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*J Immunol* 2007; 178:5802-5811; doi: 10.4049/jimmunol.178.9.5802

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*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852

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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Antigen Presentation by Nonhemopoietic Cells Amplifies Clonal Expansion of Effector CD8 T Cells in a Pathogen-Specific Manner

Sunil Thomas, Ganesh A. Kolumam, and Kaja Murali-Krishna

Professional APCs of hemopoietic-origin prime pathogen-specific naive CD8 T cells. The primed CD8 T cells can encounter Ag on infected nonhemopoietic cell types. Whether these nonhemopoietic interactions perpetuate effector T cell expansion remains unknown. We addressed this question in vivo, using four viral and bacterial pathogens, by comparing expansion of effector CD8 T cells in bone marrow chimeric mice expressing restricting MHC on all cell types vs mice that specifically lack restricting MHC on nonhemopoietic cell types or radiation-sensitive hemopoietic cell types. Absence of Ag presentation by nonhemopoietic cell types allowed priming of naive CD8 T cells in all four infection models tested, but diminished their sustained expansion by ~10-fold during lymphocytic choriomeningitis virus and by ≤2-fold during vaccinia virus, vesicular stomatitis virus, or Listeria monocytogenes infections. Absence of Ag presentation by a majority (>99%) of hemopoietic cells surprisingly also allowed initial priming of naive CD8 T cells in all the four infection models, albeit with delayed kinetics, but the sustained expansion of these primed CD8 T cells was markedly evident only during lymphocytic choriomeningitis virus, but not during vaccinia virus, vesicular stomatitis virus, or L. monocytogenes. Thus, infected nonhemopoietic cells can amplify effector CD8 T cell expansion during infection, but the extent to which they can amplify is determined by the pathogen. Further understanding of mechanisms by which pathogens differentially affect the ability of nonhemopoietic cell types to contribute to T cell expansion, how these processes alter during acute vs chronic phase of infections, and how these processes influence the quality and quantity of memory cells will have implications for rational vaccine design. The Journal of Immunology, 2007, 178: 5802–5811.

The CD8 T cells play an important role in clearing intracellular viral and bacterial infections. Naive CD8 T cells that are specific to the pathogen, once primed, undergo clonal expansion, acquire effector functions, alter circulatory properties, and go on to recognize a wide variety of infected targets in both lymphoid and nonlymphoid organs (1, 2). Clonal expansion is important not only for efficient clearance of the pathogen, but also for the generation of a higher number of memory cells that are qualitatively superior compared with their naive counterparts (3, 4). An understanding of the factors that contribute to clonal expansion in response to live infection is critical for rational vaccination and therapeutic strategies.

Intracellularly replicating pathogens can infect a wide variety of cells. However, not every infected cell is capable of priming CD8 T cell responses. Optimal priming of naive CD8 T cells requires presentation of pathogen-derived peptides by specialized professional APCs. The well-characterized professional

APCs, such as dendritic cells and macrophages, are of hemopoietic origin (1, 5–10). These cells have the ability to process and present pathogen-derived peptides, to express costimulatory and inflammatory molecules, and to carry pathogen-derived peptides to the lymphoid tissues through which the naive T cell precursors generally circulate, thereby acting as initiators of adaptive immune responses during infection.

Infected cells of nonhemopoietic origin, despite displaying pathogen-derived peptides, are thought to be inefficient in initiating T cell responses due to their inability to express costimulatory molecules and traffic to lymphoid organs (11).

The primed effector T cells, in contrast to their naive counterparts, are generally less stringent in costimulatory requirements (11–14). This, combined with their ability to circulate through both lymphoid and nonlymphoid organs (2, 15), raises the question of whether effector CD8 T cell interaction with infected targets of nonhemopoietic origin further influences clonal expansion in vivo.

In this study, we addressed this question in vivo using four different pathogens, all of which are known to infect cells of both hemopoietic and nonhemopoietic origin. We found that when we ablated one of the MHC restriction elements specifically in the nonhemopoietic compartment, the expansion of virus-specific CD8 T cells restricted to that particular MHC was diminished by ~90% during lymphocytic choriomeningitis virus (LCMV)3 infection.

1 This work was supported in part by National Institutes of Health Grants 1R01AI053146 and R21AI051386 (to K.M.-K.) and by funds from the Washington National Primate Center and the University of Washington Department of Immunology.

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3 Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; β2m, β2-microglobulin; Lm, Listeria monocytogenes; VSV, vesicular stomatitis virus; VV, vaccinia virus.

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Received for publication December 5, 2006. Accepted for publication February 9, 2007.

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Additional experiments confirmed that cognate interaction of the effector CD8 T cells with nonhemopoietic targets greatly amplified clonal expansion, thereby contributing to the generation of massive numbers of effector CD8 T cells following LCMV infection. By contrast, the ability to sustain the expansion of the primed CD8 T cells was mostly limited to hemopoietic cells during vaccinia virus (VV), vesicular stomatitis virus (VSV), or *Listeria monocytogenes* (Lm) infections.

**Materials and Methods**

**Mice**

C57BL/6 (B6) H-2D\(^{b}\), H-2K\(^{d}\), CD8\(^{b}\), K\(^{b}\), rb-microglobulin (\(\beta_{2}\)-m), B6 Ly5.1 P14 TCR transgenic mice whose CD8 T cells recognize gp33–41 epitope of LCMV in a D\(^{b}\)-restricted fashion, and B6 Thy1.1 OT-1 TCR transgenic mice whose CD8 T cells recognize OVA epitope in a K\(^{b}\)-restricted fashion (18), and B6 mice were used at 4 mo after bone marrow reconstitution. Mice were used at specific pathogen-free conditions at the University of Washington under the guidelines of the Institutional Animal Care and Use Committee.

**Generation of bone marrow chimeras**

B6→B6, B6→D\(^{b}\)/+, and B6→K\(^{b}\)/– chimeric mice were generated by transferring i.v. 7×10\(^{7}\) T and B cell-depleted bone marrow cells from B6 mice into lethally irradiated (950 rad) recipient B6, D\(^{b}\)/+, or K\(^{b}\)/– mice, respectively. D\(^{b}\)/+→B6, K\(^{b}\)/+→B6, or D\(^{b}\)/+→K\(^{b}\)/+→B6 chimeric mice were generated by transferring T and B cell-depleted bone marrow cells from D\(^{b}\)/+, K\(^{b}\)/+, or D\(^{b}\)/+→K\(^{b}\)/+→B6 chimeric mice were generated by transferring T and B cell-depleted bone marrow cells from D\(^{b}\)/+, K\(^{b}\)/+, or D\(^{b}\)/+→K\(^{b}\)/+→B6, respectively, into lethally irradiated recipient B6 mice. Bone marrow chimeric mice were used at 4 mo after bone marrow reconstitution.

**T and B cell depletion**

Bone marrow cells were isolated from tibia and femurs of adult mice by flushing with RPMI 1640 medium with 1% FCS, subjected to RBC lysis, followed by T and B cell depletion by incubating with a mixture of anti-Thy-1 and B220 Abs for 30 min, and followed by washing and incubating with low toxicity guinea pig complement (Cedarlane Laboratories).

**Abs, staining, and tetramers**

All Abs were purchased from either BD Biosciences or eBioscience, except the anti-human granzyme B Ab (clone GB12), which was purchased from Caltag Laboratories. MHC tetramer D\(^{b}\)-gp33–41 was produced, as described (20, 21). Intracellular staining was performed after stimulating 2×10\(^{5}\) spleen cells with 0.1 \(\mu\)g/ml peptide in 96-well flat-bottom plates for 5 h in the presence of brefeldin A, followed by surface and intracellular staining. Intracellular staining was performed by using the intracellular staining kit (BD Biosciences), as per the manufacturer’s instructions (20). Intracellular staining for granzyme B was performed exactly as described above, except that these cells were not in vitro stimulated with peptide.

**CFSE labeling and cell transfers**

Cells were labeled with CFSE by incubating with 5 \(\mu\)M CFSE for 5 min, followed by quenching with fetal calf serum and washing. Total splenocytes containing the appropriate numbers of transgenic CD8 T cells, as indicated in the legends, were transferred i.v.

**Lymphocyte preparation**

Splenocyte preparation was done in RPMI 1640 medium containing 1% FCS, RBC were lysed using 0.84% ammonium chloride, washed, and suspended in RPMI 1640 containing 10% FCS before in vitro stimulation. Liver cell suspension was prepared in RPMI 1640 medium containing 1% FCS and centrifuged in 35% Percoll gradient for 30 min at 4°C at 600 × g. The pellet was subjected to RBC lysis. The cells were washed twice before FACS staining.

**Virus, bacteria, and immunizations**

LCMV (Armstrong) and VSV (Indiana) were plaque purified and grown in baby hamster kidney cells, VV were plaque purified and grown in HeLa cells. rVV expressing OVA or the LCMV glycoprotein (gp33–41) (22), and rVSV (23) and Lm (24) expressing OVA were previously described. Unless otherwise stated, 2×10\(^{3}\) PFU LCMV, 1×10\(^{4}\) PFU VV, or 1×10\(^{4}\) PFU VSV were injected i.p., and 2×10\(^{4}\) CFU Lm were injected i.v. Soluble chicken OVA (0.5 mg/mouse) was administered i.p.

**Results**

**Generation and characterization of bone marrow chimeras**

Several studies have assessed the role of hemopoietic cells in initiating CD8 T cell responses, by either removing the restricting MHC on radiation-sensitive bone marrow-derived cells or by targeted depletion of CD11c-positive cells (5–10, 25–27). These studies unanimously show that hemopoietic cells, especially CD11c-positive cells, are needed for optimal generation of detectable CD8 T cell responses both during immunization with model Ags as well as during infection with various pathogens, including VV, VSV, LCMV, Lm, and influenza A virus, although there was some confusion with reference to LCMV (see discussion in later sections). But none have addressed the consequence of primed CD8 T cell interaction with infected nonhemopoietic targets. To address this, we created radiation chimeras in which the restricting MHC is expressed on all cell types or selectively removed on nonhemopoietic cell types. This system allows us to deduce the effect of Ag presentation by nonhemopoietic cells on the already primed effector CD8 T cells. The following three types of bone marrow chimeras were generated: 1) control B6→B6 chimeras in which MHC class I molecules D\(^{b}\) and K\(^{b}\) are expressed both on hemopoietic and nonhemopoietic cells; 2) B6→D\(^{b}\)/– chimeras in which MHC K\(^{b}\) is expressed on all cell types, but D\(^{b}\) expression is selectively absent in nonhemopoietic cell types; and 3) B6→K\(^{b}\)/– chimeras in which MHC D\(^{b}\) is expressed on all cell types, but K\(^{b}\) expression is selectively absent in nonhemopoietic cell types. Chimerism was evaluated by staining spleen cells for MHC expression 4 mo after reconstitution. More than 98% of the CD11c\(^{+}\) spleen cells from the B6→B6, B6→D\(^{b}\)/+, as well as B6→K\(^{b}\)/– mice were positive for expression of both D\(^{b}\) and K\(^{b}\), confirming that the chimeras were properly reconstituted (Fig. 1A).

Lack of Ag presentation by nonhemopoietic cell types drastically reduces LCMV-specific polyclonal CD8 T cell response in an allele-specific manner

To assess D\(^{b}\)- and K\(^{b}\)-restricted polyclonal CD8 T cell responses in the chimeras, B6→B6, B6→D\(^{b}\)/+, and B6→K\(^{b}\)/– mice were infected with LCMV, and the D\(^{b}\)- and K\(^{b}\)-restricted CD8 T cell responses were analyzed at day 8 postinfection. The unmanipulated B6, D\(^{b}\)/+, or K\(^{b}\)/– mice infected with LCMV were used as controls.

On day 8 postinfection, spleen cells were stimulated with either D\(^{b}\)-restricted LCMV peptide nucleoprotein 396–404, or K\(^{b}\)-restricted LCMV peptide nucleoprotein 205–212, and then stained for intracellular IFN-\(\gamma\) and TNF-\(\alpha\). B6→D\(^{b}\)/– mice, similar to the D\(^{b}\)/– mice, elicited a much more robust K\(^{b}\)-restricted CD8 T cell response than the control B6→B6 mice (Fig. 1B, fourth panel in bottom row), whereas the D\(^{b}\)-restricted response was markedly reduced (Fig. 1B, fourth panel in middle row). This pattern was reversed in B6→K\(^{b}\)/– mice. In these mice, the D\(^{b}\)-restricted CD8 T cell response was increased compared with the control B6→B6 mice (Fig. 1B, sixth panel in middle row), whereas K\(^{b}\)-restricted CD8 T cell response was markedly reduced (Fig. 1B, sixth panel in bottom row).

A compensatory increase in D\(^{b}\)-restricted response in mice lacking K\(^{b}\), and vice versa, has been reported (16), and our results are consistent with these studies. The diminished D\(^{b}\)-restricted CD8 T cell response in the B6→D\(^{b}\)/+ mice and the diminished K\(^{b}\)-restricted CD8 T cell response in the
B6→Kb−/− mice suggest a role for nonhemopoietic cell types in determining the magnitude of CD8 T cell response to LCMV. However, previous studies showed that virus-specific CD8 T cells can be generated only against infected targets sharing the same MHC determinant as the thymic stroma, implicating that the presence of the MHC restriction element on thymic stroma is required for efficient thymic positive selection (28). Because the thymic stroma of the B6→Db−/− mice in our experiments do not express Db, the Db-restricted naive CD8 T cell repertoire may not have developed efficiently in these mice. Similarly, the Kb-restricted naive repertoire may not have developed in the B6→Kb−/− mice.

Lack of Ag presentation by nonhemopoietic cell types drastically reduces clonal expansion of adoptively transferred naive TCR transgenic T cells

To overcome the limitations posed by the impact of positive selection on repertoire development, and to stringently test the effect of viral Ag presentation by nonhemopoietic cells, we adoptively transferred congenically marked (Ly5.1+) Db-restricted LCMV-specific TCR transgenic naive CD8 T cells (P14 CD8 T cells) into B6→B6 or B6→Db−/− mice and compared their expansion following LCMV infection.

The Db-restricted donor P14 CD8 T cells, as expected, expanded massively in the spleens of the LCMV-infected B6→B6 chimeras (Fig. 2A, left panel). However, the expansion of the P14 cells was drastically diminished (~10-fold) in LCMV-infected B6→Db−/− mice compared with the B6→B6 mice, either at day 5 (Fig. 2A, right panel) or at day 8 postinfection (Fig. 2B). The diminished expansion of the Db-restricted donor CD8 T cells in B6→Db−/− chimeras is due to a specific absence of Dp on nonhemopoietic cells because P14 cell expansion was not affected in LCMV-infected B6→Kb−/− chimeras (Fig. 2B, ○). The expansion of the donor P14 CD8 T cells in B6→Db−/− chimeras was diminished at all doses of viral inocula tested (Fig. 2C). Thus, the vast majority of the robust CD8 T cell expansion that occurs in response to LCMV infection is mediated by perpetuation of clonal expansion by infected nonhemopoietic cells.
LCMV-specific donor CD8 T cells receiving Ag presentation by hemopoietic cell types only or by a mixture of hemopoietic and nonhemopoietic cell types were similar in phenotype and cytokine function

Because the D\textsuperscript{b}-restricted donor CD8 T cells are likely to receive qualitatively different signals in infected B6→B6 vs B6→D\textsuperscript{b}−/− chimeras, we asked whether these cells differ in phenotype or function. The P14 CD8 T cells responding to LCMV infection were CD43\textsuperscript{high}, CD44\textsuperscript{high}, and CD62L\textsuperscript{low} in both groups (Fig. 3A). The donor P14 effector CD8 T cells were capable of producing IFN-γ (Fig. 3B) and TNF-α (data not shown) upon in vitro peptide stimulation, irrespective of whether they were primed in LCMV-infected B6→B6 or B6→D\textsuperscript{b}−/− mice. Donor P14 effector CD8 T cells from both groups expressed the cytotoxic molecule granzyme B (Fig. 3C).

Together, the above results allow us to deduce that nearly 90% of the robust expansion of the effector CD8 T cells seen in response to LCMV is mediated by Ag presentation by virally infected cell types of the nonhemopoietic compartment. Limiting the Ag presentation to only hemopoietic cell types decreased clonal expansion by ~10-fold, but has little effect on the effector cytokine functions.

Cognate interactions with infected nonhemopoietic cells do not efficiently perpetuate clonal expansion of effector CD8 T cells during VV, VSV, or Lm infections

The level of CD8 T cell expansion is generally more robust in response to LCMV than in response to many other pathogens, including VV, VSV, and Lm in mice (29). To assess the ability of nonhemopoietic cells to perpetuate the effector CD8 T cell expansion in the context of other infections, we performed experiments using VV, VSV, or Lm. We transferred K\textsuperscript{b}-restricted OVA-specific OT-1 CD8 T cells into B6→B6 or B6→K\textsuperscript{b}−/− chimeras. The expansion of the donor OT-1 cells was analyzed after infecting the mice either with rVV expressing OVA (rVV-OVA), with rVSV expressing OVA (rVSV-OVA), or with rLm expressing OVA (rLm-OVA). In the case of rVV-OVA, lack of the MHC restriction element on nonhemopoietic cells reduced donor CD8 T cell expansion only marginally (~2-fold) (Fig. 4A). Similar trends were seen in the case of rVSV-OVA infection (Fig. 4B). In the case of Lm, no reproducible effect was observed in several experiments (Fig. 4C). Similar results as the one with rVV-OVA were obtained when we repeated the experiment with a different VV expressing the LCMV gp33 epitope (rVV-gp33). Whereas there was a substantial contribution of nonhemopoietic cells toward CD8 T cell expansion in LCMV (Fig. 4D), their contribution was <2-fold in the case of rVV-gp33 (Fig. 4E). Together, the above results show that nonhemopoietic cells greatly contribute to the sustained expansion of effector CD8 T cells in LCMV infection, but are less efficient following VV, VSV, or Lm infections.

Ag presentation exclusively by radiation-resistant cells can recruit naive CD8 T cells into proliferation early after infection with LCMV, VV, VSV, or Lm, but the continued expansion of these primed cells is sustained better during LCMV than during VV, VSV, or Lm infections

The above experiments allow us to deduce that cognate interaction of the primed effector CD8 T cells with infected nonhemopoietic
targets greatly perpetuates clonal expansion during LCMV, but such interactions do not perpetuate clonal expansion during VV, VSV, or Lm infections. Two previous studies, by using parent→F1 bone marrow chimeras, found that although expression of restricting MHC only on radiation-resistant cell types does not lead to induction of detectable CD8 T cell responses during three infections (VV, influenza A, and Lm), it does lead to induction of detectable CD8 T cell responses by day 8 LCMV infection (5, 7). These observations lead to the interpretation that Ag presentation by radiation-resistant cells does not prime naive CD8 T cells during VV, influenza, or Lm, but can somehow prime naive CD8 T cells during LCMV infection. But a more recent study, using the approach of targeted depletion specifically of D\textsuperscript{b} X11c-positive cells in B6 + D\textsuperscript{b}−/−,K\textsuperscript{b}−/−→D\textsuperscript{b}−/−,K\textsuperscript{b}−/− chimeras showed that hemopoietic cell types indeed are required for priming CD8 T cell response even during LCMV (25). The discrepancy between this and the earlier studies was explained by speculating that a small number of radiation-resistant host hemopoietic cells that persist in the parent→F1 chimeras would have contributed to efficient recruitment of naive CD8 T cells during LCMV, but not during infection with VV, VSV, or Lm. Alternatively, if this discrepancy was due to the differences in the ability of nonhemopoietic cells in perpetuating clonal expansion of the primed CD8 T cells in LCMV vs the other three pathogens (as suggested by our results above), the residual numbers of radiation-resistant hemopoietic cells should recruit naive CD8 T cells into proliferation early after infection with either LCMV, VV, VSV, or Lm, but then the continued expansion of these recruited cells should be sustained during LCMV, but not during VV, VSV, or Lm infections. We directly tested this by generating B6→B6, B6→D\textsuperscript{b}−/−, K\textsuperscript{b}−/−, and the reverse of the latter two, i.e., D\textsuperscript{b}−/−,K\textsuperscript{b}−/−→B6 or K\textsuperscript{b}−/−→B6 chimeras, and assessing the pattern of naive CD8 T cell activation and recruitment into proliferation soon after infection with various pathogens.

TCR transgenic naive CD8 T cells were transferred into bone marrow chimeric mice expressing restricting MHC on all cell types (B6→B6), on radiation-sensitive hemopoietic cell types (B6→D\textsuperscript{b}−/− or B6→K\textsuperscript{b}−/−), or on radiation-resistant nonhemopoietic cell types plus a minor fraction of hemopoietic cells (D\textsuperscript{b}−/−→B6 or K\textsuperscript{b}−/−→B6). In these reverse (D\textsuperscript{b}−/−→B6 or K\textsuperscript{b}−/−→B6) chimeras, <1% of the CD11c or CD11b-positive spleen cells stained positive for D\textsuperscript{b} or K\textsuperscript{b} expression, respectively, at 4 mo postconstitution (data not shown), and this is consistent with the residual numbers of radiation-resistant host hemopoietic cells seen in radiation chimeras in other studies (5, 6, 7, 9).

Conceivably marked CFSE-labeled D\textsuperscript{b}-restricted P14 cells were transferred into B6→B6, B6→D\textsuperscript{b}−/−, or D\textsuperscript{b}−/−→B6 chimeras at 4 mo post marrow reconstitution. These chimeras were then infected with LCMV. Mice were sacrificed either at 35 or 60 h postinfection. The transferred donor CD8 T cells were assessed for cell size (based on forward scatter), IL-2R α-chain (CD25) expression, and cell division. By 35 h postinfection, there was minimal cell division, but marked CD25 up-regulation and increase in cell size. These activation events were seen in all chimeras, albeit with less efficiency in the D\textsuperscript{b}−/−→B6 chimeras (Fig. 5, middle panel).

By 60 h post-LCMV, all the transferred cells had undergone at least three rounds of cell division, up-regulated CD25, and increase in size in all the chimeras (Fig. 5, lower panel), and in vitro stimulation with P14 peptide-pulsed D\textsuperscript{b}+ feeder cells for 5 h induced IFN-γ production in P14 donor CD8 T cells, irrespective of whether they were primed in B6→B6, B6→D\textsuperscript{b}−/−, or D\textsuperscript{b}−/−→B6 chimeras (data not shown). These results make the following three points: 1) absence of the restricting MHC on nonhemopoietic cell types (as seen in B6→D\textsuperscript{b}−/− chimeras) does not diminish initial recruitment of LCMV-specific naive CD8 T cells (Fig. 5); 2) these recruited cells, however, expand inefficiently at later stages (Fig. 2), because of the lack of nonhemopoietic cell-mediated perpetuation of clonal expansion in B6→D\textsuperscript{b}−/− chimeras; 3) radiation-resistant cell types can also cause initial activation and recruitment of LCMV-specific naive CD8 T cells.

With reference to the initial activation or recruitment of naive CD8 T cells into proliferation, our results with VV and VSV followed trends that were very similar to the ones seen in LCMV. For these experiments, we transferred D\textsuperscript{b}-restricted P14 CD8 T cells into B6→B6, B6→D\textsuperscript{b}−/−, or D\textsuperscript{b}−/−→B6 chimeras and infected them with rVV-gp33. In a different set of experiments, we transferred K\textsuperscript{b}-restricted OT-I CD8 T cells into B6→B6, B6→K\textsuperscript{b}−/−, or K\textsuperscript{b}−/−→B6 chimeras and infected them with rVV-OVA, rVSV-OVA, or rLm-OVA. Data for rVV-gp33 is shown in Fig. 6. Cell division, CD25 up-regulation, and increase in cell size at 35 h postinfection were similar in B6→B6 and B6→D\textsuperscript{b}−/− chimeras, and less efficient, but clearly evident in D\textsuperscript{b}−/−→B6 chimeras. By 60 h post-rVV-gp33, all the transferred cells had undergone at least three rounds of cell division, up-regulated CD25, and increased in size in all the chimeras (Fig. 6, lower panels). Similar results were seen with rVSV-OVA (Fig. 7) and rVV-OVA (data not shown). These results make the following three points: 1) Absence of the restricting MHC on nonhemopoietic (radiation-resistant) cell types as seen in B6→D\textsuperscript{b}−/− or B6→K\textsuperscript{b}−/− chimeras does not diminish initial recruitment of VV or VSV-specific naive CD8 T cells (Figs. 6 and 7). 2) The continued expansion of the primed cells in these chimeras was similar to that seen in B6→B6 chimeras at later...
stages (Fig. 4, A and B), because during VV or VSV infections, the nonhemopoietic cells were anyway inefficient in perpetuating the clonal expansion of the primed CD8 T cells. 3) Surprisingly, the ability of radiation-resistant cell types to cause initial activation and recruitment of naive CD8 T cells is seen not only during LCMV, but also during VV or VSV infections.

In the case of Lm, the initial recruitment events followed the same trends that were seen with VV and VSV; however, we found a much more severe defect in all three parameters tested, i.e., CFSE dilution, CD25 up-regulation, and forward scatter (Fig. 4). This result makes the following two points: 1) Radiation-resistant cell types in DBA/2 mice that express restricting MHC on all cell types (B6→B6) vs mice expressing restricting MHC only on radiation-resistant cell types (DBA/2→B6 or K10→B6). We transferred DBA-restricted P14 CD8 T cells into DBA→B6 or B6→B6 chimeric mice, and compared their expansion following LCMV infection. Despite the delayed kinetics of early recruitment of donor P14 CD8 T cells seen in DBA→B6 chimeras (Fig. 5, middle panel), the sustained expansion of these recruited cells was largely unaffected (Fig. 9A). This result makes the following two points: 1) Radiation-resistant cell types in DBA→B6 chimeras recruit LCMV-specific naive CD8 T cells with somewhat delayed kinetics. 2) These recruited cells expand nearly as much in DBA→B6 chimeras compared with B6→B6 chimeras at later stages because of the perpetuation of their expansion by nonhemopoietic cell types.

By contrast, K10-restricted OVA-specific OT-1 donor CD8 T cells transferred into K10→B6 chimeras did not expand well (compared with B6→B6 chimeras) after infection with either rVV-OVA (Fig. 9B), rLM-OVA (Fig. 9C), or rVSV-OVA (data not shown). Similarly, DBA-restricted LCMV gp33-41 epitope-specific P14 donor CD8 T cells transferred into DBA→B6 chimeras did not expand well (compared with B6→B6 chimeras) after infection with rVV-gp33-41 or rLM-gp33 (data not shown). Together, these experiments demonstrate that radiation-resistant cell types can recruit naive CD8 T cells into response in all the four infection models tested, although with delayed kinetics, but their ability to perpetuate the expansion of the recruited cells was more efficient during LCMV, but less efficient during VV, VSV, or Lm infections. This may perhaps be a contributing factor for the most robust expansion of the effector CD8 T cells typically seen during LCMV, but not during infection with the other three pathogens (29).
Radiation-resistant cell types are also capable of presenting exogenously administered OVA to naive CD8 T cells and causing their initial recruitment into response, but are incapable of sustaining their proliferation.

The above experiments suggested that Ags presented by radiation-resistant cell types can cause early recruitment of naive CD8 T cells into proliferation. Whether these radiation-resistant cell types arise from hemopoietic or nonhemopoietic compartments was unclear. Only <1% of the hemopoietic APCs in these mice was comprised of radiation-resistant host cells expressing the relevant restricting MHC. It was not clear whether such residual numbers were sufficient to cause efficient naive CD8 T cell recruitment. To address this, we transferred Kb-restricted OT-1 cells into Kb

FIGURE 10. Recruitment of CD8 T cells is similar during immunization with LCMV plus soluble OVA, regardless of whether only hemopoietic cells, only nonhemopoietic cells, or a mixture of both cell populations participate in priming. A total of 1 × 10⁶ CFSE-labeled, congenically marked OT1 CD8 T cells was transferred into B6→B6, B6→Kb⁻/+→B6 chimeric mice. Mice were immunized with LCMV along with soluble OVA 24 h later. CFSE dilution, CD25 up-regulation, and forward scatter on donor OT1 CD8 T cells at 35 h or 60 h post-LCMV plus OVA immunization in spleen. Similar results were obtained in a different experiment.

FIGURE 11. Sustained expansion of cross-primed OVA-specific CD8 T cells is deficient in the absence of Ag presentation by a majority of hemopoietic cells, whereas expansion of LCMV-specific cells does not require hemopoietic cells. A total of 100,000 OT1 and 25,000 P14 CD8 T cells was transferred into B6→B6 or D⁻/+→Kb⁻/+→B6 chimeric mice. Mice were immunized with LCMV along with soluble OVA 24 h later. Analysis was done on day 7 postimmunization. Dot plots are gated on CD8 T cells. Numbers within plots indicate fraction of CD8 T cells.
the same host, under conditions in which both the cell populations are initially primed to proliferate by radiation-resistant APCs. For this, we generated D\(^{b}\)/K\(^{b}\)−/−/β\(^{m}\)−/−→B6 chimeras and transferred a mixture of D\(^{b}\)-restricted P14 cells and K\(^{b}\)-restricted OT1 cells into these or B6 mice as controls and immunized them with LCMV plus soluble OVA.

Mice were analyzed on day 7 postimmunization. We found that the OT-1 donor CD8 T cells expanded only in B6 mice, but failed to expand in D\(^{b}\)/K\(^{b}\)−/−/β\(^{m}\)−/−→B6 chimeras. By contrast, the cotransferred LCMV-specific P14 donor CD8 T cells expanded well in both B6 mice as well as in the D\(^{b}\)/K\(^{b}\)−/−/β\(^{m}\)−/−→B6 chimeras (Fig. 11). The presence of P14 response, but the lack of OT-1 response in D\(^{b}\)/K\(^{b}\)−/−/β\(^{m}\)−/−→B6 chimeras makes the following two points: 1) The residual numbers of bone marrow-derived, but radiation-resistant APCs expressing the restricting MHC in the D\(^{b}\)/K\(^{b}\)−/−/β\(^{m}\)−/−→B6 chimeras were sufficient to recruit naive CD8 T cells into response, but insufficient to mediate the sustained expansion of the recruited cells. Thus, despite efficient initial recruitment, no visible level of OVA-specific CD8 T cell response was observed in D\(^{b}\)/K\(^{b}\)−/−/β\(^{m}\)−/−→B6 chimeras at day 7 postimmunization. 2) The LCMV-specific P14 CD8 T cells in the same D\(^{b}\)/K\(^{b}\)−/−/β\(^{m}\)−/−→B6 chimeras, however, continued to expand, resulting in a robust response by day 7, due to the ability of LCMV-infected nonhemopoietic cell types in perpetuating the expansion of the primed effector CD8 T cells.

Discussion

Our study makes the following contributions toward understanding the role of nonhemopoietic cells in CD8 T cell expansion in infections: First, our experiments show that Ag presentation by cells of nonhemopoietic origin can contribute markedly to the clonal expansion of effector CD8 T cells. Second, the ability of nonhemopoietic cells to perpetuate clonal expansion was drastically different depending upon the infectious agent. Nonhemopoietic cells were strikingly efficient in perpetuating effector CD8 T cell expansion during LCMV infection, but are highly inefficient during VV, VSV, or Lm infections. Third, our study shows that the effector CD8 T cells that receive TCR signals exclusively from hemopoietic cells were similar in their phenotypic and cytokine functional aspects to the effector CD8 T cells receiving a mixture of TCR signals from both hemopoietic and nonhemopoietic cells.

It was originally thought that under the conditions of LCMV infection, nonhemopoietic cells are capable of priming naive CD8 T cells. Sigal et al. (6) compared VV-specific polyclonal responses in B6→B6 vs TAP−/−→B6 radiation chimeras, and showed that the TAP−/−→B6 mice failed to generate vaccinia-specific responses. Lenz et al. (5) compared VV, Vm, and LCMV-specific responses in parent→F\(_{1}\) bone marrow chimeric mice, and showed that when the restricting MHC was lacking on radiation-sensitive cells, VV- or Vm-specific polyclonal CD8 T cells were undetectable, whereas LCMV-specific polyclonal CD8 T cells were readily detectable. Three possible, mutually nonexclusive interpretations were offered to explain the difference between VV or Vm vs LCMV infections in these findings, as follows: One was that the LCMV-specific naive CD8 T cell repertoire is somewhat at a higher frequency in these mice. The second was that hemopoietic cells are the only ones that initiate naive CD8 T cells into response during VV or Vm, but nonhemopoietic cells can also initiate naive CD8 T cell response during LCMV. The third explanation was that features of LCMV (e.g., its noncytopathic nature) enable it to selectively use the residual radiation-resistant (but hemopoietic)professional APCs of the host that persist in low numbers following irradiation and reconstitution. Sigal and Rock (7) subjected parent→F\(_{1}\) bone marrow chimeric mice to a second round of radiation and reconstitution (parent→(parent→F\(_{1}\)) and showed that these mice still have <1% residual host bone marrow-derived professional APCs (although this is much lower than the ~5% residual bone marrow-derived APCs seen in single radiation chimeras) and that these (parent→(parent→F\(_{1}\)) chimeras still elicit detectable, but much less of polyclonal CD8 T cell response against LCMV compared with the parent→F\(_{1}\) chimeras, although there were differences in this pattern dependent upon which LCMV epitope-specific responses they were looking at.

One limitation of assessing endogenous polyclonal responses in the above mentioned studies is the possibility of differences in repertoire selection in different chimeras. Another limitation is the lack of knowledge, whether the difference in LCMV vs other infections was due to differences in early naive CD8 T cell recruitment. More recently, Probst and van den Broek (25) used transgenic CD11c/DTR mice that allow targeted depletion of CD11c-positive cells. These authors generated (CD11cDTR\(^{+}\) plus H-2Db\(^{+}\)→H-2Db\(^{+}\)) chimeras, into which they later transferred H-2Db\(^{+}\)-restricted P14 cells, followed by specific depletion of H-2Db\(^{+}\)-bearing CD11c-positive cells and LCMV infection. This study showed that the depletion of D\(^{b}\) CD11c\(^{+}\) cells specifically diminishes the expansion of the D\(^{b}\)-restricted donor P14 CD8 T cells, but does not diminish expansion of endogenous K\(^{b}\)-restricted CD8 T cells, at least in the peripheral blood compartment that was analyzed. However, the early recruitment of naive CD8 T cells was not analyzed in this study either. All these studies, together, demonstrate the following: 1) bone marrow chimeras do have some residual numbers of radiation-resistant host-derived hemopoietic cells; 2) these residual numbers of radiation-resistant host-derived hemopoietic cells were unable to elicit detectable response either in VV or Lm, or influenza virus infections, but are able to elicit detectable responses during LCMV for reasons that were unclear. Our study, in addition to showing for the first time that nonhemopoietic cells perpetuate primed CD8 T cell expansion during LCMV, also clarifies the possible reasons for the discrepant observations between earlier studies with reference to LCMV.

Our study is not designed to address whether nonhemopoietic cells prime naive CD8 T cells or not. But some of our experiments presented in this study suggest that radiation-resistant cell types can prime initial naive CD8 T cell activation and early proliferation not only during LCMV, but also during VV, VSV, and Lm infections (Figs. 5–11). Moreover, our results show that these radiation-resistant cell types can also cross-prime. However, we do not know whether these so-called radiation-resistant cells that are capable of priming naive CD8 T cells under these different conditions represent truly nonhemopoietic cell types, or represent the small numbers of the host-derived hemopoietic cell types that resist radiation, or a combination of both. It is also possible that entirely different, hitherto less appreciated radiation-resistant APCs that can prime naive CD8 T cells may exist, but these cells may not be able to sustain the clonal expansion of such primed cells. Because bone marrow chimeras contain a small number of host-derived APCs that resist radiation, and because the APCs are highly efficient in priming naive CD8 T cells even in small numbers, we favor the idea that these radiation-resistant cell types capable of priming naive CD8 T cells under the conditions tested in this study are most likely of hemopoietic origin, but additional experiments are needed to test this stringently.

Why are nonhemopoietic cells more efficient in causing the expansion of effector cells in LCMV compared with the other three infections? All four pathogens used in this study infect cells of nonhemopoietic origin (31–34). Hence, the diminished contribution by nonhemopoietic cell types in VV, VSV, and Lm cannot
simply be attributed to lack of infection. For T cells, the requirement for costimulation, the capacity to respond to various types of APCs, the propensity to live or die, and the decision to proliferate or to undergo tolerization are largely determined by a combination of two major factors, as follows: 1) the differentiation state of the T cell, and 2) the type and status of the cell that presents Ag to the T cell. There are significant differences in the type of cells each pathogen infects, the inflammatory cytokines they induce, and the level of cytopathicity. LCMV, VV, and VSV infect a wide variety of hemopoietic and nonhemopoietic cell types. Lm, in contrast, predominantly infects macrophages, dendritic cells, and a limited repertoire of nonhemopoietic cells, especially hepatocytes, which are generally known to provide a strong tolerization signal to activated T cells (35), although under some conditions the liver may be an excellent priming site for naive CD8 T cells (36).

Recent studies from our laboratory showed that the effector CD8 T cells require direct IFN-I signals for proper survival and expansion (20, 37), and that the level of IFN-I dependence is highest in the case of LCMV and lower in the case of VV, VSV, and Lm (29). IFN-I is made not only by professional APCs, but also by virtually all infected cells (38–40). LCMV is not known to produce any molecules that interfere with the action of IFN-I, whereas VV induces soluble IFN-I receptor-like molecules that neutralize IFN-I (41). LCMV is noncytopathic to most cells that it infects, and hence, LCMV Ags are most likely to be presented by infected cells for a longer time. In contrast, VV and VSV are highly cytopathic to the cells they infect. We think some or all of these factors would determine the infection milieu and the Ag loads in the four pathogens that we have tested. We expect these to be different not only across different pathogens, but also in chimeras responding to the same pathogen, but different in the kind of cell population involved in the priming/perpetuation of CD8 T cells. Indeed, the early kinetic differences we see in cell size, CD25 up-regulation, and division we believe are wholly or in part a consequence of differences in the kinetics/magnitude of such parameters as Ag load/inflammation.

In summary, our studies demonstrate that expansion and effector programs initiated by hemopoietic APCs can be perpetuated by nonhemopoietic cells during some, but not all infections. This has implications for designing immune therapies for chronic diseases nonhemopoietic cells during some, but not all infections. This has implications for designing immune therapies for chronic diseases that involve virus-infection and cytosol in effector and memory T cells after viral infection. Eur. J. Immunol. 29: 291–299.


Thompson, L. J., G. A. Kolumam, S. Thomas, and K. Murali-Krishna. 2006. Innate inflammatory signals induced by various pathogens differentially dictate the IFN-I dependence of CD8 T cells for clonal expansion and memory forma-