACGATATCC-3’) and a reverse primer encoding an EcoRI site (5’-TGCAAGATTCGGGCTGAACCTTCC-3’); restriction sites are underlined. The resulting PCR product was cloned into a unique BspI/EcoRI site of a pUC19 plasmid containing the small fragment of a BamHI/EcoRI-digested PyV genome. The large fragment was also cloned into pUC19. The small and large fragments were excised from pUC19, ligated to generate full-length PyV DNA, and transfected (using Lipofectamine 2000 [Invitrogen]) into baby mouse kidney cells. Recombinant vesicular stomatitis virus encoding the D^3^-restricted LT359-368 epitope (rVSV-LT359) was generated as described previously (2). The tail veins of the mice were injected intravenously (i.v.) with 1 × 10^6 PFU of rVSV-LT359.

**Synthetic peptides.** The LT359-368Abu (SAVKNY[Abu]SKL) peptide, in which the cysteine residue at position 7 was replaced with α-aminoisobutyric acid, a thiol-less cysteine analog residue, was synthesized by the solid-phase method using F-moc chemistries (Emory University Microchemical Core Facility) and high-pressure liquid chromatography purified to more than 90% purity. For simplicity, the LT359-368Abu peptide is referred to as LT359 peptide.

**TaQMan real-time PCR to quantitate PyV DNA.** DNA isolation and TaqMan PCR were performed as described previously (21). PyV DNA quantity is expressed in genome copies per milligram of tissue and is calculated based on a standard curve of known PyV genome copy number versus the threshold cycle of detection. The detection limit with this assay is 10 copies of genomic viral DNA.

**Flow cytometry.** One million cells per sample were stained in phosphate-buffered saline containing 2% fetal bovine serum and 0.1% sodium azide. Antibody staining was performed at 4°C for 30 min; allophycocyanin-conjugated D^3^-LT359 tetramer (21) staining was performed at room temperature for 30 min. Cells were surface stained with a fluorescein isothiocyanate-conjugated anti-CD4 (clone GK1.5) (BD Biosciences), phycoerythrin-Cy7-conjugated monoclonal antibody (MAb) specific for CD3ε (eBioscience), and phycoerythrin-conjugated MAb specific for TCRβ (clone H57-597), KLRG1, CD127, PD-1, and rat immunoglobulin G1 isotype controls (eBioscience). TCR ββ-domain usage was determined by intracellular staining using a fluorescein isothiocyanate-conjugated panel of MAbS (BD Pharmingen). This approach was developed due to MHC tetramer-induced downregulation of TCRs on CD8KO T cells. After staining with D^3^-LT359 tetramer and CD3 and CD4 MABs, cells were permeabilized with Cytofix/Cytoperm and stained intracellularly with anti-Vβ MABs. Samples were acquired on a FACSCalibur (BD Biosciences), and data were analyzed using FlowJo software (Tree Star).

**Intracellular staining.** Cells were stimulated directly ex vivo with the indicated concentration of LT359 peptide and then stained for surface CD3, CD4, and intracellular cytokines as described previously (21).

**In vivo cytotoxicity assays.** B6 mice infected with PyV 21 days or 6 weeks previously were tested in vivo for killing of LT359-368Abu peptide-pulsed naive B6 spleen cells over a 4-h period, as described previously (7).

**Adaptive transfer.** B6 and CD8KO mice were injected in the footpads with 2 × 10^6 PFU PyV. Three weeks after infection, spleen cells were incubated for a total of 40 min at 37°C in flasks coated with goat anti-mouse immunoglobulin G (Jackson ImmunoResearch Laboratories). Nonadherent cells were labeled with 2.5 μM 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) (Invitrogen) for 10 min at 37°C. A total of 40 × 10^6 cells was injected i.v. into infection-matched B6 or CD8KO hosts. CD3^+ D^3^-LT359 tetramer~ cells in the blood were tracked over time for CFSE fluorescence intensity and as a frequency of total donor CD3^+ cells.

**Statistics.** Statistical significance was determined by unpaired Student’s t test, assuming unequal variances. A P value of ≤0.05 was considered statistically significant. Linear and nonlinear regression analyses were performed using GraphPad Prism, version 4.0e (GraphPad Software, Inc.).

**RESULTS**

PyV infection elicits an MHC-I-restricted T-cell response in CD8KO mice. Because B6 and CD8KO mice are both resistant to PyV-induced tumors (13), we compared their abilities to control PyV replication. Quantitative PCR assays for PyV DNA in spleen (Fig. 1A) and kidney (data not shown) revealed no differences in viral DNA load between B6 and CD8KO mice through acute and persistent phases of infection. Thus, CD8 deficiency has no overt effect on the capacity of the host to control PyV load.

We next explored the possibility that virus-infected CD8KO mice might mount an MHC-I-restricted antiviral T-cell response. As shown in Fig. 1B, PyV-infected CD8KO mice indeed generated a T-cell response directed toward the dominant D^3^-restricted viral epitope, LT359-368, albeit one of lower magnitude than that of B6 mice. D^3^-LT359 tetramer staining was specific, as D^3^-gpl33 tetramers (which detect lymphocytic choriomeningitis virus [LCMV]-specific CD8 T cells [31]) did not bind T cells from either PyV-infected B6 or CD8KO mice (data not shown). In addition, small populations of CD3^+ CD4^- D^3^-LT359 tetramer~ cells were detected in all
B6 mice and infrequently in CD8KO mice at days 8 and 21 postinfection (p.i.), respectively (data not shown). These cells are likely an activated population that has upregulated CD4 upon costimulation (35).

The pattern of LT359-specific T-cell responses in CD8KO mice, however, departed dramatically from that seen in infected B6 mice. CD3^+ CD4^- D^bLT359 tetramer^+ T cells progressively expanded to reach peak magnitude by 3 weeks p.i. and, as a population, did not contract (Fig. 1B, top panel). Although CD3^+ CD4^- D^bLT359 tetramer^+ T cells were detected in all PyV-infected CD8KO mice, the magnitude of this Ag-specific T-cell response varied among individual animals. Approximately two-thirds of CD8KO mice, designated high responders (HRs), exhibited this anti-PyV MHC-I-restricted T-cell response, while the remaining mice showed a much lower number of LT359-specific T cells; we termed these remaining mice low responders (LRs). LR-CD8KO mice had low frequencies (8%) and numbers (1 \times 10^5) of LT359-specific T cells at all time points examined (Fig. 1B). Subdominant MHC-I-restricted anti-PyV T cells (21) were not observed in either HR- or LR-CD8KO mice (data not shown), presumably because their levels fell below detection by tetramer staining or peptide-stimulated intracellular cytokine staining and enzyme-linked immunospot assays for gamma interferon (IFN-\gamma) production. Failure to detect these subdominant T cells may also reflect an increased dependence on CD8 for binding pMHC than is needed for stabilizing D^bLT359 tetramer binding. Notably, at their maximal point of expansion in HR-CD8KO mice, LT359-specific T cells comprised nearly a third of all splenic CD3^+ CD4^- T cells (Fig. 1B, bottom panel). By 5 months p.i., B6 and HR-CD8KO mice had similar numbers of LT359-specific T cells (Fig. 1B, top panel). While numbers of LT359-specific T cells remained stable over time (Fig. 1B, bottom panel), the decline in the percentage of LT359-specific T cells in CD8KO mice at day 150 p.i. (Fig. 1B, bottom panel) can largely be accounted for by an observed twofold increase in total D^bLT359 tetramer-negative CD3^+ CD4^- frequencies in the day 150 p.i. mice compared to day 42 and day 21 p.i. mice; MHC-I tetramers for the two known subdominant PyV-specific CD8 T cells (21) did not stain spleen cells from these day 150 p.i. mice (data not shown). Acutely and persistently infected B6 mice, interestingly, did not have detectable CD3^- CD4^- CD8^- D^bLT359 tetramer^- T cells in the spleen or in the bone marrow, which is a site enriched in double-negative T cells (29, 36). Taken together, these data indicate that CD8 coreceptors are not essential for generating an MHC-I-restricted antiviral T-cell response.

Acutely cleared rVSV infection elicits an Ag-specific T-cell response in CD8KO mice. We next asked whether the ability of CD8KO mice to mount MHC-I-restricted T-cell responses required repetitive Ag encounter, as in persistent virus infection, or was a unique feature of PyV infection. To address these questions, we compared the LT359-specific T-cell responses in B6 and CD8KO mice infected either by PyV or by rVSV-LT359, which is cleared after acute infection. As shown in Fig. 2A, circulating LT359-specific T cells in PyV-infected CD8KO mice approached roughly half of the total CD3^+ CD4^- pool by 6 weeks p.i. This increase was not due to the attrition of
non-PyV-specific CD3⁺CD4⁻ T cells, as the frequency of this population remained stable through persistent infection (data not shown). As a fraction of total circulating lymphocytes, the frequency of LT359-specific T cells in HR-CD8KO mice eventually reached that of B6 mice by 6 weeks p.i. (Fig. 2B). A sizeable number of PyV-infected CD8KO mice fell into the LR category (Fig. 2A and B). Interestingly, this mouse-to-mouse variability in LT359-specific T-cell responses in PyV-infected CD8KO mice was recapitulated by rVSV-LT359 infection (Fig. 2C and D). Such individual variability in Ag-specific T-cell responses, in the context of heterologous viral infections, points toward peripheral TCR repertoire differences among CD8KO mice. Also, in contrast to the “inflationary” LT359-specific T-cell response in PyV-infected CD8KO mice, the response in HR-CD8KO mice to rVSV-LT359 infection declined over time (Fig. 2C). This result suggests that persistent Ag is required for the progressive increase of LT359-specific T cells.

We next asked whether the capacity of CD8KO mice to generate an MHC-I-restricted response was limited to the LT359 epitope. CD8KO mice were inoculated with a recombinant PyV (encoding amino acids 257 to 264 of chicken ovalbumin [SIINFEKL]), which efficiently elicits a K*+-restricted T-cell response in B6 mice (Fig. 2E and data not shown). As shown in Fig. 2E, K*SIINFEKL tetramers stained CD3⁺ splenic T cells in CD8KO mice infected with this recombinant PyV. Thus, CD8KO mice are capable of mounting MHC-I-restricted T-cell responses to infection with unrelated viruses and directed to different specificities.

**Functional deficits of MHC-I-restricted, PyV-specific T cells in CD8KO mice.** MHC-I-restricted PyV-specific T cells in CD8KO mice exhibited a defect in cytokine effector capability. At both day 21 (Fig. 3A, top panel) and 6 weeks p.i. (Fig. 3A, bottom panel), only 50 to 60% of D*LTLT359 tetramer⁺ splenic CD3⁺CD4⁻ T cells in CD8KO mice produced IFN-γ after in vitro peptide stimulation, while nearly all D*LTLT359 tetramer⁺ T cells in B6 mice did so. Moreover, of those CD8KO T cells making IFN-γ, per-cell production was significantly lower than that in B6 mice at day 21 p.i. (Fig. 3B, top panel), although differences in IFN-γ production at 6 weeks p.i. were not statistically significant (Fig. 3B, bottom panel). The fraction of peptide-stimulated IFN-γ⁺ T cells coproducing either interleukin-2 or tumor necrosis factor alpha was the same for both CD8KO and B6 mice at both of these time points (data not shown). PyV-infected CD8KO mice were also capable of eliminating LT359 peptide-pulsed splenic target cells in vivo during both acute and persistent phases of infection, but with less efficiency and greater intermouse variability than those of infected B6 mice (Fig. 4). Individual differences in cytotoxic capabilities among CD8KO mice generally correlated with the magnitude of the LT359-specific T-cell response (Fig. 4B). Despite these differences in effector function, similar frequencies of CD127 (interleukin-7Rα), KLRG1, and PD-1 expression on D*LTLT359-specific T cells were obtained for B6 and CD8KO mice at day 21 (Fig. 5) and 6 weeks p.i. (data not shown).

**PyV-specific T cells from wild-type and CD8KO mice have similar avidities.** The efficiency with which a given Ag-specific CD8 T-cell population binds its cognate pMHC represents one measure of T-cell avidity (6, 9, 43). Interestingly, CD8-deficient T cells were better able to bind D*LTLT359 tetramers under conditions of ligand saturation, as shown by higher mean fluorescence intensities (MFI) (Fig. 6A, left and middle panels). TCR expression levels do not explain this difference, however, because D*LTLT359 tetramer⁺ T cells in B6 and CD8KO mice showed equivalent levels of staining by CD3ε (data not shown) and TCRβ chain MAb (Fig. 6A, right panel). Moreover, D*LTLT359 tetramers stain CD3⁺CD4⁻ cells from both HR-CD8KO and LR-CD8KO mice with similar MFI (data not shown), suggesting that LT359-specific T cells in these mice are not qualitatively different. Despite this difference in tetramer binding, when normalized to the number of spleen cells stained by saturating amounts of tetramer, spleen cells from PyV-infected CD8KO and B6 mice stained equivalently with D*LTLT359 tetramers over a broad titration range (Fig. 6B). Using intracellular IFN-γ as a readout of T-cell activation, we found that graded doses of LT359 peptide stimulated similar proportions of Ag-specific T cells in wild-type and CD8KO mice (Fig. 6C). Anti-CD8a inhibited IFN-γ production by LT359 peptide-stimulated spleen cells from B6 mice, indicating that CD8 molecules operate as coreceptors for these PyV-specific T cells (data not shown). An important caveat to these functional assays, however, is that only ∼50% of the LT359-specific T cells in CD8KO mice produce IFN-γ (Fig. 3A). Taken together, these data suggest that the presence or absence of CD8 does not appear to contribute to the avidity of the LT359-specific T-cell population, although a lack of coreceptor engagement is associated with impaired IFN-γ effector activity (Fig. 3).

**D*LTLT359-specific T-cell turnover rates are similar in B6 and CD8KO mice.** CFSE-labeled spleen cells from PyV-infected B6 and CD8KO mice at day 21 p.i. were transferred to infection-matched B6 and CD8KO recipients, respectively, and...
their survival and proliferation were monitored in the blood of individual mice. Based on the absence of CFSE dilution, neither wild-type nor CD8-deficient donor LT359-specific T cells underwent cell division over the 5-week period of examination (data not shown). As we have previously seen (39), there was progressive attrition of donor LT359-specific T cells in persistently infected B6 mice (Fig. 7). The same fate held for donor LT359-specific T cells in persistently infected CD8KO mice, with the survival curves for the transferred D\(^\text{L}\)LT359 tetramer\(^+\) cells being statistically indistinguishable between CD8KO and B6 mice (Fig. 7). Thus, the absence of CD8 does not alter the turnover kinetics of chronic memory, MHC-I-restricted PyV-specific T cells.

**LT359-specific T cells from PyV-infected CD8KO mice have highly restricted V\(\beta\) TCR usage.** Because thymic output of MHC-I-restricted TCR\(\beta^+\)CD4\(^+\)CD8\(^-\) cells is reduced in CD8KO mice (15), we asked whether the absence of CD8 coreceptors would constrain peripheral T-cell repertoire diversity for PyV-specific MHC-I-restricted T cells. In naïve mice, V\(\beta\) expression profiles of CD4 T cells in B6 and CD8KO mice were diverse and largely overlapped (Fig. 8A, right panel), but the V\(\beta\) expression pattern of CD3\(^+\)CD4\(^-\) T cells in CD8KO mice departed significantly from that of B6 mice (Fig. 8A, left panel). This skewing of the TCR repertoire in CD8KO mice was dramatically revealed by V\(\beta\) MAb staining of LT359-specific T cells. As we previously showed (21), LT359-specific T cells in B6 mice displayed a diverse TCR V\(\beta\) profile that narrowed somewhat over the course of persistent infection (Fig. 8B and C, left panels). In marked contrast, during the expansion phase, the vast majority of D\(^\text{L}\)LT359 tetramer\(^+\) T cells in CD8KO mice already expressed a very limited range of V\(\beta\) elements (Fig. 8B, right panel), which differed among individual mice and was still evident through late times after infection (Fig. 8C, right panel). These data suggest that CD8 deficiency limits TCR diversity either at the thymocyte selection stage or through preferential expansion of certain LT359-specific T-cell clonotypes during PyV infection.

**DISCUSSION**

In this study, we demonstrate that CD8KO mice infected by PyV generate a virus-specific, MHC-I-restricted, T-cell response. In contrast to the case for wild-type mice, PyV-specific MHC-I-restricted T cells in CD8KO mice were detectable only after a considerable lag and then progressively expanded in the majority of CD8KO mice. Ag persistence appeared to drive this expansion of MHC-I-restricted T cells, since such “inflation” did not occur following acute infection with an rVSV carrying the dominant CD8 T-cell PyV epitope. During the persistent phase of PyV infection, however, CD8KO and wild-type B6 mice maintained similar numbers of MHC-I-restricted dominant epitope-specific T cells. Interestingly, unlike the generally diverse TCR repertoire of the anti-PyV CD8 T-cell response in B6 mice, the virus-specific D\(^\text{L}\)-restricted T-cell re-
response in CD8KO mice was typically comprised of cells expressing only a single TCR Vβ domain. In light of the importance of virus-specific CD8 T cells in protection against PyV tumorigenesis (5, 13, 26, 28), the ability of CD8KO mice to mount a virus-specific, MHC-I-restricted, T-cell response provides a possible explanation for the resistance of these mice to PyV-induced tumors.

Early studies were unable to detect MHC-I-restricted T-cell responses ex vivo against LCMV or VSV in CD8KO mice by using 51Cr release cytotoxicity assays (3, 14, 15). The low sensitivity of this assay generally fails to detect memory cytotoxic T cells in wild-type mice that are readily visualized by MHC-I tetramers and in vivo cytotoxicity assays (7). Additionally, given the delayed expansion of virus-specific MHC-I-restricted T cells in CD8KO mice (Fig. 1B), investigators in these previous studies (3, 14) may have assayed for LCMV-specific cytotoxic T lymphocytes (CTLs) too early after infection. Low-level, alloreactive MHC-I-restricted responses in CD8KO mice have previously been described for a skin transplantation model (8). As in persistent viral infection, allogeneic skin grafts provide chronic antigenic stimulation that may help drive the expansion of the low number of naïve MHC-I-restricted T cells in CD8KO mice to detectable levels.

Despite variability among individual CD8KO mice in the size of PyV-specific, MHC-I-restricted, T-cell responses, these mice exhibited comparable efficiencies in controlling infection. Sera from infected B6 and CD8KO mice, irrespective of...
DbLT359 T-cell response levels, had similar titers of hemagglutination inhibition activity, indicating no differences in virus-neutralizing antibody levels (data not shown). PyV elicits both CD4 T-cell-dependent and -independent antiviral humoral responses, which, while promoting viral clearance, are not sufficient to prevent PyV tumorigenesis (1, 13, 27, 37, 38). We recently reported that MHC-II-deficient B6 mice, which maintain a small, chronic memory PyV-specific CD8 T-cell population, do not develop PyV-induced tumors (22); thus, even a small functional MHC-I-restricted T-cell response, as seen in the LR-CD8KO mice, would also likely be sufficient for PyV tumor resistance. Nevertheless, 20 to 30% of PyV-infected CD8KO mice have previously been reported to develop hind limb paralysis (13), which is associated with PyV-induced vertebral tumors (19, 41). Coincidentally, this frequency is similar to that of DbLT359-specific LR-CD8KO mice, raising the possibility that there may be some breakthrough tumors in the LR-CD8KO mice months after infection.

Previous work suggests that MHC-I-restricted T cells in CD8KO mice may compensate for the absence of CD8 by positively selecting thymocytes having high-affinity TCRs (18, 33). In support of this idea, CD8KO T cells bound D plethora tetramers more efficiently on a per-cell basis (Fig. 6A). However, these T cells did not exhibit greater sensitivity to Ag (Fig. 6C) or enhanced tetramer binding under conditions of limited ligand availability (Fig. 6B), two characteristics of high-avidity T cells (9, 11, 43). These data suggest that CD8-deficient, LT359-specific T cells do not possess higher-affinity TCRs. While the higher DbLT359 tetramer MFI in CD8KO mice cannot be explained by elevated TCR surface expression, the topological arrangement of TCRs has been shown to enhance MHC-I tetramer binding (12). Such compensatory mechanisms may be required to permit positive selection of MHC-I-restricted thymocytes in the absence of CD8.

The absence of CD8 coreceptor engagement may also be responsible for the depressed effector competence of virus-specific MHC-I-restricted T cells in the CD8KO mice. Stimulation with pMHC lacking the CD8 binding site results in

![FIG. 8. Vβ TCR repertoire analysis of naïve and LT359-specific T cells from B6 and CD8KO mice. Spleen cells were stained with DβLT359 tetramer, anti-CD3, anti-CD4, and the indicated Vβ TCR MAbs. (A) Naïve CD3⁺CD4⁻ (left panel) or CD3⁺CD4⁺ (right panel) T cells. (B) CD3⁺CD4⁻ T cells at day 21 post-PyV infection from B6 (left panel) or CD8KO (right panel) mice. All commercially available MAbs specific for TCR Vβ chains were tested. For the sake of clarity, Vβ chains 8.3 through 17 are not shown, as they were not expressed by CD8-deficient LT359-specific T cells at day 21 p.i. (C) CD3⁺CD4⁺ T cells at 6 weeks post-PyV infection from B6 (left panel) or CD8KO (right panel) mice. * P value was ≤0.05. Data for naïve mice are the means for three animals per group. For PyV-infected mice, each bar pattern represents an individual animal.]
incomplete TCR zeta-chain phosphorylation and only partial activation of T-cell effector functions (24, 32), and TCR signaling is handicapped by the absence of CD8-associated p56^lck^-mediated amplification (34). Similarly, during an infection by vaccinia virus or *Listeria monocytogenes*, the transient down-regulation of CD8 results in decreased sensitivity to Ag (42). In our system, only approximately half of LT359-specific T cells from CD8KO animals were competent to produce IFN-γ (Fig. 3A). Although T-cell effector integrity is degraded in chronically infected hosts (16, 17, 25, 40), impaired IFN-γ production by CD8-deficient T cells was also seen after acute infection with rVSV-LT359 infection (data not shown). In vivo CTL assays showed that CD8-deficient T cells are less sensitive to low levels of cognate peptide during the acute phase of infection, a characteristic of low-avidity CTLs (13); a confounding variable, though, is that there were fewer D^3^LT359-specific T cells in CD8KO mice at this time point. In persistently infected CD8KO mice, however, T cells were much less efficient killers despite the presence of equivalent numbers of splenic LT359-specific T cells in B6 and CD8KO mice. The cytotoxicity effector apparatus appeared to be unaffected, as LT359-specific T cells in B6 and CD8KO mice had equivalent levels of granzyme B staining and abilities to moblize CD107, a marker of degranulation (data not shown). Our data point toward an autonomous defect in TCR signaling by CD8-deficient virus-specific T cells.

A central finding was the profound oligoclonality of the MHC-I-restricted PyV-specific response in CD8KO mice. The availability of only a few clonotypes among naive, anti-PyV, MHC-I-restricted T cells is a likely consequence of inefficient thymic selection for MHC-I-restricted thymocytes in the absence of CD8 (15). Without the contribution of the CD8 coreceptor, only a limited number of TCRs may possess sufficient affinity to transmit a positive selection signal. Stochastic selection of such a constrained repertoire of PyV epitope-reactive T-cell clonotypes would fit the mouse-to-mouse variability of Vβ expression profiles by antiviral MHC-I-restricted T cells in CD8KO mice. In support of this scenario, Jenkins and coworkers recently demonstrated that the Vβ expression profile of CD4 T cells that expand in response to a defined immunogen can be traced to that of the naive Ag-specific CD4 T-cell population and, interestingly, that low frequencies of naive epitope-specific CD4 T cells are associated with narrow and variable Vβ TCR expression profiles among individual mice (30). Moreover, small differences in numbers of naive Ag-specific CD4 T cells were found to translate to marked differences in the size of the effector T-cell response. The observed variability in PyV-specific, MHC-I-restricted, T-cell responses among CD8KO mice may similarly reflect individual host differences in the number of antiviral naive T cells. Another, not mutually exclusive, possibility is that the oligoclonality of MHC-I-restricted anti-PyV T cells in CD8KO mice may result from preferential expansion and maintenance of particular clonotypes during acute and persistent phases of PyV infection.

We recently demonstrated that naive virus-specific CD8 T cells are continuously primed during persistent infection (21, 39). Ongoing recruitment, but drawing upon a limited number of naive virus-specific T cells, may account for the delay in detecting D^3^LT359 tetramer^+^ T cells in CD8KO mice. As was seen in B6 mice, PyV-specific MHC-I-restricted T cells in CD8KO mice failed to proliferate and underwent progressive deletion when transferred to persistently infected recipients. Based on reduced virus-specific CD8 T-cell numbers in persistently infected thymectomized B6 mice, we proposed that re-supply of the naive T-cell compartment by recent thymic emigrants contributed to the maintenance of anti-PyV CD8 T cells during persistent infection (39). If thymic output were essential for the maintenance of chronic memory CD8 T cells in CD8KO mice, we would expect the marked oligoclonality of the PyV-specific T-cell population to diminish over time, as recent thymic emigrants expressing different Vβ TCRs were recruited into the preexisting MHC-I-restricted, antiviral T-cell pool. Yet, in CD8KO mice, MHC-I-restricted PyV-specific T cells retain a highly focused Vβ profile late into persistent infection. This finding raises the possibility that the trickle of MHC-I-restricted recent thymic emigrants in CD8KO mice, with the resulting small reserve of CD3^+^CD4^-^ naive T cells, might direct these hosts to rely on a self-renewing, extrathymic nonsplenic source to maintain chronic memory, MHC-I-restricted PyV-specific T cells. Alternatively, the naive T-cell compartment in CD8KO mice may be populated with a sufficient number of PyV-specific MHC-I-restricted T cells, albeit clonotypically constrained, to permit ongoing de novo recruitment of antiviral T cells that also give rise to effector/memory T cells having a narrow Vβ expression profile.

Our description of MHC-I-restricted T-cell responses in CD8-deficient mice provides an explanation for the relative resistance to intracellular microbial infection of mice and humans lacking this T-cell coreceptor (10, 13). Moreover, the results of this study have practical implications for the use of CD8KO mice to investigate the contribution of MHC-I-restricted T-cell responses in tumor and infection models.

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**REFERENCES**


