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The mechanisms by which CD8+ T cells mediate immunity against bacterial pathogens remain largely unknown. Perforin-dependent cytolysis plays a role, but is not required for CD8+ T cell-mediated immunity against *Listeria monocytogenes*. TNF is essential for CD8+ T cell immunity to *L. monocytogenes*, but the cellular source of TNF is undefined. TNF-deficient and TNF/perforin double-deficient mice were used to generate CD8+ T cells specific for an *L. monocytogenes*-derived Ag, Wild-type and TNF-deficient CD8+ T cells mediated antilisterial immunity in wild-type but not TNF-deficient host mice, revealing that CD8+ T cell-derived TNF is not required for CD8+ T cell-mediated antilisterial immunity, but demonstrating a role for TNF derived from other cell types. TNF/perforin double-deficient CD8+ T cells mediated antilisterial immunity in the liver, but not in the spleen, of wild-type recipient mice, suggesting that perforin-independent immunity in the spleen requires CD8+ T cell-derived TNF. 

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**Cutting Edge: Antilisterial Activity of CD8+ T Cells Derived from TNF-Deficient and TNF/Perforin Double-Deficient Mice**

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CD8+ T cells are potent mediators of immunity against the intracellular bacterial pathogen *Listeria monocytogenes* (LM)1 (1). In vitro, Ag-stimulated CD8+ T cells express an array of effector functions including cytolysis and elaboration of cytokines such as IFN-γ and TNF. However, the mechanisms by which CD8+ T cells provide antilisterial immunity in vivo remain poorly understood. Studies by Kagi et al. indicated that a perforin-dependent pathway, presumably cytolytic, participates in optimal CD8+ T cell-mediated immunity against LM, especially in the spleen (2). However, while studies with LM Ag-specific CD8+ T cell lines derived from perforin-deficient (P0) mice confirmed a role for perforin in the spleen, they also revealed a perforin-independent pathway for antilisterial immunity (3, 4).

Numerous studies have demonstrated a role for TNF in resistance to primary listeriosis (5–8). Neutralization of TNF with mAbs in WT mice also inhibited secondary responses against LM, while neutralization of IFN-γ did not (9). Additionally, antilisterial immunity mediated by P0-derived CD8+ T cell lines is inhibited by pretreatment of host animals with anti-TNF mAbs (3). Because mAb-mediated neutralization cannot identify the cellular source of the required TNF, it has not been clear whether TNF derived from LM Ag-specific CD8+ T cells, or some other cell type, is required for effective CD8+ T cell-mediated immunity in vivo. To address this issue, we analyzed the capacity of TNF-deficient (T0) and TNF/perforin double-deficient (T0P0) CD8+ T cells to provide immunity against LM.

**Materials and Methods**

**Mice**

C57BL/6 (B6) (H-2b MHC) mice were obtained from the National Cancer Institute (Frederick, MD). T0 (H-2b MHC) mice on the B6/129 background and B6/129 controls have been described (10). T0P0 (H-2b MHC) mice were generated by appropriate cross and backcross of T0 mice with P0 (H-2b MHC) mice (11).

**Bacteria and cell lines**

LM strain 10403s (12) and attenuated LM strain DP-L1942 (ActA−) (13) were maintained and used for animal injection as described (14). Recombinant LM XFL204 was kindly provided by Dr. H. Shen (University of Pennsylvania, Philadelphia, PA). XFL204 is derived from 10403s and was engineered using previously described strategies (15) to secrete a fusion protein consisting of dihydrofolate reductase and amino acids 396–404 of the nucleoprotein (NP) of lymphocytic choriomeningitis virus (LCMV). NP396–404 is a well characterized H-2Dd-restricted CD8+ T cell epitope from LCMV (16). EL4, a B6-derived thymoma cell line, and EL4 expressing the LM listeriolysin O (LLO) gene (EL4-LLO) were maintained as described (17).

**Generation of CD8+ T cell lines**

CD8+ T cell lines specific for LLO were derived from LM immune B6 (wild type: WT), P0, T0, and T0P0 mice by in vitro restimulation with EL4-LLO cells as described (17). WT and P0 mice were immunized by i.v. injection with 106 to 107 CFU of virulent LM strain 10403s, while T0 and T0P0 mice were immunized with 103 to 105 attenuated LM DP-L1942. In the case of T cell lines specific for NP396–404 T0 mice were immunized...
with XFL204 and EL4 cells supplemented with 100 nM synthetic NP\textsubscript{396–404} peptide were used as stimulators as described (18).

**Intracellular cytokine staining and \textsuperscript{51}Cr release assays**

Intracellular cytokine staining was performed as described (19). Briefly, CD\textsuperscript{8\textsuperscript{+}} T cells specific for LLO were incubated for 4 h in the presence of EL4 or EL4-LLO cells and stained with FITC-labeled anti-CD8 (53-6.7; PharMingen, San Diego, CA). The cells were then fixed, permeabilized, and stained with PE-conjugated anti-TNF (MP6-XT22; PharMingen) or PE-conjugated anti-IFN-\(\gamma\) (XMG1.2; PharMingen). List mode data was acquired on a FACScan (Becton Dickinson, Mountain View, CA) using FlowJo software (Tree Star, San Carlos, CA). \textsuperscript{51}Cr release assays were performed as described (3).

**Adoptive transfer and survival assays**

LLO- or NP\textsubscript{396–404}-specific CD\textsuperscript{8\textsuperscript{+}} T cells were washed in antibiotic-free buffer and resuspended in pyrogen-free normal saline. Cells were delivered i.v. in 0.2- to 0.5-ml volumes into naive host mice. Within 2 h, host mice were challenged i.v. with the indicated dose of bacteria. CFU per spleen and liver were determined 3 days postchallenge as described (14). Data are presented as mean log$_{10}$ CFU ± SD per spleen or per gram of liver. Student’s t test was used for statistical analysis; values of \(p\) are shown for each group compared with the control group that did not receive T cells. The susceptibility of B6, B6/129, T\textsuperscript{0}, and T\textsuperscript{0} P\textsuperscript{0} mice to infection with LM was estimated as described (14).

**Results and Discussion**

**Resistance of T\textsuperscript{0} and T\textsuperscript{0} P\textsuperscript{0} mice to primary infection with virulent and ActA\textsuperscript{−}LM**

While P\textsuperscript{0} and WT mice exhibit similar resistance to primary infection with virulent LM (2–4), T\textsuperscript{0} (8) and TNF receptor type I (p55)-deficient (20–22) mice are extremely susceptible to infection. Consistent with these studies, the estimated LD$_{50}$ of virulent LM 10403s in WT, T\textsuperscript{0}, and T\textsuperscript{0} P\textsuperscript{0} mice was 10$^{-4}$, 10$^{-9}$, and 10$^{-3.3}$, respectively. This result confirms the critical role played by TNF in resistance to primary infection with virulent LM (8). Furthermore, this result indicates that a lack of perforin does not increase the susceptibility of mice already profoundly immunocompromised due to a lack of TNF.

We have previously elicited potent CD\textsuperscript{8\textsuperscript{+}} T cell responses in immunocompromised mice (18, 23) by immunizing with attenuated LM DP-L1942 (13). Both T\textsuperscript{0} and T\textsuperscript{0} P\textsuperscript{0} mice survived at least 4 wk following high-dose challenges with ActA\textsuperscript{−}LM (>10$^6$ CFU), suggesting that this strategy might be used to generate CD8$^+$ T cell responses in the absence of TNF.

**Generation and characterization of CD8$^+$ T cells from T\textsuperscript{0} and T\textsuperscript{0} P\textsuperscript{0} mice**

Adaptive immunity to LM in WT mice involves CD8$^+$ T cells (24). To address the effector functions involved in CD8$^+$ T cell immunity to LM, we analyzed the protective capacity of CD8$^+$ T cell lines by in vitro re-stimulation of P\textsuperscript{0} and WT CD8$^+$ T cells from T\textsuperscript{0} and T\textsuperscript{0} P\textsuperscript{0} mice. Consistent with previous in vitro studies of P\textsuperscript{0} CD8$^+$ T cells (3, 4), this cytosis was inhibited by anti-CD95 mAbs (Fig. 2). These results demonstrate Ag-specific, CD95-dependent cytosis in vitro by CD8$^+$ T cells derived from LM-immune T\textsuperscript{0} P\textsuperscript{0} mice.

T\textsuperscript{0} CD8$^+$ T cells mediated Ag-specific cytosis of EL4-LLO cells that was comparable to WT CD8$^+$ T cells (Fig. 2). CD8$^+$ T cells from T\textsuperscript{0} P\textsuperscript{0} mice mediated Ag-specific cytosis of LLO-expressing target cells, which was delayed compared with WT and T\textsuperscript{0}-derived CD8$^+$ T cells. Consistent with previous in vitro studies of P\textsuperscript{0} CD8$^+$ T cells (3, 4), this cytosis was inhibited by anti-CD95 mAbs (Fig. 2). These results demonstrate Ag-specific, CD95-dependent cytosis in vitro by CD8$^+$ T cells derived from LM-immune T\textsuperscript{0} P\textsuperscript{0} mice.

T\textsuperscript{0} CD8$^+$ T cells mediate immunity against LM in WT, but not T\textsuperscript{0}, host mice

WT and P\textsuperscript{0} LLO-specific CD8$^+$ T cells mediate antilisterial immunity in adoptive transfer assays (3, 17). Immunity mediated by P\textsuperscript{0} CD8$^+$ T cells is abrogated by treatment of host mice with neutralizing anti-TNF mAbs (3). Although this approach does not identify the cellular source of the biologically relevant TNF, these experiments suggest that TNF is required for CD8$^+$ T cell-mediated immunity against LM. To address this hypothesis, we transferred T\textsuperscript{0}-derived LLO-specific CD8$^+$ T cells into WT host mice, which were subsequently challenged with virulent LM. LLO-specific CD8$^+$ T cells from T\textsuperscript{0} mice provided antilisterial immunity as measured by a ≥100-fold decrease in LM CFUs in the spleen (Fig. 3A) and liver (Fig. 3B). These results demonstrate that CD8$^+$ T cell-derived TNF is not required for antilisterial immunity.

In the same experiment, LLO-specific CD8$^+$ T cells from T\textsuperscript{0} donors were transferred into T\textsuperscript{0} hosts. Due to the importance of TNF in the innate response to LM, the dose of LM administered to T\textsuperscript{0} hosts was reduced by 100-fold. This challenge resulted in comparable levels of infection in control T\textsuperscript{0} and WT hosts (3). However, even with these lower challenge doses, T\textsuperscript{0} hosts were not significantly protected by T\textsuperscript{0} CD8$^+$ T cells (Fig. 3, C and D). This
suggests that antilisterial immunity mediated by LLO-specific T-cell lines requires host cell-derived TNF.

To determine whether host cell-derived TNF is also required for immunity mediated by WT CD8\(^+\) T cells, T\(^0\) or WT hosts were injected with WT CD8\(^+\) T cells and then challenged with the low dose or high dose, respectively, of virulent LM. WT CD8\(^+\) T cells mediated dramatic reductions in bacterial numbers in the spleen (>100-fold) and liver (>1000-fold) of WT hosts (Fig. 4, A and B) but were unable to provide significant protection in T\(^0\) hosts (Fig. 4, C and D).

While both strains of mice are H-2\(^b\), the possibility exists that minor Ag differences between B6 and T\(^0\) (B6/129 background) strains could stimulate host responses that nonspecifically decrease the in vivo bacterial counts. This is unlikely because the degree of
mice were injected i.v. with 10^7 LLO-specific CD8^+ T cells into WT host mice. Naive B6 mice, but not the spleen, of WT host mice. CD8^+ T cells provided significant antilistemal immunity in the liver, but not in the spleen (Fig. 5, E and F). These results suggest that CD8^+ T cells provide immunity in the liver by a pathway that is independent of both CD8^+ T cell-derived perforin and TNF. This result also indicates that perforin-independent CD8^+ T cell immunity in the spleen requires CD8^+ T cell-derived TNF.

Together, these studies demonstrate an in vivo pathway of CD8^+ T cell-mediated immunity against LM that is independent of CD8^+ T cell-derived TNF. This result underscores the apparent versatility of activated CD8^+ T cells in terms of their ability to fight listeriosis. TNF joins the arsenal of perforin, CD95, and IFN-γ (3, 4, 23) as the fourth effector function that is individually expendable for effective CD8^+ T cell immunity to LM. However, CD8^+ T cell immunity is severely hampered when two effector functions (perforin and TNF) are simultaneously deactivated. The relevance of CD8^+ T cell-derived TNF in this process is underscored by our recent results demonstrating that deletion of IFN-γ and perforin does not additionally compromise CD8^+ T cell-mediated antilisterial immunity compared with perforin deficiency alone (27). The present results also uncover a pathway of CD8^+ T cell-mediated antilisterial immunity in the liver that is independent of both perforin and CD8^+ T cell-derived TNF. While it is well appreciated that the molecular mechanisms of effective CD8^+ T cell responses are pathogen specific (1), the present results emphasize that effective CD8^+ T cell responses may be expressed in a tissue- or organ-specific fashion as well. Finally, while the role of a number of CD8^+ T cell effector functions have been examined, the importance of as yet untested (28) or unknown effector functions should not be overlooked.

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References


