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Priming of CTLs by Lymphocytic Choriomeningitis Virus Depends on Dendritic Cells

Hans Christian Probst and Maries van den Broek

Appropriate activation of naive CD8+ T cells depends on the coordinated interaction of these cells with professional APC that present antigenic peptides in the context of MHC class I molecules. It is accepted that dendritic cells (DC) are efficient in activating naive T cells and are unique in their capacity to prime CD8+ T cell responses against exogenous cell-associated Ags. Nevertheless, it is unclear whether epitopes, derived from endogenously synthesized proteins and presented by MHC class I molecules on the surface of other APC including B cells and macrophages, can activate naive CD8+ T cells in vivo. By infecting transgenic CD11c-DTR/GFP mice that allow conditional depletion of DC with lymphocytic choriomeningitis virus (LCMV), which infects all types of APC and elicits a vigorous CTL response, we unambiguously show that priming of LCMV-specific CD8+ T cells is crucially dependent on DC, despite ample presence of LCMV-infected macrophages and B cells in secondary lymphoid organs.


The CD8+ CTL recognize pathogen-derived peptides that are presented on the surface of infected host cells by MHC class I molecules. Because MHC class I molecules are expressed on virtually all nucleated cells, appropriately activated CTL can lyse all infected host cells and thus crucially contribute to the elimination of intracellular pathogens. The activation of CD8+ T cells is thought to depend on a coordinated interaction of naive CD8+ T cells with professional APC that present the antigenic peptide in the context of host MHC class I molecules. There is now substantial evidence that dendritic cells (DC) are the key APC for the priming of naive CD8+ T cells (1) and that DC alone are sufficient for this (2, 3). DC reside in peripheral tissues where they efficiently take up Ag for subsequent transport into secondary lymphoid organs where priming of naive T cells takes place (4, 5).

Lafferty et al. (6, 7), who noted that the rejection of histoincompatible organ grafts was dependent on donor leukocytes trapped in the graft, first demonstrated the requirement for specialized stimulator cells for T cell activation in a series of classical experiments. Based on their ability to express MHC class II and costimulatory molecules and to take up Ags, B cells, macrophages, and DC are thought to have the capability to stimulate naive T cells and are thus referred to as professional APC. However, previous experiments question an important role for B cells with respect to priming of naive CD8+ T cells (8, 9). Using confocal microscopy of sectioned lymph nodes after local infection with vaccinia virus, it could be shown that, although DC and macrophages both were infected with vaccinia virus, only DC seemed to productively present Ag to naive T cells (10). It has been suggested that other types of APC might be able to prime CD8+ T cell responses if they are virally infected and if the Ag is synthesized within the APC. For instance, it has been observed that presentation of Ag by non-professional APC is sufficient to prime CD8+ T cells as long as this takes place in secondary lymphoid organs (11, 12).

Recently, a novel diphtheria toxin (DT)-based system was developed, allowing the inducible ablation of DC in vivo (1). Depletion of the DC compartment resulted in a complete inability to prime CD8+ T cell responses against Listeria monocytogenes and sporozoites of Plasmodium yoelii. The authors concluded from their experiments that DC are the only APC capable of priming CD8+ T cells to exogenous, cell-associated Ags. However, because priming against exogenously derived Ags requires cross-presentation, the unique role of DC in these situations might be explained by their effectiveness to cross-present Ags derived from phagocytosed material (5, 13–15).

Lymphocytic choriomeningitis virus (LCMV) infection of mice that allows conditional depletion of DC (CD11c-DTR mice; Ref.1) with LCMV is a particularly suitable system to investigate which cell type really primes naive CD8+ T cells in vivo, because it meets two criteria that are relevant to this question: 1) LCMV infects different cell types of the immune system including APC such as macrophages, B cells, and DC (16–18); and 2) LCMV replicates efficiently in secondary lymphoid organs (19). It has been shown that bone marrow-derived cells are crucial for the priming of LCMV-specific CD8+ T cells (20, 21), but it is still unclear which cell type is required for the induction of LCMV-specific CTL responses in vivo. We infected CD11-DTR mice (1) with LCMV and analyzed the priming of naive LCMV-specific CD8+ T cells in the presence and in the absence of DC. We found that naive CD8+ T cells were not primed after depletion of DC, despite ample presence of LCMV in secondary lymphoid organs. This clearly shows that the mere presence of LCMV in any cell in the secondary lymphoid organs is insufficient for CD8+ T cell priming but that this critically depends on Ag presentation by DC.

Materials and Methods

Mice

CD11c-DTR mice carry a transgene encoding the simian DT receptor (DTR)-GFP fusion protein under control of the murine CD11c promoter...
Injection of diphtheria toxin (DT) results in transient depletion of CD11c+ cells (e.g., DC). CD11c-DTR mice were backcrossed to C57BL/6 mice for 10 generations. Transgenic mice expressing the P14 TCR (Vβ2, Vβ8.1) that recognizes LCMV GP33–41/Dd (318 mice) have been described (22). CD11c-DTR, H-2Db, and anti-CD11c (N418, Biomedicals), rat anti-DEC205 (NLDC-145; Grube (Heinrich Pette Institute, Hamburg, Germany) (23) and was propagated by Neosystem SA: GP33–41/Dd KAVYNFATC; GP36–34/Dd SVGVENPGGYCL. NP36–34/Ro4 FPQPQNGQFT. EL-4 cells are dimethylbenzanthrene-induced, C57BL/6-derived thymoma cells. MC57G are methylcholanthrene-induced, C57BL/6-derived fibrosarcoma cells. LCMV-WE was originally obtained from Dr. F. Lehmann-Grube (Heinrich Pette Institute, Hamburg, Germany) (23) and was propagated on L929 cells at a low multiplicity of infection.

Reagents, cells, and viruses

DT was obtained from Sigma-Aldrich. The following peptides were synthesized by Neosystem SA: GP33–41/Dd KAVYNFATC; GP36–34/Dd SVGVENPGGYCL. NP36–34/Ro4 FPQPQNGQFT. EL-4 cells are dimethylbenzanthrene-induced, C57BL/6-derived thymoma cells. MC57G are methylcholanthrene-induced, C57BL/6-derived fibrosarcoma cells. LCMV-WE was originally obtained from Dr. F. Lehmann-Grube (Heinrich Pette Institute, Hamburg, Germany) (23) and was propagated on L929 cells at a low multiplicity of infection.

Treatment of mice

CD11c-DTR mice were injected i.p. with 100 ng of DT in PBS to deplete DC or with PBS as control at day −1 and were infected with 100 PFU of LCMV-WE i.v. at day 0. Alternatively, CD11c-DTR/H-2Db−/− mice were injected i.p. with 100 ng of DT or with PBS on days −1, +1, and +3 and were infected with 100 PFU of LCMV-WE i.v. at day 0. In some experiments, 3 × 10^5 318 splenocytes, corresponding to ~3 × 10^9 GP33–41/Dd-specific CD8+ T cells, were adoptively transferred into mixed bone marrow chimeras on day −2, followed by treatment as above.

Staining with tetrameric MHC class I-peptide complexes

Tetrameric complexes containing biotinylated H-2Db or -Kb, the relevant peptide, and extravidin-PE were generated, and stained cells were adoptively transferred into mixed bone marrow chimeras on day −2, followed by treatment as above.

Cytotoxicity assay

To measure direct ex vivo LCMV-specific CTL responses, splenocytes were isolated 8 days after infection with LCMV-WE and were tested for cytolytic activity toward peptide-loaded (10−7 M) or unloaded 51Cr-labeled EL-4 target cells in a 5-h assay. The percentage specific lysis was calculated as [100 × (experimental release − spontaneous release)/ (maximal release − spontaneous release)] × 100%.

Expansion of adoptively transferred 318 CD8+ T cells

Mice were bled at different time points after infection, and the percentage of Vα2/Vβ8.1+ T cells within CD8+ cells was determined by FACS analysis, using anti-Vβ8.1-FITC, anti-Vα2-PE, and anti-Vβ8.1-biotin/streptavidin-APC (all BD Pharmingen). Tetramer+ cells were determined after gating on live CD8+ cells.

Immunohistology

At indicated time points after DT injection and LCMV infection, organs were removed and snap frozen in liquid nitrogen. Cryosections of 5 μm were fixed in acetone for 10 min and subsequently incubated with rabbit anti-GFP (RDI Research Diagnostics), rat-anti-LCMV NP (V14 (25), hamster anti-CD11c (N418, Biomedicals), rat anti-DEC205 (NLDC-145; American Type Culture Collection), rat anti-marginal zone (MZ) macrophages (ERTR-9; Ref. 26), rat anti-red pulp macrophages (F4/80; HB-198, American Type Culture Collection), or rat anti-metallophilic macrophages (MOMA-1; Ref. 27). Goat anti-rat Ig (Caltag) in 5% normal mouse serum was used as a secondary reagent, and alkaline phosphatase-labeled donkey anti-goat Ig (Jackson ImmunoResearch Laboratories) in 5% normal mouse serum was used as tertiary reagent. Alternatively, alkaline phosphatase-labeled rabbit anti-hamster Ig or goat anti-rabbit IgG was used as secondary reagent. The substrate for the red color reaction was naphthol AS-BI phosphate/new fuchsin. Endogenous alkaline phosphatase activity was quenched by levamisole. Sections were counterstained with hemalum.

Results

Injection of DT transiently depletes CD11c+ cells from CD11c-DTR mice

CD11c-DTR mice were injected i.p. with 100 ng of DT or with PBS, and spleens were removed for immunohistology 18, 42, and 66 h later. Eighteen hours after DT injection, virtually all DC were depleted as visualized by the absence of CD11c+ and of CD205+ cells as well as by the absence of transgene expressing GFP+ cells (Fig. 1). However, 42 h after DT injection some CD11c+ and GFP+ cells were detectable again, and after 66 h the numbers were comparable with those in untreated mice (Fig. 1). Neither injection of PBS in CD11c-DTR mice nor injection of DT in B6 mice depleted CD11c+ cells (data not shown).

Administration of DT to CD11c-DTR mice did not affect the number and localization of CD4+CD8− and CD19+ cells (data not shown).

Delayed CTL priming by LCMV in mice that were transiently depleted of DC

To investigate whether DC are required for CTL priming by LCMV, we injected CD11c-DTR mice with 100 ng of DT or with PBS at day −1 and infected them with 100 PFU of LCMV-WE at day 0. We analyzed the induction of LCMV-specific CTL by tetramer staining at days 7 and 10 after infection. Untreated CD11c-DTR mice showed expansion of LCMV-specific CTL at day 7, which increased until day 10 (Fig. 2A). In contrast, injection of DT...
resulting in DC depletion prevented detectable priming at day 7 after infection. Nevertheless, analysis of CTL priming at day 10 revealed that an LCMV-specific CTL response was induced in CD11c-DTR mice that were transiently depleted of DC, albeit with delayed kinetics compared with control mice (Fig. 2A). Acute control of LCMV crucially depends on CD8+ T cells (28, 29). Therefore, delayed priming of virus-specific CTL will delay viral clearance. By the time the DC become detectable again, virus is still present, which can infect the emerging DC leading to priming. This is illustrated by the fact that a transient absence of DC at the time of infection resulting in delayed CTL priming has consequences for virus control: Nondepleted mice were virus-free by day 15, whereas transiently depleted mice still had high titers of LCMV in the spleen (Fig. 2B).

Continued absence of DC completely prevents the priming of CTL by LCMV

The experimental setup as described above has two disadvantages that complicate the interpretation of the results: 1) repeated injections of DT resulted in death of CD11c-DTR mice (Ref 1 and own unpublished findings) by a yet unknown mechanism, probably due to ectopic expression of the transgene; 2) infection of mice with LCMV under conditions where DC are transiently absent results in delayed viral clearance (Fig. 2C). This obscures comparison of LCMV-specific CTL responses in these two situations, as it was shown before that LCMV-specific CTL are exhausted by high antigenic load (30). To circumvent these problems, we generated mixed bone marrow chimeras. H-2Db−/− recipients were lethally irradiated and reconstituted with a 1:1 mixture of CD11c-DTR and H-2Db−/− bone marrow. Injection of DT will deplete H-2Db−/− expressing DC originating from CD11c-DTR bone marrow but leave the H-2Kb-expressing DC from H-2Db−/− bone marrow. LCMV contains four major CTL epitopes in H-2b mice, GP33-41/Db, GP34-41/Kb, GP276-286/Db, and NP396-404/Db; therefore, the viral control will in any case be guaranteed through GP34-41/Kb-specific CTL. Moreover, because only bone marrow-derived cells will express the transgenic DTR, we expected that we could repeatedly administer DT without lethality.

We injected DT or PBS into mixed bone marrow chimeras and infected them with LCMV 1 day later. Analysis of ex vivo cytotoxic activity 8 days after infection clearly showed that priming of LCMV-specific CTL depended completely on Ag presentation by DC. There was no effect of DC depletion on the GP34-41/Kb-restricted response, whereas both Db-restricted responses were virtually absent in depleted mixed bone marrow chimeras (Fig. 3). To have a maximally sensitive assay for CTL priming, we adaptively transferred 3 × 105 sex-matched 318 TCR transgenic splenocytes, containing ~5 × 104 GP34-41/Db-specific naive CD8+ T cells into CD11c-DTR/H-2Db−/− mice at day −2. We treated these mice with DT or with PBS every second day starting from day −1 and infected them with LCMV at day 0. We followed the expansion of TCR-transgenic GP34-41/Db-restricted T cells in the blood as a measure of productive priming. Even with this highly sensitive readout that allows detection of minimal priming, we found no expansion of GP34-41/Db-restricted T cells in the absence of DC (Fig. 4A). This shows that, even although many other cells including macrophages and B cells are infected by LCMV (17, 18), only DC can induce priming of LCMV-specific CTL in vivo. The GP34-41/Kb-restricted response in the same chimeras was highly in the DC-depleted mice, showing that they could mount CTL responses upon LCMV infection (Fig. 4B). The rather small percentage of GP34-41/Kb-specific T cells in nondepleted chimeras is explained by the fact that up to 80% of the CD8+ T cells consist of the massively expanded, adaptively transferred TCR-transgenic T cells (31).

DT treatment does not affect splenic architecture or LCMV localization in CD11c-DTR/H-2Db−/− mice

Efficient control of LCMV depends not only on virus-specific CD8+ T cells but also on intact splenic architecture (32). To investigate whether the splenic architecture and LCMV localization after infection in CD11c-DTR/H-2Db−/− mice was affected by DC depletion, we analyzed bone marrow chimeras by histological means, directly comparing nondepleted and DT-treated mixed bone marrow chimeras. We found that the spleens of nondepleted chimeras were larger and had a more prominent trabecular architecture (Fig. 5A). After infection, however, this difference vanished and the spleens of the chimeras were indistinguishable, regardless of DC presence or absence. Therefore, the general conclusions that can be drawn from histological analysis are that the absence of DC in the spleen has no effect on virus control and that priming of virus-specific CTL is independent of splenic architecture.

FIGURE 2. Delayed induction of LCMV-specific CTL due to transient DC depletion. CD11c-DTR mice were injected with DT at day −1 and were infected with 100 PFU of LCMV-WE at day 0. A, The expansion of LCMV-specific CTL was measured using staining of PBL with tetrameric MHC class I-peptide complexes after gating on live CD8+ cells at 7 and 10 days after infection (p.i.). B, Viral titers were determined in the spleen 15 days after infection using a focus-forming assay. ○, DT-treated CD11c-DTR mice; □, PBS-treated CD11c-DTR mice. Each data point represents an individual mouse. One representative experiment of two is shown.

FIGURE 3. No priming of LCMV-specific CTL in the absence of DC. Mixed bone marrow chimeras (CD11c-DTR + H-2Db−/−→H-2Db−/−) were treated with DT at days −1, +1, and +3 and were infected with 100 PFU of LCMV-WE at day 0. Eight days after infection, ex vivo cytotoxic activity of H-2Db- and of Kb-restricted CTL was determined in the spleen using a standard 5-h 51Cr release assay and peptide-loaded EL-4 cells as targets. Lysis of unloaded EL-4 cells was <5%, and spontaneous lysis was <17%. ○, DT-treated mixed bone marrow chimeras; □, PBS-treated mixed bone marrow chimeras.
mature chimeras were normal, we performed immunohistology on DT-treated mixed bone marrow chimeras 2 days after infection with LCMV. We found normal localization of nontransgenic (D8−/−) CD11c+ cells but complete depletion of the GFP expressing CD11c-DTR-transgenic DC (Fig. 5) and normal localization and numbers of CD4+ , CD8+ , and CD19+ cells (data not shown). Moreover, the MZ in mixed bone marrow chimeras after DT injection was comparable with that in untreated chimeras, with the obvious exception that GFP+ cells were absent from DT-treated chimeras (Fig. 5). Importantly, LCMV was equally well concentrated to the MZ in DT-treated chimeras as were untreated mice. This shows that the functions of Ag sampling and immediate virus control, which were shown to depend on an intact MZ (32), were still intact in depleted chimeras.

**Discussion**

Through the elimination of infected cells, CD8+ T cells crucially contribute to the immune defense against intracellular pathogens, such as viruses. Although every possible cell that presents the specific peptide-MHC class I complex on its surface can be detected and eliminated by activated effector CTL, only a few cell types meet the requirements for activation of naive CD8+ T cells. A recent study identified CD11c+ DC as the only cell type able to prime CD8+ T cell responses against L. monocytogenes and P. yoelii (1), showing that activation of CD8+ T cells by exogenous, cell-associated Ags depends on cross-presentation by DC. Whereas this paper clearly demonstrated the unique role of DC in cross-presentation, it does not necessarily prove that CD8+ T cell priming against endogenously synthesized Ags relies on presentation by DC as well. LCMV infection of mice that can be conditionally depleted of DC (1) is an ideal experimental system to address this issue, given that LCMV infects different kinds of APC including DC, macrophages, and B cells and replicates in lymphoid organs (16–19).

Previous experiments have been performed to investigate on which cell type the priming of LCMV-specific CD8+ T cells depends (20, 21, 33), and conflicting data were obtained. Zinkernagel et al. (33) found absolute dependence of priming on hemopoietic cells. In contrast, two studies by Lenz et al. (20) and by Sigal et al. (21) reported a partial independence of bone marrow-derived cells for priming against LCMV. Both studies analyzed LCMV infection of parent→F1 bone marrow chimeras and reported that responses restricted to the recipient haplotype also could be detected.

However, our results reveal that even under very sensitive conditions, i.e., after transfer of TCR-transgenic T cells, priming against LCMV is entirely dependent on bone marrow-derived cells and, more specifically, on a certain type of bone marrow-derived APC, the CD11c+ DC.

Our results suggest that the bone marrow-independent priming observed by Lenz et al. and by Sigal et al. was actually priming by residual host APC in the irradiation chimeras brought to light by the extremely potent CD8+ T cell stimulus LCMV. This notion is supported by the fact that priming of CD8+ T cells restricted to the MHC of the recipient was observed only for the most immunodominant epitope, but not for weaker responses against other epitopes (20, 21). We have avoided the complication of residual
host APC in this study by the use of H-2D\textsuperscript{b}+/− recipients and the analysis of the H-2D\textsuperscript{b}-restricted vs the H-2K\textsuperscript{b}-restricted response. Using LCMV infection of mice in which CD11c\textsuperscript{+} DC can conditionally be depleted, we have shown that priming of LCMV-specific CD8\textsuperscript{+} T cells in vivo crucially depends on the presence of CD11c\textsuperscript{+} DC, even though LCMV is present in the secondary lymphoid organs in ample amounts and although it is known to infect, besides DC, other presumable professional APC including B cells and macrophages. Our results imply that only DC can activate naïve CD8\textsuperscript{+} T cells in vivo, independent of whether they use cross-presentation of exogenous, cell-associated Ags (1) or direct presentation of endogenously synthesized Ags.

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Disclosures
The authors have no financial conflict of interest.

References


