Differential requirements for the chemokine receptor CCR7 in T cell activation during *Listeria monocytogenes* infection

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Effective priming of T cell responses depends on cognate interactions between naive T cells and professional antigen-presenting cells (APCs). This contact is the result of highly coordinated migration processes, in which the chemokine receptor CCR7 and its ligands, CCL19 and CCL21, play a central role. We used the murine *Listeria monocytogenes* infection model to characterize the role of the CCR7/CCR7 ligand system in the generation of T cell responses during bacterial infection. We demonstrate that efficient priming of naive major histocompatibility complex (MHC) class Ia–restricted $CD8^+$ T cells requires CCR7. In contrast, MHC class Ib–restricted $CD8^+$ T cells and MHC class II–restricted $CD4^+$ T cells seem to be less dependent on CCR7; memory T cell responses are independent of CCR7. Infection experiments with bone marrow chimeras or mice reconstituted with purified T cell populations indicate that CCR7 has to be expressed on $CD8^+$ T cells and professional APCs to promote efficient MHC class Ia–restricted T cell priming. Thus, different T cell subtypes and maturation stages have discrete requirements for CCR7.

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Abbreviations used: CFSE, carboxyfluorescein succinimidyl ester; f-met, formyl-methionine; LLO, listeriolysin O; LmOVA, recombinant *Listeria monocytogenes* strain secreting a truncated OVA protein; MACS, magnetic cell sorting; TSB, tryptic soy broth.

Close contact between naive T cells and professional APCs is a prerequisite for effective T cell priming. This contact does not occur randomly, but rather is a consequence of highly coordinated migration processes which involve different adhesion molecules and chemokine/chemokine receptor systems. The chemokine receptor CCR7, and its ligands, CCL19 (EBV-induced gene 1 ligand chemokine, macrophage inflammatory protein-3β) and CCL21 (secondary lymphoid tissue chemokine, 6Ckine), seem to play a central role (1, 2). CCR7 is expressed on naive T cells, a subpopulation of memory T cells, and B cells (1). Immature dendritic cells do not express CCR7; however, during maturation, CCR7 is up-regulated on their surface (3). CCL19 and CCL21 are secreted by stromal cells that are located in the T cell zones of secondary lymphoid organs. In addition, CCL21 is expressed by high endothelial venules and the lymphatic endothelium (1, 2, 4). Standard laboratory mouse strains express at least two isoforms of CCL21—CCL21-Ser and CCL21-Leu—which are encoded by independent genes. In contrast to CCL21-Ser, expression of CCL21-Leu is restricted to the lymphatic epithelium of peripheral tissues (4). After chemokine attraction, naive T cells and activated dendritic cells enter lymphoid tissues and migrate along the CCL19/CCL21 gradients into T cell zones, where cognate interactions eventually occur (1, 2, 5).

The concept of chemokine-controlled T cell priming is supported by observations in CCR7-deficient mice and in *plt* (paucity of lymph node T cell) mice, which fail to express CCL19 and CCL21-Ser and show impaired expression levels of CCL21-Leu (4, 6–9). In both mouse strains, T lymphocytes home poorly into lymph nodes and Peyer's patches, and inside lymph nodes, T cells are distributed aberrantly (4, 6, 8). Although T cells can still enter the spleen, these mice fail to develop distinct T cell zones in the white pulp (4, 6). Furthermore, migration of mature dendritic cells into secondary lymphoid tissues is impaired (3, 6). Consequently, both mouse strains demonstrate altered acquired immune responses. In CCR7-deficient mice, delayed-type hypersensitivity reactions are reduced, and T cell dependent antibody production is delayed (6). CCR7 mice fail to reject allogeneic tumors and rejec-

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tion of grafted allogeneic hearts is delayed (9). In *plt* mice, acquired immune responses seem to be affected less severely. Although, *plt* mice are more susceptible to infection with murine hepatitis virus (7), immune responses after infection with lymphocytic choriomeningitis virus, vesicular stomatitis virus, and different strains of vaccinia virus are similar to that

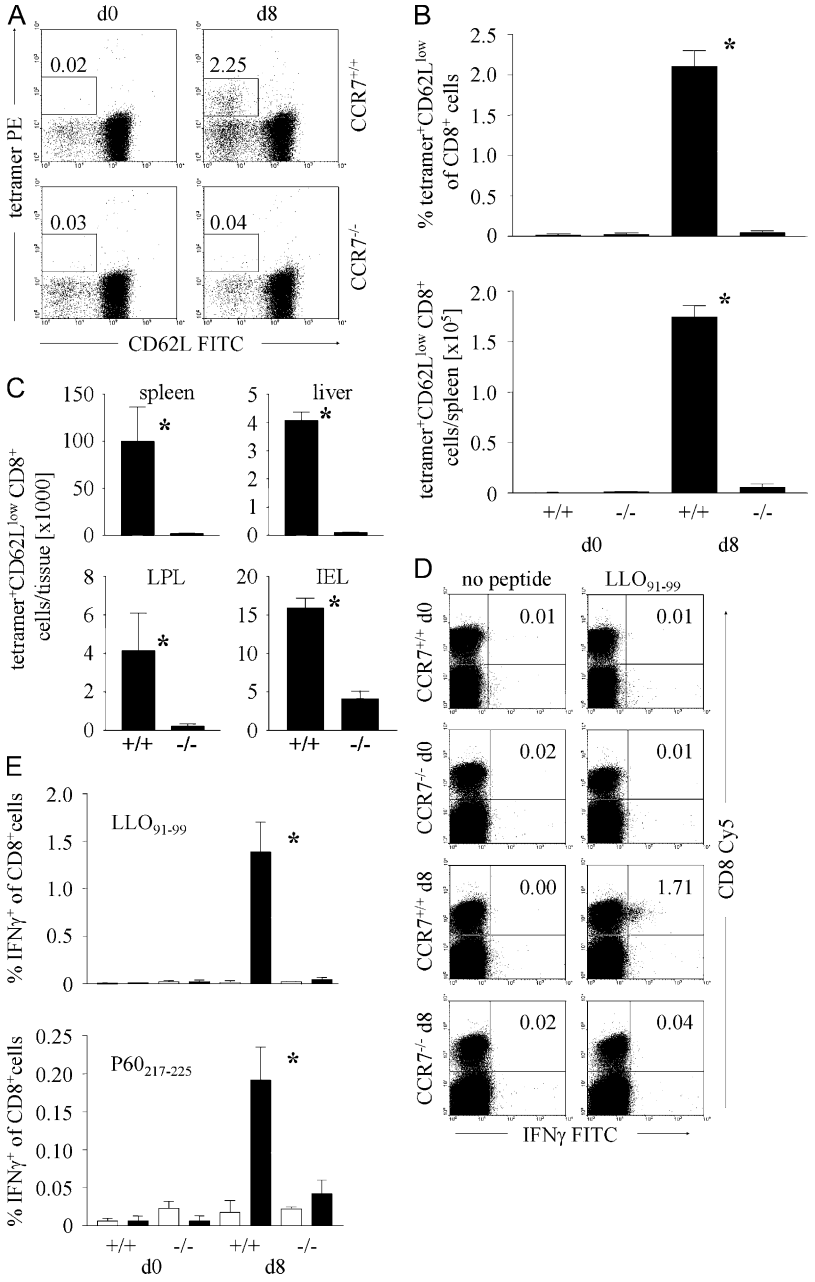

Figure 1. CD8$^+$ T cell response to primary *L. monocytogenes* infection. CCR7$^{+/+}$ and CCR7$^{-/-}$ mice on BALB/c background were infected i.v. with 10^3 (A, B, D, E) or orally with 10^9 *L. monocytogenes* organisms (C). After 8 d, spleen cells (A, B) or cells isolated from liver, lamina propria, and epithelium of the small intestine (C) were stained with FITC-conjugated anti-CD62L mAb, Cy5-conjugated anti-CD8α mAb, and PE-conjugated LLO_{91-99} tetramers. Cells were analyzed by flow cytometry after the addition of propidium iodide. (A) CD8-gated representative results. Numbers give the percent of cells within the region and are calculated for CD8$^+$ T cells only. (B, C) The percent of LLO_{91-99}-tetramer$^+$ CD62L^{low} of CD8$^+$ T cells and absolute number of LLO_{91-99}-tetramer$^+$ CD62L^{low} CD8$^+$ T cells per spleen (mean ± SD of three independently analyzed mice per group). In addition, spleen cells were incubated for 5 h with the peptides LLO_{91-99} or $p60_{217-225}$ (D, E). Cells were stained extracellularly with anti-CD8α mAb and intracellularly with anti-IFNγ mAb and analyzed by flow cytometry. (D) Representative results. Numbers give the frequencies for IFNγ$^+$ of CD8$^+$ cells only. (E) Frequencies of IFNγ$^+$ cells among CD8$^+$ T cells after in vitro incubation without (white bars) or with peptides LLO_{91-99} or $p60_{217-225}$ (black bars; mean ± SD of three individually analyzed mice per group). *Difference between cells from infected CCR7$^{+/+}$ and CCR7$^{-/-}$ mice was statistically significant ($P < 0.05$).

in control mice (8). Despite an aberrant T cell migration pattern in infected *plt* mice, antiviral T cell and antibody responses are virtually normal, and *plt* mice can control viral infections (8). Furthermore, immunization of *plt* mice with ovalbumin results in delayed, but otherwise normal or even stronger priming of $CD4^+$ T cells (4). Overall, these studies question the general requirement for CCR7/CCR7 ligands interactions for T cell priming and point to more complex functions during acquired immune responses.

Infection of mice with the intracellular bacterium *Listeria monocytogenes* represents a valuable model to study T cell responses in vivo (10, 11). After infection, *L. monocytogenes* migrate to their main target organs—spleen and liver—where they invade macrophages and hepatocytes. In these cells, *L. monocytogenes* penetrate the phagosomal membrane and enter the cytoplasm. Because of their intracytoplasmic habitat, *L. monocytogenes*–derived antigens are presented efficiently via the MHC class I pathway; this results in the induction of a potent $CD8^+$ T cell response. $CD8^+$ T cells are crucial for the control of *L. monocytogenes* infection and are responsible for protection against reinfection. In addition, a considerable $CD4^+$ T cell response is induced, although the role of this cell population is less clear (12, 13). Several *L. monocytogenes*–derived T cell epitopes have been characterized, including MHC class Ia–, MHC class Ib–, and MHC class II–presented peptides (10, 14). The respective peptides have been used extensively to study the kinetics and regulation of *L. monocytogenes*–specific T cell responses during infection. These analyses yielded extensive information on the effector and memory phases of *L. monocytogenes*–specific $CD4^+$ and $CD8^+$ T cell responses. However, the early events of T cell priming remain largely unknown. Only limited information is available on the sites and the APC populations that are involved in T cell priming and the requirements for T cell differentiation into effector and memory cells (15–18).

We analyzed CCR7-deficient mice using the murine *L. monocytogenes* infection model. We demonstrate that CCR7 has to be expressed on $CD8^+$ T cells and professional APCs to allow efficient MHC class Ia–restricted priming. In contrast, MHC class Ib–restricted $CD8^+$ T cells and MHC class II–restricted $CD4^+$ T cells are less dependent, and memory T cell responses are independent of CCR7.

RESULTS

$CCR7^{-/-}$ mice backcrossed onto the BALB/c background and $CCR7^{+/+}$ BALB/c control mice were i.v. infected with 10^3 *L. monocytogenes* organisms, and the $CD8^+$ T cell response was analyzed using H-$2K^d$ tetramers loaded with the immunodominant peptide listeriolysin O $(LLO)_{91–99}$ (19). 8 d after infection, approximately 2% of all $CD8^+$ splenocytes from $CCR7^{+/+}$ mice were $LLO_{91–99}$ tetramer$^+$ (Fig. 1, A and B). Spleens of $CCR7^{+/+}$ mice contained a total of 1.5–2.0×10^5 $LLO_{91–99}$ tetramer$^+$ $CD8^+$ T cells. In contrast, frequencies and numbers of $LLO_{91–99}$ tetramer$^+$ $CD8^+$ T cells in infected $CCR7^{-/-}$ mice were reduced and were close to background levels that were observed in noninfected animals (Fig. 1, A and B). The impaired response in $CCR7^{-/-}$ mice was not restricted to the spleen. After oral infection, we observed reduced cell numbers of $LLO_{91–99}$ tetramer$^+$ $CD8^+$ T cells in spleen, liver, and lamina propria and epithelium of the small intestine (Fig. 1 C). Similar results were obtained at d 9, 10, and 17 after infection, excluding that the low frequencies of $LLO_{91–99}$ tetramer$^+$ $CD8^+$ T cells were the result of a delayed T cell response in $CCR7^{-/-}$ mice (unpublished data).

In parallel to the tetramer assay, splenocytes from infected $CCR7^{+/+}$ and $CCR7^{-/-}$ BALB/c mice were restimulated in vitro with the peptides $LLO_{91–99}$ and $p60_{217–225}$, an independent immunodominant *L. monocytogenes*–derived $CD8^+$ T cell epitope (19). Stimulation with both peptides induced IFNγ production in $CD8^+$ spleen cells from infected control mice (Fig. 1, D and E). In contrast, only marginal frequencies of IFNγ^+ $CD8^+$ T cells were observed in spleens of $CCR7^{-/-}$ mice; this confirmed the results from the $LLO_{91–99}$ tetramer assay and indicated that the impaired response was not restricted to the $LLO_{91–99}$ epitope.

Despite the marginal proportion of *L. monocytogenes*–specific $CD8^+$ T cells, $CCR7^{-/-}$ mice tolerated the *L. monocytogenes* inoculum that was used in our experiments, and susceptibility to infection was not increased substantially. $CCR7^{-/-}$ mice showed impaired clearance of *L. monocytogenes* with slightly higher bacterial titers at late time points after infection (Fig. 2). However, at later time points (>10 d after infection), $CCR7^{+/+}$ and $CCR7^{-/-}$ mice usually had cleared *L. monocytogenes* from spleen and liver (unpublished data).

Infection of mice with *L. monocytogenes* results in the generation of specific $CD8^+$ memory T cells, which mount an accelerated and elevated anti–*L. monocytogenes* T cell response to reinfection. To assess the role of CCR7 in the

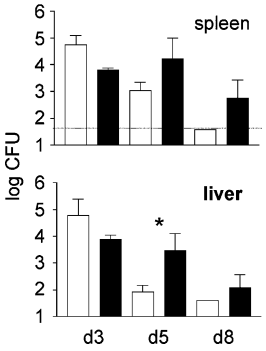

Figure 2. Bacterial burdens in spleen and liver of $CCR7^{-/-}$ mice after *L. monocytogenes* infection. $CCR7^{+/+}$ (white bars) and $CCR7^{-/-}$ mice (black bars) were i.v. infected with 10^3 *L. monocytogenes* organisms. On the indicated days, mice were killed and bacterial titers in spleen and liver were determined. Results represent the mean ± SD of four or five mice per group. The dotted line gives the detection limit of our assay (40 bacteria/organ). *Difference between titers from infected $CCR7^{+/+}$ and $CCR7^{-/-}$ mice was statistically significant ($P < 0.05$).

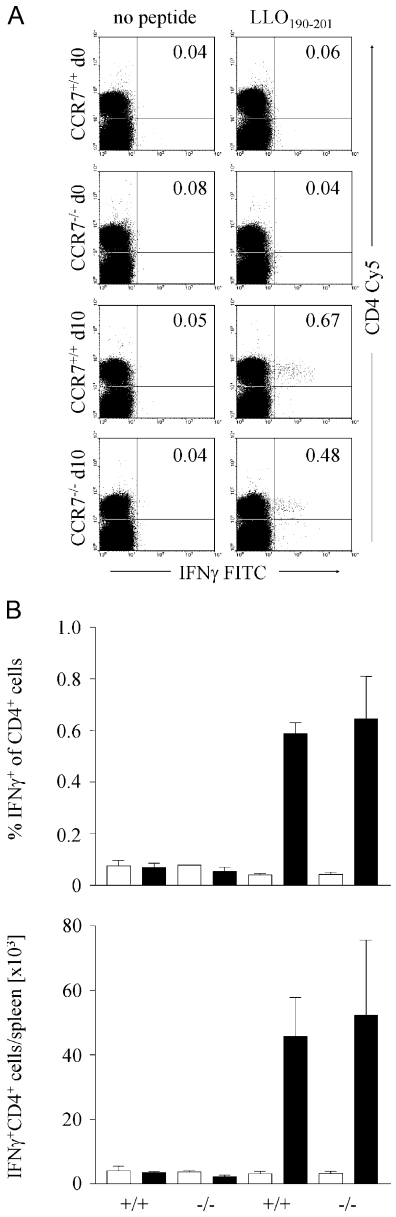

Figure 6. CD4$^+$ T cell response to LLO$_{190–201}$ during primary infection. CCR7$^{+/+}$ and CCR7$^{-/-}$ mice on C57BL/6 background were i.v. infected with 2×10^3 *L. monocytogenes* organisms. After 10 d, spleen cells were incubated for 5 h with the peptide LLO$_{190–201}$. Cells were stained extracellularly with anti-CD4 mAb and intracellularly with anti-IFNγ mAb, and analyzed by flow cytometry. (A) Representative results. (B) Frequencies of IFNγ^+ cells among CD4$^+$ T cells and absolute numbers of IFNγ^+ CD4$^+$ cells per spleen after incubation without (white bars) and with peptide LLO$_{190–201}$ (black bars; mean $\pm$ SD of three individually analyzed mice per group). There was no significant difference ($P > 0.05$) between IFNγ^+ cells from infected CCR7$^{+/+}$ and CCR7$^{-/-}$ mice after restimulation with peptide.

mogenous response in all six experiments, in which we analyzed CD4$^+$ T cell responses. In contrast, CD4$^+$ T cells from CCR7$^{-/-}$ mice demonstrated some variation. We observed individual mice, in which the response was reduced up to one third of the mean response of wild-type animals. However, in none of the experiments did the difference between the mean response in CCR7$^{+/+}$ and CCR7$^{-/-}$ mice reached a statistically significant level ($P < 0.05$). In summary, our results demonstrate that MHC class II–restricted CD4$^+$ T cell responses to *L. monocytogenes* are far less dependent on CCR7 than are the corresponding MHC class Ia–restricted CD8$^+$ T cell responses.

Because CCR7 is expressed on naive CD8$^+$ T cells and on activated dendritic cells, we determined the role of CCR7 on CD8$^+$ T cells in an adoptive T cell transfer model. CD8$^+$ T cells from the TCR-transgenic mouse strain OT1 recognize OVA$_{257–264}$, the immunodominant CD8$^+$ T cell epitope that is included in the truncated ovalbumin protein expressed by LmOVA (20). To determine whether CCR7 expression on CD8$^+$ T cells is sufficient for the induction of a response, purified CD8$^+$ T cells from OT1 donors were labeled with carboxyfluorescein succinimidyl ester (CFSE) and adoptively transferred into CCR7$^{+/+}$ and CCR7$^{-/-}$ recipients. After 24 h, mice were infected with LmOVA; 72 h later, proliferation of transferred CD8$^+$ T cells was analyzed by flow cytometry (Fig. 7). In noninfected CCR7$^{+/+}$ animals, transferred T cells maintained the CFSE^{high} phenotype. Infection of CCR7$^{+/+}$ recipients induced proliferation of CD8$^+$ donor cells as indicated by the stepwise loss of CFSE staining. In noninfected CCR7$^{-/-}$ recipients, we detected a low level of donor cell proliferation for which we currently have no explanation. However, proliferation did not increase further upon LmOVA infection. As was expected from the analysis of the endogenous CD8$^+$ T cell response to LmOVA infection (Fig. 4 A), the block in activation of transferred CD8$^+$ T cells was incomplete in CCR7$^{-/-}$ recipients. At day 4 after infec-

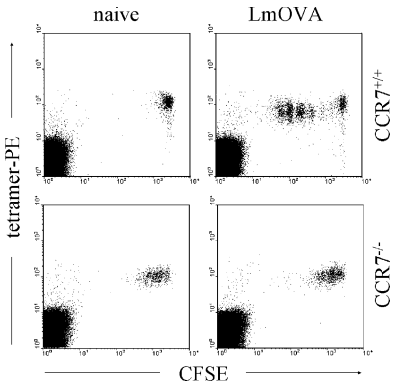

Figure 7. Expression of CCR7 on CD8$^+$ T cells is insufficient for T cell priming. CCR7$^{+/+}$ and CCR7$^{-/-}$ mice on C57BL/6 background received 4×10^6 purified CFSE-labeled CD8$^+$ T cells from OT1 mice. After 24 h, recipients were i.v. infected with 5×10^3 LmOVA organisms, or were left untreated (naive). 3 d later, spleen cells were stained with PE-conjugated OVA$_{257–264}$ tetramers and Cy5-conjugated anti-CD8α mAb, and CFSE expression on transferred CD8$^+$ T cells was analyzed by flow cytometry. Figures show CD8-gated T cells only and are representative for two individually analyzed mice per group.