Cutting Edge: Shift in Antigen Dependence by an Antiviral MHC Class Ib-Restricted CD8 T Cell Response during Persistent Viral Infection

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The CD8 T cells response to acutely cleared viral infections are characterized by rapid expansion followed by dramatic contraction and differentiation into memory cells that self-renew in a cytokine-dependent, Ag-independent manner (1–5). In contrast, memory CD8 T cells in persistent viral infections may suffer defects in homeostatic proliferation, with the severity of this dysfunction associated with level, duration, and pathogenesis of the infection (6, 7). For example, maintenance of antiviral memory CD8 T cells in high-level systemic chronic lymphocytic choriomeningitis virus (LCMV) infection requires cognate Ag but not IL-7 and IL-15 (8). Low-level systemic viral infections, however, appear to inflict a different insult on antiviral CD8 T cell responses. Depending on their epitope specificity, antiviral CD8 T cell numbers in mice infected by murine CMV (MCMV) increase over the course of infection and then stabilize at high frequencies, a phenomenon termed memory inflation (9, 10). Similarly, CMV-specific CD8 T cells in humans accumulate throughout an individual’s lifetime (11). Conventional MHC class Ia-restricted antiviral CD8 T cells in mice persistently infected by polyoma virus (PyV) fail to divide and are gradually lost, with the maintenance of stable numbers of antiviral CD8 T cells requiring ongoing recruitment of virus-specific, naive CD8 T cell progenitors (12).

Using the PyV infection mouse model, we recently uncovered a novel protective MHC class Ib-restricted CD8 T cell response whose expansion profile differs dramatically from that of conventional class Ia-restricted anti-PyV CD8 T cells (13). These unconventional CD8 T cells recognize a peptide derived from aa 139–148 of the PyV VP2 capsid protein (VP2,139) presented by the nonpolymorphic molecule Q9, a member of the Qa-2 family of class Ib molecules. In PyV-infected MHC class Ia-deficient mice, the Q9/VP2,139-specific CD8 T cell response progressively expands for ~12 wk, then enters a long-term plateau phase. In this study, we tested the hypothesis that cognate Ag regulates the inflation of these MHC class Ib-restricted antiviral CD8 T cells.

Materials and Methods

Mice

C57BL/6Gr (B6) female mice were purchased from the National Cancer Institute (Frederick, MD), B6.Kb−/−Dª−/− (Kb−/−Db−/−) mice (Thy1.2) were obtained from Taconic Farms; Kb−/− Db−/− Thy1.1 (14) mice were provided by P. Jensen (University of Utah, Salt Lake City, UT). Mice were bred and housed by the Division of Animal Resources at Emory University (Atlanta, GA) in accordance with the guidelines of the Institutional Animal Care and Use Committee of Emory University. Mice were 6–8 wk old at the time of infection.

Viruses and cell transfers

Kb−/−Db−/− and B6 mice were infected s.c. with 1 × 106 PFU of PyV. A recombinant vaccinia virus (VV) carrying the PyV VP2 gene (VV-VP2) (15) was provided by R. Consigli (Kansas State University, Manhattan, KS); Kb−/−Db−/− mice received 1 × 106 PFU of VV-VP2 i.p. The A2.H145A mutant virus was created as described (13). Splenocytes from PyV-infected mice were injected intravenously into normal B6 mice.

Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; MCMV, mouse CMV; p.i., postinfection; PyV, mouse polyomavirus; VV, vaccinia virus.
Kb−/−D3−/− Thy1.1 mice were B cell depleted, labeled with 5 μm CFSE, and injected i.v. (50 × 10^6 cells) into infected Kb−/−D3−/−Thy1.2 mice.

Flow cytometry

Anti-CD3ε, anti-CD8α, anti-Thy1.1, anti-Ki67, annexin V, propidium iodide, 7-aminoactinomycin D (7-AAD) and a TCR Vβ Ab panel were purchased from BD Biosciences and used as described (16). Q9/VP2.139 tetramers were constructed using either cloned full-length Q9 cDNA (17) or Q9 cDNA encoding the α3 domain of H-2Db in place of that of Q9. Both tetramers stained equivalent percentages of splenocytes from PyV-infected Kb−/−D3−/− mice with the same mean fluorescence intensity and were used interchangeably as described (13). Samples were acquired on a FACSCalibur flow cytometer (BD Biosciences) and data were analyzed using FlowJo software (Tree Star).

Bone marrow chimera

Persistently infected Kb−/−D3−/− mice given 600 μg of busulfan (Busulfex; Otsuka America Pharmaceutical) i.p. were injected i.v. 24 h later with 25 × 10^6 CD3-depleted bone marrow cells from Kb−/−D3−/−Thy1.1 mice. After CD3 depletion using anti-CD3ε and MACS sorting, only 0.4% of mononuclear cells expressed CD3.

Results and Discussion

Persistently infected with the Q9/VP2.139-specific CD8 T cell inflammatory response

PyV-infected Kb−/−D3−/− mice generate a VP2.139-specific CD8 T cell response that progressively increases over the first 3 mo after infection (13). Inflationary CD8 T cell responses have been observed in several different persistent viral infections, with one report showing that persistent infection is necessary for the prolonged expansion of Ag-specific cells (16). To determine whether persistent viral infection was necessary for the protracted expansion of VP2.139-specific CD8 T cells, we compared the Q9/VP2.139-specific CD8 T cell response longitudinally in the blood of individual Kb−/−D3−/− mice infected by either PyV, which establishes a persistent infection.

FIGURE 1. VP2.139-specific CD8 T cell expansion is associated with persistent viral infection. Percentage of Q9/VP2.139 tetramer−/− CD8 T cells in the blood of PyV or VV-VP2-infected Kb−/−D3−/− mice ± SEM over time (n = 3 mice). Data are representative of two independent experiments.

FIGURE 2. De novo priming of VP2.139-specific CD8 T cells during persistent infection. Representative dot plots of lymphocytes isolated from the indicated organs of Thy congenic mice 50 days after bone marrow transfer (n = 3–4 mice) are shown. Plots are gated on CD8 T cells and the numerical values indicate the percentage of donor tetramer+ cells of the total tetramer+ population. Data are representative of two independent experiments.

FIGURE 3. Dynamic phenotype of VP2.139-specific cells over the course of persistent PyV infection. Splenic Q9/VP2.139 tetramer−/− cells from PyV-infected Kb−/−D3−/− mice were analyzed for Kb67 (A) or annexin V and 7-AAD coexpression (B) at 1 and 3 mo p.i. A, Plots are gated on CD8+ cells and numerical values indicate the percentages of Kb67+ tetramer−/− cells. B, Plots are gated on tetramer+ cells and numerical values indicate the percentage of cells in the indicated quadrant. Splenic D3+IL359 tetramer−/− cells from B6 mice at day 8 p.i. were analyzed for Annexin V and propidium iodide staining as a positive control. C, Dot plots in A and B are representative of mice in these panels, which indicate the percentage of Kb67+ (left) or annexin V+ 7-AAD+ (right) of tetramer+ CD8 T cells at the indicated time points (n = 5 mice). D, TCR Vβ expression by splenic CD8 T cells from uninfected Kb−/−D3−/− mice (left panel) and by Q9/VP2.139 tetramer+ CD8 T cells from Kb−/−D3−/− mice on day 35 p.i. (right panel). Each bar pattern represents an individual mouse. Two independent experiments were performed.
Two independent experiments were performed. During persistent PyV infection in wild-type B6 mice, de novo primed CD8 T cells resupply the short-lived MHC class Ia-restricted CD8 T cells and thereby maintain stable numbers of these antiviral T cells (12). We asked whether naive Q9/VP2.139-specific CD8 T cells similarly contribute to the inflationary response of VP2.139-specific CD8 T cells. To do this, Kb<sup>B</sup>−/−D<sup>b</sup>−/− mice underwent minimal myeloablative busulfan conditioning midway through the Q9/VP2.139-specific CD8 T cell expansion phase (day 35 postinfection (p.i.)), followed by an injection of T cell-depleted, Thy congenic K<sup>B</sup>−/−D<sup>b</sup>−/− bone marrow. Fifty days after bone marrow transfer (which just precedes the plateau phase), donor-derived Thy1.1<sup>+</sup>Q9/VP2.139 tetramer<sup>+</sup> CD8 T cells were detected, but they accounted for only a small fraction of the total VP2.139-specific CD8 T cell response (Fig. 2). In contrast, virus-specific CD8 T cells recruited in persistently infected wild-type B6 mice constitute 10–14% of the total dominant, PyV epitope-specific, MHC-Ib-restricted CD8 T cell population (18). These results indicate that naive Q9/VP2.139-specific CD8 T cells are indeed recruited during the protracted expansion phase but that this process does not fully account for the dramatic inflation of this antiviral MHC-Ib-restricted CD8 T cell response.

Expansion phase VP2.139-specific CD8 T cells are highly proliferative

We next investigated the relative contributions of proliferation and survival of VP2.139-specific CD8 T cells over the course of their long-term expansion phase. Previously, we had observed that 3 mo p.i. VP2.139-specific CD8 T cells no longer expand but are maintained at high numbers (13). We therefore compared inflation phase VP2.139-specific CD8 T cells (1 mo p.i.) to those from the plateau phase (3 mo p.i.) for the expression of molecules marking cell proliferation and survival. A larger fraction of inflation phase VP2.139-specific CD8 T cells expressed Ki67, a cell cycle-related nuclear protein, than those in the plateau phase (Fig. 3, A and C). In contrast, few VP2.139-specific CD8 T cells in either phase of the response stained with annexin V, a marker of apoptosis (Fig. 3, B and C); the anti-apoptotic protein Bcl-2 was expressed by similar frequencies of Q9/VP2.139 tetramer<sup>+</sup> CD8 T cells and at comparable mean fluorescence intensity at 1 and 3 mo p.i. (P. A. Swanson, unpublished observations). These phenotypic data indicate that VP2.139-specific CD8 T cells survive long term in a nonproliferative or low proliferative state without appreciable cell death during the plateau phase.

The strikingly narrow expression of different TCR Vβ families by Q9/VP2.139 tetramer<sup>+</sup> CD8 T cells in individual
Kb<sup>b</sup>−/−D<sup>b</sup>−/− mice compared with the diverse Vβ family usage by CD8 T cells in uninfected Kb<sup>b</sup>−/−D<sup>b</sup>−/− mice (Fig. 3D) further suggests that a particular public clonotype of Q9/VP2.139-specific CD8 T cells does not preferentially expand and dominate this antiviral T cell population. Interestingly, CD8α<sup>b</sup>−/− mice mount an MHC class Ia-restricted, PyV-specific T cell response having a similar dramatic narrowing of Vβ expression, with Vβ usage differing between individual mice (16). A salient feature of the Q9 structure, which is otherwise highly homologous to MHC-Ia molecules, is the deviated orientation of an αβ domain loop that renders CD8αα-like binding inefficient (19, 20). Weak to absent (as in CD8α<sup>b</sup>−/− mice) CD8 coreceptor engagement may permit only a trickle of MHC-I-restricted thymic emigrants, with a consequent small oligoclonal reserve of naïve anti-PyV T cell precursors.

Ag is required for VP2.139-specific CD8 T cell proliferation, but not maintenance

To directly investigate the proliferative state and survival of Q9/VP2.139-specific CD8 T cells during PyV infection, we longitudinally monitored the fate of CFSE-labeled Q9/VP2.139 tetramer<sup>+</sup> CD8 T cells from donor Kb<sup>b</sup>−/−D<sup>b</sup>−/− mice at 1 mo p.i. (inflation phase) or 3 mo p.i. (plateau phase) following transfer into infection-matched Thy congenic Kb<sup>a</sup>-restricted recipients (Fig. 4A). For the 1 mo p.i. donor-to-recipient adoptive cell transfers, the frequency of donor VP2.139-specific CD8 T cells steadily increased over the 30-day post-transfer observation period (Fig. 4A) and this was accompanied by substantial cell division as indicated by CFSE dilution (Fig. 4C). In contrast, the donor Q9/VP2.139 tetramer<sup>+</sup> CD8 T cells exhibited minimal expansion in the 3 mo p.i. donor-to-recipient adoptive cell transfers (Fig. 4A) and failed to divide (Fig. 4C). To exclude the possibility that VP2.139-specific CD8 T cells from the plateau phase suffer a cell-intrinsic proliferation defect, we transferred CFSE-labeled splenocytes from 3 mo p.i. mice to 1 mo p.i. mice. In this experimental setup, VP2.139-specific CD8 T cells from the plateau phase recapitulated the expansion profile and cell division seen by the inflation phase cells (Fig. 4A). These data further suggest that the failure of the plateau phase cells to proliferate is due to insufficient numbers of Q9/VP2.139 epitope<sup>b</sup> APCs. To test this possibility, splenocytes from Kb<sup>b</sup>−/−D<sup>b</sup>−/− mice infected by wild-type PyV (strain A2) 1 mo earlier were transferred to Thy congenic Kb<sup>b</sup>−/−D<sup>b</sup>−/− mice infected by either the A2 virus or a mutant A2 virus, A2.H145A, in which the dominant Q9-anchoring histidine in the seventh position (from the amino terminus) of the VP2.139 epitope was replaced by alanine. An H145A VP2.139<sub>195-148</sub> analog synthetic peptide fails to compete with the wild-type VP2.139<sub>195-148</sub> peptide in Q9 peptide-binding assays (A. R. Hofstetter and P. A. Swanson, unpublished observations), and infection by the A2.H145A mutant virus does not induce a Q9/VP2.139-specific CD8 T cell response in Kb<sup>b</sup>−/−D<sup>b</sup>−/− mice (13). Unlike VP2.139-specific CD8 T cell transfers from donors 1 mo p.i. with A2 to A2-infected recipients, those donor anti-PyV cells transferred to A2.H145A-infected recipients did not proliferate (Fig. 4C) and yet are stably maintained (Fig. 4B). These findings demonstrate that Ag is required for VP2.139-specific CD8 T cell expansion but is dispensable for cell survival.

The phenotype and longevity of expansion phase PyV-specific, MHC class Ib-restricted CD8 T cells differ from that of the inflationary epitope-specific CD8 T cells in MCMV infection. Those epitope-specific CD8 T cells that undergo progressive expansion during persistent MCMV infection are mostly short-lived effector cells that are replenished primarily from memory cells primed during the early stages of infection (21, 22). These MCMV-specific CD8 T cells do not express costimulatory molecules such as CD27 and CD28, nor do they express receptors for the homeostatic cytokines IL-7 and IL-15, which could account for their inability to survive long term (21, 22). In contrast, the inflationary VP2.139-specific CD8 T cells are long lived and express CD127, CD122, and Bcl-2 (Ref. 13 and P. A. Swanson, unpublished observations). Of note, VP2.139-specific CD8 T cells eventually reach stable high frequencies and are maintained in the absence of homeostatic proliferation. The long-term nonproliferative state of these T cells is reminiscent of LCMV-specific memory CD8 T cells that reside in the intestinal epithelium (23). Whether the differences between inflationary VP2.139-specific and MCMV-specific CD8 T cell responses reflect differences at the level of virus-host interaction or MHC class Ia vs Ib Ag presentation remains to be determined.

The mechanism by which Ag controls memory CD8 T cell responses may also differ depending on the level of persistent infection. Ag appears to play a dual role in the CD8 T cell response in high-level LCMV clone 13 infection. High levels of Ag during early stages of LCMV clone 13 infection results in the selective culling of antiviral CD8 T cells of particular specificities (6, 24, 25), whereas CD8 T cells directed to other viral epitopes are maintained by Ag-driven proliferation (8). During a low-level persistent viral infection such as MCMV, stable memory virus-specific CD8 T cell responses do not require Ag for homeostatic proliferation or survival, but those that undergo inflation are highly dependent on Ag for expansion (21). Conventional MHC Ia-restricted, PyV-specific memory CD8 T cells do not homeostatically proliferate and are short lived, with the antiviral response maintained during persistent infection by the recruitment of PyV-specific naïve CD8 T cells (12). The Ag-dependent inflation and Ag-independent maintenance of the PyV-specific, MHC class Ib-restricted CD8 T cell response described here reveal a novel pattern of memory CD8 T cell responses to persistent viral infection.

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Disclosures

The authors have no financial conflict of interest.

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