Requirements for Bone Marrow–derived Antigen-presenting Cells in Priming Cytotoxic T Cell Responses to Intracellular Pathogens

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Abstract

Bone marrow (BM)-derived antigen-presenting cells (APCs) are potent stimulators of T cell immune responses. We investigated the requirements for antigen presentation by these cells in priming cytotoxic T lymphocyte (CTL) responses to intracellular bacterial and viral pathogens. [Parent→F1] radiation BM chimeras were constructed using C57BL/6 donors and (C57BL/6 × BALB/c)F1 recipients. Infection of chimeric mice with either Listeria monocytogenes or vaccinia virus expressing the nucleoprotein (NP) antigen from lymphocytic choriomeningitis virus (LCMV) primed H2-Db–restricted, but not H2-Kd–restricted CTL responses, demonstrating the requirement for BM-derived APCs for successful priming of CTL responses to these pathogens. Surprisingly, this did not hold true for chimeric mice infected with LCMV itself. LCMV-infected animals developed strong CTL responses specific for both H2-Db– and H2-Ld–restricted NP epitopes. These findings indicate that in vivo priming of CTL responses to LCMV is remarkably insensitive to deficiencies in antigen presentation by professional BM-derived APCs.

Key words: cross-presentation • cytotoxic T cell • bacterial immunity • viral immunity • radiation chimera

Introduction

CD8+ CTLs recognize pathogen-derived peptides that are presented on the surface of infected host cells by MHC class I molecules. As MHC class I molecules are expressed on nearly all nucleated cell types, appropriately stimulated CTLs can lyse a variety of infected host cell types. Such CTLs also produce cytokines such as IFN-γ and TNF-α, which contribute to effective immune responses. A crucial step in the generation of immunity to viral and bacterial intracellular pathogens is thus the priming and activation of pathogen-specific CTL responses.

Antigen presentation by specialized bone marrow (BM)-derived APCs can prime CTL responses in vitro and in vivo. In particular, dendritic cells (DCs) are extremely efficient at presenting antigens and priming T cell responses (for a review, see reference 1). The efficiency of CTL priming by DCs has been attributed in part to the expression of costimulatory molecules, including members of the B7 (CD80/CD86) family (2). Expression of CD80/CD86 molecules is upregulated in cells treated with bacterial components (3). Furthermore, the ability of APCs to costimulate CD8+ T cell activation is enhanced after signals delivered by activated CD4+ helper T cells or by infection of DCs with viral pathogens (4–6). Thus, DCs or other BM-derived APCs infected with intracellular viral pathogens appear to present antigens and prime CTL responses in vivo.

Uninfected BM-derived APCs also prime CTL responses in infected or tumor-bearing animals. This phenomenon, termed cross-priming, was first illustrated in experiments in which CTL responses to minor histocompatibility antigens were primed in animals immunized with cells that expressed the minor H antigens, but not the restricting MHC alleles (7, 8). During cross-priming, antigens from the immunizing cells are acquired and “cross-presented” by the MHC class I molecules on the surface of BM-derived APCs (9, 10). Cross-priming has been implicated in the activation of CTL responses after immunization.
tion of radiation BM chimeras via intramuscular injection of naked DNA (11–13), and via inoculation with nonhematopoietic tumor cells (14, 15). Thus, abundant evidence implicates BM-derived APCs in the priming and cross-priming of CTL responses to a variety of nonreplicating antigens.

Nonprofessional APCs have also been shown to be capable of priming CTL responses under some circumstances. This conclusion stems from experiments demonstrating that fibroblast cell lines can prime murine CTL responses (16–18). In one set of experiments, fibroblasts that expressed lymphocytic choriomeningitis virus (LCMV) nucleoprotein (NP) or glycoprotein antigens, but not known costimulatory molecules, were shown to directly immunize CTL responses to the LCMV antigens (18). Such priming correlated with the fibroblasts reaching the spleen of inoculated animals, suggesting that priming of CTL responses by nonprofessional APCs selectively occurs within cytokinerich lymphoid environments (18). These results suggest that infection with viral and intracellular bacterial pathogens that induce inflammation within lymphoid tissues may prime CTL responses independently of antigen presentation by BM-derived APCs.

In this study, we sought to address the role of BM-derived professional APCs in the in vivo priming of CTL responses to bacterial and viral pathogens that replicate within the cytosol of non–BM-derived cell types. To this end, the priming of CTL responses to pathogen-delivered antigens in [Parent→F₃] radiation BM chimeras was evaluated after immunization with Listeria monocytogenes, LCMV, or vaccinia virus. Our results indicate that antigen presentation by BM-derived APCs is essential for the priming of CTL responses to L. monocytogenes and vaccinia virus. In contrast, CTLs restricted by both BM and parenchymal MHC class I haplotypes were primed during infection with LCMV. These data indicate that LCMV infection uniquely facilitates priming of CTL responses in situations in which there are profound deficiencies in antigen presentation by BM-derived, professional APCs. We discuss potential mechanisms to account for the radiation-resistant, host cell presentation of LCMV.

Materials and Methods

Mice and Construction of Radiation Chimeras. 5–6-wk-old female C57BL/6 (B6) mice were purchased from Taconic Farms. (BALB/c × B6)F₁ (CB6) mice were purchased from The Jackson Laboratory. All mice were housed under specific pathogen-free conditions. To establish chimeras, recipient mice were irradiated with a single dose (1,050 rads) from a Cs source. Within 24 h, irradiated recipient mice were reconstituted with 3 × 10⁸ T cell–depleted BM cells isolated from the femurs of donor mice. T cells were depleted from BM preparations by incubation with a cocktail of anti-Thy1.2 (clone 30-H12), anti-CD8α (clone 53–6.7), and anti-CD4 (clone GK1.5), followed by lysis with low-tox-M rabbit complement (Cedarlane Labs). Chimeric animals were provided antibiotic water containing 13 mg/liter polymixin B sulfate and 0.025 mg/liter neomycin sulfate for 4 wk after irradiation. At 4 wk after reconstitution, chimerism was evaluated by the staining of PBls with fluorochrome-labeled antibodies. Similar levels of chimerism were obtained in all three sets of chimeric animals used for the experiments described here. Infections of chimeric animals were done between 6 and 12 wk after BM reconstitution.

Cell Staining and FACS. Fluorochrome- or biotin-conjugated monoclonal antibodies to H-2-Kb (AF6–88.5), H-2-K¹ (SF1.1.1), and CD3e (145–2C11) were purchased from BD PharMingen, as was an Fc receptor–blocking antibody (anti-CD16/32; 2.4G2). Streptavidin–Tricolor was used as a secondary reagent to detect biotinylated antibodies (Caltag). After osmotic lysis of erythrocytes, samples of 10⁶ cells were incubated on ice with saturating amounts of antibody in staining buffer (PBS, 3% FCS, and 0.02% NaN₃). Stained cells were fixed in a PBS/1% paraformaldehyde solution before analysis on a FACSScan™ (Becton Dickinson).

Infectious Agents. L. monocytogenes EJL243 was provided by Drs. Eric R. Jensen and Jeff F. Miller (University of California at Los Angeles, Los Angeles, CA). This strain secretes the full-length LCMV NP antigen and is derived from L. monocytogenes 10403S (19). For infections, bacteria were thawed from stocks stored at −70°C and grown to mid-log phase (A₆₀₀nm ∼ 0.1) in triplicate soy broth (Difco/Becton Dickinson). Log phase bacteria were diluted in PBS for intravenous infection of chimeric mice. CFUs present in inocula were determined by absorbance at 600 nm and by plating on triplicate soy broth/agar plates. LCMV Armstrong 53b was originally obtained from Dr. Peter Southern (University of Minnesota, Minneapolis, MN). Virus was grown on BHK–21 cells (CCL-10; American Type Culture Collection) and titred on Vero Cl008 cells (CRL-1586; American Type Culture Collection) as described previously (20). Recombinant vaccinia virus encoding the full-length LCMV NP (VacNP) was obtained from Jonathan Yewdell (National Institutes of Health, Bethesda, MD). VacNP was grown on HeLa cells and titred on Vero cells. Mice were infected intravenously with LCMV or vaccinia virus diluted to the indicated PFU/animal in 200 μl PBS.

Peptides. Peptides were synthesized on a Synergy apparatus (Applied Biosystems/PerkinElmer), except for p60(217–225), which was synthesized by Research Genetists. Peptides were stored in water or RPMI 1640 at −20°C as 100–200 μM stocks.

Immunization with DCs. DCs were enriched from spleens of CB6 animals by low density centrifugation and an adherence/deadherence procedure as described previously (21). DCs were pulsed with synthetic listeriolysin O (LLO)₁⁶₀₋₁₇₁ peptide for 1 h, and washed with RP10 media (RPMI 1640 with 10% heat-inactivated FCS, 2 mM l-glutamine, and antibiotics). DCs were resuspended in PBS and administered intravenously into naive chimeric animals.

CTL Assays. For experiments with L. monocytogenes–infected animals, responder splenocytes were harvested from immunized animals and expanded 6 d ex vivo in cultures containing peptide-pulsed stimulator cells. Stimulator cells were splenocytes harvested from naive CB6 mice pulsed for 2 h at 37°C in 1 ml RP10 containing a 1 μM concentration of the indicated stimulatory peptide. Stimulator cells were irradiated (2,500 rads), washed in RP10, and added to 3 × 10⁷ cells/flask to T–25 Falcon tissue culture flasks (Becton Dickinson) containing 3 × 10⁶ responder splenocytes. Flasks were incubated upright for 6 d in a 7% CO₂ environment at 37°C in 10 ml RP10. Viable lymphoblastoid cells, as determined by trypan blue exclusion, were used at indicated E/T ratios in 4-h ⁵¹Cr-release assays. For the assays, P815 (H₂ᵈ) and EL4 (H₂⁰) target cells were labeled with ⁵¹Cr by a
Results

Preliminary Analysis of Chimeras. We reconstituted the hematopoietic system of lethally irradiated CB6 recipient mice with T cell–depleted BM from B6 donors. Radiation-resistant cells in such chimeric animals express both MHC haplotypes of the recipient (H2d and H2b), whereas radiation-sensitive BM-derived cells express only the donor haplotype (H2b). The chimerism in BM–derived cell types from naive, chimeric animals was evaluated by fluorescent antibody staining of PBLs 4 wk after reconstitution. The PBLs of irradiated CB6 animals reconstituted with CB6 BM ([CB6→CB6] chimeras) were 98.4 ± 0.4% positive for staining with a monoclonal antibody to H2-Kd. In contrast, this antibody failed to stain 87.3 ± 2.2% of PBLs from animals reconstituted with BM from B6 donors ([B6→CB6] chimeras). The difference between these numbers indicates the approximate proportion of radiation-resistant, recipient-derived PBLs which persisted in the spleens of infected [B6→CB6] chimeras (see Fig. 5 and below). These data indicate that BM-derived cell types in the chimeric animals were predominantly donor derived and express only the donor H2b antigen.

Parenchymal Cell Types Fail to Prime CTL Responses to L. monocytogenes–delivered Antigens. H2-Db– or H2-Kb–restricted CTL epitopes have not been identified in endogenous L. monocytogenes proteins. Thus, to determine the effects of chimerism on priming of CTL responses to this intracellular pathogen, we used a recombinant L. monocytogenes strain. The strain used, EJL243, expresses a secreted form of LLO and p60, and an H2-Db–restricted epitope from the LCMV NP antigen. Lysis of P815 (H2b) target cells coated with the LLO(91–99) or p60(217–225) epitopes was readily apparent when effector cells were prepared from [CB6→CB6] animals (Fig. 1, D and E). Thus, CTL responses to these H2-Kd–restricted epitopes were efficiently primed in mice whose BM-derived APCs express both H2b and H2d MHC alleles. CTL responses to an H2-Db–restricted epitope from the NP antigen were also observed in [CB6→CB6] animals, as judged by specific lysis of EL4 (H2b) target cells coated with the NP(396–404) peptide (Fig. 1 F). However, such H2-Db–restricted responses were observed in only a subset of the [CB6→CB6] animals tested (Fig. 1 F, and data not shown), suggesting that responses to H2-Kd–restricted or other endogenous L. monocytogenes epitopes dominate the response in such animals.

An inverse pattern of CTL responses was observed in [B6→CB6] chimeric animals. In these animals, immunization with the recombinant L. monocytogenes consistently failed to prime H2-Kd–restricted CTL responses to either...
of H2-Db– and H2-Ld–restricted CTL responses in presentation by BM-derived APCs, we assayed for priming CTL responses to viral infection also requires antigen pre-

To investigate whether priming of Donor BM Haplotype.

Antigen Presentation by BM-derived APCs Is Required for Priming of CTL Responses. A potential caveat to the above experiments is the possibility that [B6→CB6] animals have reduced numbers of H2Ld-restricted CTL precursors. A paucity of such precursors might arise from differences in thymic selection in [B6→CB6] compared with [CB6→CB6] chimeras. Alternatively, given the requirement for MHC expression in the maintenance of naive T cells (for a re-

Figure 2. Exogenous CB6 APCs can prime CTL responses to an H2-Kd-restricted Listeria epitope in [B6→CB6] chimeras. DCs isolated from spleens of CB6 mice were pulsed with 1 μM LLO91–99 and used to immunize two naive [B6→CB6] chimeras intravenously. After 3 wk, sple-

APCs, and was predicted from the results of above experiments using L. monocytogenes (Fig. 1, D–F). However, the response pattern of the LCMV-infected [B6→CB6] chimeric animals was surprising. Such animals responded to both the H2-Db–restricted epitope and the H2-Ld–rellen.

Figure 3. CTL priming during infection with LCMV is not dictated by BM MHC haplotype. Three [B6→CB6] (A–C) and two [CB6→CB6] (D and E) chimeras were infected intravenously with 106 PFU LCMV-Armstrong. On day 9 after infection, ex vivo CTL activity was measured directly using P815 target cells pulsed with PBS (open symbols) or 50 nM of the corresponding peptide (filled symbols).

the LLO91–99 or the p60217–225 epitopes (Fig. 1, A and B), whereas CTL responses to the H2-Db-restricted NP396–404 epitope were readily detected (Fig. 1 C). These data indi-
cate that chimeric animals whose BM-derived APCs express only H2b fail to mount detectable H2-Kd-restricted CTL responses to L. monocytogenes–delivered antigens. As parenchymal cells of these animals are H2dXb, the selective lack of responsiveness to the H2d epitopes suggests that such parenchymal cells are quite ineffective at priming CTL responses to L. monocytogenes.

CTL Responses to LCMV Infection Are Not Dictated by the Donor BM Haplotype. To investigate whether priming of CTL responses to viral infection also requires antigen pre-

sentation by BM-derived APCs, we assayed for priming of H2-Db– and H2-Ld–restricted CTL responses in [CB6→CB6] and [B6→CB6] chimeras immunized with LCMV. Splenocyte effector cells from animals immunized with 106 PFU LCMV-Armstrong were tested directly ex vivo for lysis of peptide-pulsed H2-Db–transfected P815 target cells (P815-Db). The effector cells from LCMV-immune [CB6→CB6] chimeras efficiently lysed target cells coated with either the H2Ld-restricted NP118–126 peptide or the H2-Db-restricted NP396–404 peptide (Fig. 3, D and E). This result is consistent with priming of both H2b- and H2d–restricted CTL populations by H2bXd BM-derived APCs.
restricted epitope (Fig. 3, A–C). Identical results were observed when mice were infected with lower doses (~200 PFU) of LCMV. These data indicate that LCMV infection elicits priming of H2d-restricted CTL responses in animals whose BM-derived cells are H2b. Thus, in sharp contrast to the situation described above for responses to L. monocytogenes infections, the CTL responses of LCMV-infected chimeric animals are not dictated by the MHC haplotype of the donor BM.

Nonprofessional Antigen Presentation Is Not a Ubiquitous Feature of Viral Infections. The data above suggest that LCMV infection of [B6→CB6] chimeric animals leads to priming of CTL responses that are restricted by parenchymal cell MHC alleles. To determine whether infection with a different viral pathogen could also induce similar priming, we evaluated CTL responses after the infection of chimeric animals with VacNP. As with LCMV infection, CTL activity in spleens of VacNP-infected [B6→CB6] and control [CB6→CB6] animals was assayed directly ex vivo. Expectedly, [CB6→CB6] animals responded well to the Ld-restricted NP118–126 epitope (Fig. 4, C and D). These animals responded poorly or not at all to the subdominant H2-Dd–restricted NP epitope. In contrast, in [B6→CB6] animals, responses to the H2-Dd epitope were clearly seen but in no case did we observe responses to the H2-Ld–restricted NP118–126 epitope (Fig. 4, A and B). This indicates that priming of CTL responses by parenchymal cell types is not detected in the VacNP-infected animals, similar to our findings with L. monocytogenes–infected animals (Fig. 1). Furthermore, these results indicate that the apparently BM-independent priming of CTL responses after LCMV infection is not a general feature of infections with viral pathogens and may rather be a specific feature of LCMV infection.

Host-derived APC Populations Are Depleted Over 20-fold in Chimeric Animals and Are Not Enriched upon LCMV Infection. The above data suggest that LCMV infection facilitates priming of CTL responses to antigen expressed by nonprofessional APCs. However, the ability of [B6→CB6] animals to generate strong CTL responses to the H2-Ld-restricted NP epitope might also reflect presentation by residual BM-derived host APCs or selective recruitment or expansion of host-derived professional APCs in the spleens of LCMV-infected animals. To address these issues, we first analyzed H2-Kd expression on CD3+ and CD3− splenocytes from LCMV-infected or VacNP-infected chimeric mice. Representative results of this analysis appear in Fig. 5. As expected, nearly all cells from infected [CB6→CB6] animals stained positive with an antibody to H2-Kd. In contrast, only 6.5% of splenocytes from the VacNP-infected [B6→CB6] animal expressed H2-Kd. A similar proportion (5.5%) of cells was H2-Kd+ in the LCMV-infected spleen. Furthermore, the majority of host-derived H2-Kd+ cells in both sets of infected [B6→CB6] animals were CD3+ T cells. The reduced size (0.2%) of the H2-Kd+, CD3− population in the LCMV-infected [B6→CB6] animal may be explained by dilution of these cells by the three- to fourfold greater expansion of T cells occurring in the spleens of LCMV-infected animals. After accounting for this expansion, the populations of H2-Kd+, CD3− splenocytes are approximately equal in the LCMV-infected and VacNP-infected animals. Thus, we fail to demonstrate any enrich-
Discussion

We used radiation chimeras to investigate the requirement for professional, BM-derived APCs in the priming of CTL responses to antigenic epitopes from three different intracellular pathogens. Our data indicate that [B6→CB6] chimeric animals infected with two of these pathogens generated only CTL responses restricted by the BM MHC haplotype (H2b). Chimeric [B6→CB6] animals infected with recombinant L. monocytogenes bacteria or vaccinia virus mounted strong H2-Db-restricted responses to noncompetently expressed LCMV NP antigen, but were consistently unresponsive to H2d-restricted epitopes from the same NP antigen or from two endogenous L. monocytogenes proteins (LLO and p60). The unresponsiveness was not attributable to holes in the T cell repertoire of the radiation chimeras used, as CTL responses to an H2-Kd-restricted L. monocytogenes-derived epitope could be induced in the [B6→CB6] chimeras after in vivo priming with peptide-pulsed DCs, and immunization with LCMV showed that the repertoire was intact.

With regard to CTL priming after vaccinia virus infection, our results confirm those reported in a recent study by Sigal et al. (25), who illustrated the importance of BM-derived APCs in the priming and cross-priming of CTL responses to recombinant vaccinia virus and poliovirus infections. Furthermore, older studies had indicated that priming of CTL responses to influenza (26) and vesicular stomatitis virus (27) were abrogated in mice whose macrophages were depleted by in vivo treatment with carrageenan, silica, or liposome-encapsulated toxins. Thus, BM-derived APCs have now been implicated as essential elements in the priming of CTL responses to at least four viral pathogens.

It is well known that wild-type mice infected with L. monocytogenes develop robust CTL responses to epitopes from a small number of secreted endogenous or recombinantly expressed bacterial antigens (19, 28). In contrast to the above-mentioned viral infections, mechanisms for priming of CTL responses to this and other bacterial pathogens are largely obscure. However, a study by Shen et al. has shed some light on this subject (29). This study illustrated that a model antigen localized to the L. monocytogenes cytosol efficiently primed antigen-specific CTL responses in infected mice. Conversely, this antigen had to be secreted into the cytosol of infected cells to provide CTL-mediated protection during the effector phase of anti-Listeria immunity. The authors explained these differences in the requirement for antigen secretion by demonstrating that processing of nonsecreted bacterial antigens can occur within macrophages. Thus, these and other professional APCs are postulated to cross-prime CTL responses to nonsecreted L. monocytogenes antigens. In contrast, nonprofessional APCs are unable to access and/or present antigens that are not secreted by cytosolic L. monocytogenes, explaining the requirement for antigen secretion in the effector phase of CTL immunity (29). Our results with L. monocytogenes–infected chimeric animals are consistent with a central role for BM-derived APCs in the priming of CTL responses to L. monocytogenes, as they provide the first direct evidence that antigen presentation by BM-derived APCs is essential for the priming of CTL responses to an intracellular bacterial pathogen.

In sharp contrast to our results with L. monocytogenes and vaccinia virus, the priming of CTL responses in LCMV-infected [B6→CB6] chimeric animals shows little requirement for antigen presentation by donor BM-derived APCs. Both H2b- and H2d-restricted CTL responses were invariably observed after infection of [B6→CB6] chimeric animals, even with very low (200 PFU/animal) viral inocula (data not shown). These findings are somewhat at odds with the conclusions of a previous report in which effector cells from chimeric [k×b→d×b] animals failed to lyse LCMV-infected H2d target cells after in vivo priming with LCMV (30). However, the failure to detect H2d-restricted responses in this case may have been due to other factors, such as the use of infected (as opposed to peptide-pulsed) target cells. In line with this explanation, adoptive transfer experiments in that same study are more consistent with the possibility that H2d-restricted CTL were indeed primed to some extent in the [k×b→d×b] chimeras. Furthermore, our findings with LCMV are confirmed and complemented by results from Sigal and Rock reported in this issue (31). As in our work, they find that CTL responses to NP118/Ld in [b→d×b] BM chimeras are readily detected in LCMV-infected animals (31). This response persisted even after supralethal irradiation of host animals, although even such extreme measures failed to completely eradicate host-derived BM APCs. Thus, it becomes clear that CTL priming during an LCMV infection, particularly to the NP118/Ld epitope, is remarkably insensitive to the level of antigen presentation by BM-derived APCs. Our own staining results indicate that the population of host-derived APCs that persist in [B6→CB6] chimeras is between 1/29 and 1/18 of that seen in [CB6→CB6] chimeras, and that the ratio of chimerism is not altered after infection with LCMV versus VacNP (see Fig. 5, and Results). These data argue against enhanced survival or expansion of recipient-
derived (CB6) professional APCs in the LCMV-infected chimeric mice. However, we cannot yet differentiate between priming of NP118/Ld responses by the small population of host BM-derived APCs versus non–BM-derived host cell types. Such priming is, however, remarkably efficient. Thus, although the proportion of B220+CD3+ splenocytes expressing Kd is reduced by at least 20-fold in [B6→CB6] animals, there is no observed reduction in the level of CTL priming after LCMV infection (Fig. 3). This contrasts sharply with the situation after L. monocytogenes and vaccinia infections and leads us to conclude that unique features of the LCMV infection make this pathogen more efficient at stimulating CTL responses. What might these features be?

First, LCMV may uniquely infect a subset of non–BM-derived cells that constitutively prime host CTL responses. However, this possibility seems unlikely because each of the intracellular pathogens used in this study infects a broad range of non–BM-derived host cell types in vitro. L. monocytogenes, for example, has been shown to infect hepatocytes, epithelial, endothelial, and other adherent cell types, such as fibroblasts, in vitro. Furthermore, bacteria have been observed in splenic barrier cells (a fibroblastoid cell type [32]) as well as in hepatocytes in tissue sections from L. monocytogenes–infected mice (33). Although the precise nature of all cell types infected by each of these pathogens in vivo remains to be determined, the available evidence suggests that infection of non–BM host cell types, even within lymphoid organs, does not by itself facilitate BM-independent priming of CTL responses to an intracellular pathogen.

Second, LCMV infection may enhance the priming ability of H2d-expressing cells or otherwise lower the threshold for activation of CTL precursors. Such enhancement could affect priming by either BM-derived or parenchymal cell types, perhaps by inducing expression of costimulatory factors that enhance their ability to prime naive CTLs. It is clear from previous work that the priming of CTL responses to LCMV can occur independently of CD28 expression (34). We have also observed CD28-independent priming of CTL responses restricted by both donor and recipient haplotypes after LCMV infection of CB6 radiation chimeras that were reconstituted with CD28-deficient (H2b) BM (data not shown). These observations suggest that additional, CD28-independent, costimulatory factors contribute to priming of CTL responses during LCMV infections, whether restricted by donor or host H2. We hypothesize that selective upregulation of such factors during LCMV infection, but not during L. monocytogenes or vaccinia infections, may account for the remarkably efficient priming of CTL responses seen during LCMV infection. Such novel costimulatory factors might act to modify either the non–BM-derived APCs or the T cell. This hypothesis is supported by recent data from Ochsenbein et al. (35). These authors showed that mice immunized with LCMV mount a protective memory response upon secondary challenge with LCMV that is more rapid and robust than the memory response induced by immunization with a recombinant, NP epitope–expressing L. monocytogenes. This effect was also seen upon adoptive transfer of equal numbers of immune CTLs from animals primed with LCMV or with NP-expressing L. monocytogenes. These results suggest that CTLs immunized during an LCMV infection are more protective on a per cell basis than are CTLs immunized to the same antigen during a L. monocytogenes infection. Thus, infection of mice with LCMV, as opposed to L. monocytogenes (and presumably vaccinia virus), may induce a prolonged alteration in the threshold required for activation or survival of antigen-specific CTLs. Such mechanisms may also account for the profound differences in the burst size of the primary, CTL response seen after LCMV infection (36, 37) versus L. monocytogenes infection (28) of wild-type animals. Further experiments will be required to test the validity of this hypothesis.

The features of host–pathogen interactions that govern the efficiency of CTL priming in vivo are not well understood. Even when antigen is present in central lymphoid organs, in most cases it must be presented by professional APCs to prime effectively. LCMV appears to bend or perhaps even break this rule, as CTL priming to LCMV-delivered antigen is completely insensitive to the profound reduction in antigen presentation by BM APC populations. It will be important to better define the mechanisms for “BM-insensitive” CTL priming during LCMV infection. A better understanding of the mechanisms for CTL priming during infections should facilitate the development of vaccines and immune therapies for important human pathogens.

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References

1. Banchereau, J., and R.M. Steinman. 1998. Dendritic cells and the control of immunity. Nature. 392:245–252.
2. Steinman, R.M. 2000. DC-SIGN: a guide to some mysteries of dendritic cells. Cell. 100:491–497.
3. Lenschow, D.J., T.L. Walunas, and J.A. Bluestone. 1996. CD28/B7 system of T cell costimulation. Annu. Rev. Immunol. 14:233–258.
4. Ridge, J.P., F. Di Rosa, and P. Matzinger. 1998. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature. 393:474–478.
5. Bennett, S.R., F.R. Carbone, F. Karamalis, R.A. Flavell, J.F. Miller, and W.R. Heath. 1998. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. Nature. 393:478–480.
6. Schoenberger, S.P., R.E. Toes, E.J. van der Voort, R. Offringa, and C.J. Melief. 1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40–CD40L interactions. Na-
7. Bevan, M.J. 1976. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. J. Exp. Med. 143: 1283–1288.

8. Bevan, M.J. 1976. Minor H antigens introduced on H-2 different stimulating cells cross-react at the cytotoxic T cell level during in vivo priming. J. Immunol. 117:2233–2238.

9. Carbone, F.R., C. Kurts, S.R. Bennett, J.F. Miller, and W.R. Heath. 1998. Cross-presentation: a general mechanism for CTL immunity and tolerance. Immuno. Today. 19:368–373.

10. Heath, W.R., and F.R. Carbone. 1999. Cytotoxic T lymphocyte activation by cross-priming. Curr. Opin. Immunol. 11:314–318.

11. Doe, B., M. Selby, S. Barnett, J. Baenziger, and C.M. Walker. 1996. Induction of cytotoxic T lymphocytes by intramuscular immunization with plasmid DNA is facilitated by bone marrow-derived cells. Proc. Natl. Acad. Sci. USA. 93: 8578–8583.

12. Iwasaki, A., C.A. Torres, P.S. Ohashi, H.L. Robinson, and B.H. Barber. 1997. The dominant role of bone marrow-derived cells in CTL induction following plasmid DNA immunization at different sites. J. Immunol. 159:11–14.

13. Corr, M., D.J. Lee, D.A. Carson, and H. Tighe. 1996. Gene vaccination with naked plasmid DNA: mechanism of CTL priming. J. Exp. Med. 184:1555–1560.

14. Huang, A.Y., A.T. Bruce, D.M. Pardoll, and H.I. Levitsky. 1996. In vivo cross-priming of MHC class I-restricted antigens requires the TAP transporter. Immun. 4:349–355.

15. Huang, A.Y., P. Golumbek, M. Ahmedzadeh, E. Jaffe, D. Pardoll, and H. Levitsky. 1994. Role of bone marrow–derived cells in presenting MHC class I-restricted tumor antigens. Science. 264:961–965.

16. Sprent, J., and M. Schaefer. 1986. Capacity of purified Lyt-2+ T cells to mount primary proliferative and cytotoxic responses to La− tumour cells. Nature. 322:541–544.

17. Kosaka, H., C.D. Surh, and J. Sprent. 1992. Stimulation of mature unprimed CD8+ T cells by semiprofessional antigen-presenting cells in vivo. J. Exp. Med. 176:1291–1302.

18. Kundig, T.M., M.F. Bachmann, C. DiPaolo, J.J. Simard, M. Battegay, H. Lothen, A. Gesnur, K. Kuhlcke, P.S. Ohashi, H. Hengartner, and R.M. Zinkernagel. 1995. Fibroblasts as efficient antigen-presenting cells in lymphoid organs. Science. 268:1343–1347.

19. Shoenenbein, A.F., U. Karrer, P. Klenerman, A. Althage, A. Ciurea, H. Shen, J.F. Miller, J.L. Whitton, H. Hengartner, and R. M. Zinkernagel. 1999. A comparison of T cell memory against the same antigen induced by virus versus intracellular bacteria. Proc. Natl. Acad. Sci. USA. 96:9293–9298.

20. Bevan, M.J. 1976. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. J. Exp. Med. 143: 1283–1288.

21. Butz, E.A., and M.J. Bevan. 1998. Differential presentation of the same MHC class I epitopes by fibroblasts and dendritic cells. J. Immunol. 160:2139–2144.

22. Goldrath, A.W., and M.J. Bevan. 1999. Selecting and maintaining a diverse T–cell repertoire. Nature. 402:255–262.

23. Sigal, L.J., S. Crotty, R. Andino, and K.L. Rock. 1999. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. Nature. 398:77–80.

24. Debrick, J.E., P.A. Campbell, and U.D. Staez. 1991. Macrophages as accessory cells for class I MHC-restricted immune responses. J. Immunol. 147:2846–2851.

25. Ciavarra, R.P., K. Buhrer, N. Van Rooijen, and B. Tedeschi. 1997. T cell priming against vesicular stomatitis virus analyzed in situ: red pulp macrophages, but neither marginal metallophilic nor marginal zone macrophages, are required for priming CD+ and CD8+ T cells. J. Immunol. 158:1749–1755.

26. Buck, D.H., I.M. Plip, S. Vrij, and E.G. Pamer. 1998. Coordinate regulation of complex T cell populations responding to bacterial infection. Immunity. 8:353–362.

27. Tissue, 393:480–483.

28. Shoenenbein, A.F., U. Karrer, P. Klenerman, A. Althage, A. Ciurea, H. Shen, J.F. Miller, J.L. Whitton, H. Hengartner, and R. M. Zinkernagel. 1999. A comparison of T cell memory against the same antigen induced by virus versus intracellular bacteria. Proc. Natl. Acad. Sci. USA. 96:9293–9298.

29. Butz, E.A., and M.J. Bevan. 1998. Differential presentation of the same MHC class I epitopes by fibroblasts and dendritic cells. J. Immunol. 160:2139–2144.

30. Zinkernagel, R.M., G. Kreeb, and A. Althage. 1980. Lymphohemopoietic origin of the immunogenic, virus-antigen-presenting cells triggering anti-viral T-cell responses. Clin. Immunol. Immunopathol. 15:565–576.

31. Sigal, L.J., and K.L. Rock. 2000. Bone marrow–derived antigen presenting cells are required for the generation of cytotoxic T lymphocyte responses to viruses and use transporter associated with antigen presentation (TAP)-dependent and -independent pathways of antigen presentation. J. Exp. Med. 192:1143–1150.

32. Weiss, L. 1991. Barrier cells in the spleen. Immunol. Today. 12:24–29.

33. Conlan, J.W., and R.J. North. 1994. Neutrophils are essential for early anti-Listeria defense in the liver, but not in the spleen or peritoneal cavity, as revealed by a granulocyte-depleting monoclonal antibody. J. Exp. Med. 179:259–268.

34. Shahinian, A., K. Pfeffer, K.P. Lee, T.M. Kundig, K. Kishihara, A. Wakeham, K. Kawai, P.S. Ohashi, C.B. Thompson, and T.W. Mak. 1993. Differential T cell costimulatory requirements in CD28-deficient mice. Science. 261:609–612.

35. Murahira, K., D.J. Altman, M. Suresh, D.J. Sourdive, A.J. Zajac, J.D. Miller, J. Slansky, and R. Ahmed. 1998. Counting antigen–specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity. 8:177–187.