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Impact of CCR7 on Priming and Distribution of Antiviral Effector and Memory CTL

Tobias Junt,†* Elke Scandella,† Reinhold Förster,‡ Philippe Krebs,*† Stefan Krautwald,§ Martin Lipp,¶ Hans Hengartner,* and Burkhard Ludewig2*†

The chemokine receptor CCR7 is a key factor in the coordinate migration of T cells and dendritic cells (DC) into and their localization within secondary lymphoid organs. In this study, we investigated the impact of CCR7 on CD8+ T cell responses by infecting CCR7−/− mice with lymphocytic choriomeningitis virus (LCMV). We found that the absence of CCR7 affects the magnitude of an antiviral CTL response during the acute phase, with reduced numbers of virus-specific CTL in all lymphoid and nonlymphoid organs tested. On the single cell level, CCR7-deficient CTL gained full effector function, such that antiviral protection in CCR7-deficient mice was complete, but delayed. Similarly, adoptive transfer experiments using DC from CCR7-deficient or competent mice for the priming of CCR7-positive or CCR7-negative CD8+ T cells, respectively, revealed that ectopic positioning of DC and CTL outside organized T cell zones results in reduced priming efficacy. In the memory phase, CCR7-deficient mice maintained a stable LCMV-specific CTL population, predominantly in nonlymphoid organs, and rapidly mounted protective CTL responses against a challenge infection with a vaccinia virus recombinant for the gp33 epitope of LCMV. Taken together, the CCR7-dependent organization of the T cell zone does not appear to be a prerequisite for antiviral effector CTL differentiation and the sustenance of antiviral memory responses in lymphoid or peripheral tissues. The Journal of Immunology, 2004, 173: 6684–6693.

Naive T cells proliferate and differentiate into effector cells after Ag encounter on professional APCs within secondary lymphoid organs. Acquisition of effector cell function is accompanied by distinct changes in the expression of surface molecules, which facilitates T cell relocation from secondary lymphoid organs to peripheral tissues (1). Changes in surface markers normally comprise the modulation of adhesion molecules (2) and of receptors for inflammatory and constitutive chemokines (3). After Ag clearance, the specific effector T cell population rapidly contracts, leaving behind a stable memory T cell pool. Hyperresponsiveness of such memory T cells (4, 5) allows for the rapid generation of an effector T cell population upon Ag re-encounter. However, it is still a matter of debate as to whether memory T cells are derived only from fully differentiated effector T cells (6), or whether they represent a mixture of distinct subtypes derived from differentially stimulated precursors (7).

CCR7 and its ligands, CCL19 and CCL21, are crucial for the positioning of T cells and dendritic cells (DC) within T cell zones of secondary lymphoid organs (8–10). Naive T cells express high levels of CCR7, and its down-regulation is closely correlated with T cell activation (3, 11). Furthermore, it has been suggested that memory T cell populations can be functionally distinguished by the presence or the absence of CCR7. CCR7lowCD62Llow effector memory cells preferentially migrate to peripheral nonlymphoid organs (12, 13) and exhibit immediate effector function. It has been suggested that these cells may eventually differentiate to CCR7highCD62Lhigh central memory cells (14), which are retained within secondary lymphoid organs as Ag-experienced nonpolarized cells lacking immediate effector function.

Numerous studies support the idea that CCR7 and its chemokine ligands, CCL19 and CCL21, also have a fundamental impact on priming and maintenance of immune reactions by influencing T cell and DC migration (8, 9). We have recently shown that pltplt mice lacking the CCR7 ligands CCL19 and CCL21-Ser mount rapid antiviral T and B cell responses and exhibit normal formation of memory CTL (15). In the present study, we used CCR7-deficient mice to further dissect the role of this receptor in the distribution, migration, and function of antiviral effector and memory CTLs in lymphoid and nonlymphoid organs.

Protective immune responses against the noncytopathic lymphocytic choriomeningitis virus (LCMV) largely depend on the induction of antiviral CTL, which destroy infected cells in a contact-dependent and perforin-mediated manner (16). We examined the role of CCR7 during the effector and memory phases of antiviral immune responses using LCMV as a model infection. Our results indicate that the lack of organized T cell zones in CCR7−/− mice limits the maximal expansion of antiviral CTL. Furthermore, the CCR7 deficiency crucially affects memory T cell distribution between lymphoid and nonlymphoid organs. The absence of CCR7, however, did not preclude viral clearance and generation of protective recall responses, suggesting a role for CCR7 in the efficient expansion of antiviral CTL and the homeostatic recirculation of memory T cells.
Materials and Methods

Mice

All mice used in this study were kept at the Institut für Laborierkunde, University of Zurich (Zurich, Switzerland). CCR7+/− mice (8) were backcrossed onto the 129/Ola background for at least five generations. For adoptive transfer experiments, CCR7+/− mice were backcrossed to C57BL/6 for nine generations, then crossed to P14 (17) or H8 transgenic mice (18). Genotyping for CCR7 deficiency was performed as described previously (8). Experiments were performed with sex-matched CCR7+/− mice and control heterozygous littermates at the age of 8–12 wk. Heterozygous control mice express slightly less CCR7 on lymphocytes compared with wild-type mice; however, this does not to impinge on the ability of CCR7+/− mice to mount efficient anti-LCMV CTL responses (data not shown).

Antibodies

Anti-CD8-FITC, anti-CD3-PE, anti-IFN-γ-FITC, anti-CD62L-FITC, anti-CD8-PerCP, anti-CCR5-PE, anti-CD43-PE, anti-CD44-PE, anti-Thy1.2-PE, anti-CCR7-PE, and streptavidin-allophycocyanin were obtained from BD Pharmingen (Basil, Switzerland). To assess CCR7 expression, lymphocytes were incubated for 1 h at 4°C with 80 μl of COS cell supernatant containing 1 μg/ml CCL19-IgG, generated as previously described (19) with minor modifications (20). Cells were washed and incubated for 30 min at 4°C with biotin-SP-conjugated goat anti-human IgG described (19) with minor modifications (20). Cells were analyzed with a FACSCalibur flow cytometer using CellQuest software (BD Biosciences, Mountain View, CA). When combined with tetramer stainings, CCL19-Ig labeling followed the staining of the central hematology laboratory of University Hospital Zurich.

Intracellular cytokine staining

Spleens were removed at the indicated time points after infection with LCMV. Single cell suspensions of 1×10^6 splenocytes, lymph node cells, or liver or lung lymphocytes were incubated for 5 h at 37°C in 96-well, round-bottom plates in 200 μl of culture medium containing 25 U/ml IL-2 and 5 μg/ml brefeldin A (Sigma-Aldrich). Cells were stimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml) as the positive control or were untreated as the negative control. For analysis of peptide-specific responses, 10^6 cells were stimulated with 10^−6 M gp33 peptide and then surface stained as described previously (15). The percentage of CD8+ T cells producing IFN-γ was determined using a FACS Calibur flow cytometer.

Adoptive transfer and DC homing

Bone marrow-derived DC were generated from H8 mice as previously described (25). Assessment of DC-induced CTL priming was performed as follows. MACS-sorted P14 or P14×CCR7−/− CD8+ T cells (3×10^5) were adoptively transferred together with 2×10^6 H-2D^b-Thy1.1 recipients to reach a sufficient cell density for histomorphological evaluation. Five days postinjection of 5×10^6 cells of both cell types, spleens were sectioned, acetone-fixed, counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and stained for Thy1.2, CD8, CD62L, and CD44. The impact of CCR7 deficiency on intrasplenic positioning of DC and CTL was assessed by staining DC with the live dye 5-(and-6)-(4-(4-chloromethyl)benzoylamin)tetramethyl-rhodamine (Molecular Probes, Eugene, OR) and staining TCR transgenic CD8 T cells with CFSE (Molecular Probes) before adoptive transfer. Because only ~10% of the i.v. administered DC reach the spleen (26), both DC and T cells were injected directly into the spleens of B6 recipients to reach a sufficient cell density for histomorphological evaluation. Five hours postinjection of 5×10^6 cells of both cell types, spleens were sectioned, acetone-fixed, counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (Chemicon International, Temecula, CA), and analyzed by fluorescence microscopy using a BX61 fluorescence microscope (Olympus, Volketswil, Switzerland). To assess the proliferation of CD8+ T cells, MACS-sorted P14 CD8 T cells or P14×CCR7−/− CD8 T cells were labeled with CFSE, and 5×10^6 cells were adoptively transferred into C57BL/6 or CCR7−/− mice, respectively, before infection with 200 PFU of LCMV. Two, 4, or 7 days later, lymphocytes from spleens, MLN, and liver were stained for CD8 and Vα2 TCR and analyzed for CFSE dilution by flow cytometry. The homing efficiency of CCR7-deficient vs wild-type DC after i.v. injection was assessed by labeling with 50 μCi of 35Cr (Amersham Biosciences, Arlington Heights, IL) for 45 min at 37°C. Cells were washed three times with balanced salt solution, and the labeling efficiency per 5 million DC was determined using a Cobra II gamma counter (Canberra Packard, Mississauga, Canada). As expected, CD8+ T cells were uniformly found to be CCR7high, even in peripheral organs such as lung and liver. Furthermore, CD62L was highly expressed on CD8+ T cells in all organs tested, except in lungs, where 67% of the cells had downregulated CD62L.

Viruses and peptides

LCMV. WE strain, originally obtained from Dr. F. Lehmann-Gruube (Heinrich-Pette-Institut, Hamburg, Germany), was propagated on L929 cells at a low multiplicity of infection and titrated as previously described (21). Recombinant vaccinia virus (VV) expressing gp33 as a minigenie (VV-gp33C) was provided by Dr. M. van den Broek (University of Zurich, Zurich, Switzerland). CCR7+ mice (8) were backcrossed to C57BL/6 or CCR7+ mice (18). Genotyping for CCR7 deficiency was performed as described previously (8). Experiments were performed with sex-matched CCR7+/− mice and control heterozygous littermates at the age of 8–12 wk. Heterozygous control mice express slightly less CCR7 on lymphocytes compared with wild-type mice; however, this does not to impinge on the ability of CCR7+/− mice to mount efficient anti-LCMV CTL responses (data not shown).

Footpad swelling reaction

A delayed-type hypersensitivity (DTH) response was induced by injecting 50 PFU of LCMV-WE into the hind footpads. Footpad thickness was measured by a spring-loaded caliper. Increased footpad thickness was expressed as the percent swelling relative to the thickness before injection.

Isolation of liver, lung, and splenic white pulp lymphocytes

Perfused livers were smashed through a metal grid. Lymphocytes were purified by Ficoll (Biochrom, Berlin, Germany) gradient centrifugation (600×g, 15 min). Lungs were minced with razor blades and incubated in balanced salt solution containing 1 mg/ml DNase (Fluka, Buchs, Switzerland) and 2 mg/ml collagenase I (Sigma-Aldrich, St. Louis, MO) at 37°C for 30 min. Cell aggregates were dispersed by passing the digest through an 18-gauge syringe, and lymphocytes were isolated by Ficoll gradient centrifugation. White pulps of the spleen were isolated by digestion with collagenase V and III as previously described (23).

Construction of tetrameric class I-peptide complexes and flow cytometry

MHCl class I (H-2D^b) monomers complexed with gp33 were produced as previously described (24) and tetramerized by addition of streptavidin-PE (Molecular Probes, Eugene, OR). At the indicated time points after immunization, animals were bled, and single cell suspensions were prepared from spleens and lymph nodes. Aliquots of 5×10^5 cells or three drops of blood were stained using 50 μl of a solution containing tetrameric class I-peptide complexes at 37°C for 10 min, followed by staining with anti-CD8-PE (BD Pharmingen) at 4°C for 20 min. The cells were analyzed by flow cytometric gating on viable leukocytes. Absolute cell counts were determined by counting leukocytes in an improved Neubauer chamber and, for blood samples, using an automated Advia counter (Bayer, Germany) in the central hematology laboratory of University Hospital Zurich.

Results

CCR7 and CD62L expression on CD8+ T cells during LCMV infection

Differential expression of CCR7 and CD62L has been reported to characterize effector and memory T cells in both humans (12) and mice (7). We thus assessed the surface phenotype of virus-specific CTL in lymphoid and nonlymphoid compartments after infection of heterozygous CCR7+/- mice with LCMV (Fig. 1). Due to the very low numbers of LCMV-specific CTL in naive animals, CCR7 and CD62L expression was assessed on CD8+ T cells (Fig. 1, upper row). As expected, CD8+ T cells were uniformly found to be CCR7high, even in peripheral organs such as lung and liver. Furthermore, CD62L was highly expressed on CD8+ T cells in all organs tested, except in lungs, where 67% of the cells had downregulated CD62L.

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To characterize the phenotype of LCMV-specific effector CTL, we used MHC class I tetramers complexed with the immunodominant CTL epitope gp33 derived from the LCMV glycoprotein (tet-gp33) (24, 27). During the peak CTL response against LCMV (day 8 postinfection), the majority of CD8<sup>+</sup>_tet-gp33<sup>+</sup> effector CTL from peripheral organs, such as liver and lung, were found to be CD62L<sup>low</sup>CCR7<sup>low</sup> (Fig. 1, middle row). Surprisingly, an additional fraction of CD62L<sup>low</sup>CCR7<sup>high</sup> population of gp33-specific CTL could be observed in spleens and lymph nodes, possibly representing CD8<sup>+</sup> T cells that had not yet undergone complete differentiation to the CTL effector state. In the memory phase (day 80 postinfection), two populations, CD62L<sup>low</sup>CCR7<sup>high</sup> and CD62L<sup>low</sup>CCR7<sup>low</sup>, of CD8<sup>+</sup> cells that had not yet undergone complete differentiation to the CTL effector state. In the memory phase (day 80 postinfection), two populations, CD62L<sup>low</sup>CCR7<sup>high</sup> and CD62L<sup>low</sup>CCR7<sup>low</sup>, were determined in the indicated organs by flow cytometry (Fig. 2A). Normal effector function of CTL in the absence of CCR7

Reduced clonal burst of effector CTL in CCR7<sup>−/−</sup> mice

CCR7<sup>−/−</sup> mice show aberrantly formed lymphoid T cell zones, strongly reduced T cell numbers in lymph nodes, and homing defects of DC and naive T cells (8). To assess the functional significance of this phenotype for the generation of acute antiviral CTL responses, CCR7<sup>−/−</sup> and CCR7<sup>+/−</sup> mice were used as controls. The direct cytolytic activity of LCMV-gp33<sup>+</sup> T cells in response to cognate peptide was not impaired by the lack of CCR7 when assessed on day 14 postinfection (Fig. 3C). CCR7<sup>+/−</sup> heterozygous and CCR7<sup>+/+</sup> wild-type mice generated equivalent antiviral LCMV CTL responses (data not shown), thus excluding an impact of the slight differences in CCR7 expression between wild-type LCMV<sup>+</sup> mice and CCR7<sup>−/−</sup> mice on day 8 postinfection (Fig. 3C), probably due to the reduced numbers of effector CTL in these mice. As shown above, gp33-specific CTL continued to expand in CCR7<sup>−/−</sup> mice between days 8 and 12, eventually leading to virus clearance.

Role of CCR7 expressed by DC vs T cells for priming of CTL responses

Next, we assessed the impact of CCR7-mediated positioning of DC and T cells within secondary lymphoid organs on the efficacy of CTL priming. To this end, we used an adoptive transfer approach. CCR7<sup>−/−</sup> mice were crossed with P14 transgenic mice encoding a TCR specific for LCMV-gp33 (17) or H8 transgenic mice (18), which ubiquitously express LCMV-gp33. All mice were on the C57BL/6 background to facilitate adoptive transfer of lymphocytes into C57BL/6 and the congenic B6.PLThy1.1 strain. Tracing experiments with <sup>51</sup>Cr-labeled DC revealed that the homing patterns of CCR7-deficient and wild-type DC after i.v. injection were...
comparable (Fig. 4A), so that differential expansion of effector CTL could not be attributed to differential DC numbers within secondary lymphoid organs. Priming of adoptively transferred gp33-specific P14 or P14 × CCR7−/− CD8+ T cells by H8 or H8 × CCR7−/− DC CTL was examined by flow cytometry using the Thy1.2 marker. Expansion of P14-CCR7−/− CTL by H8CCR7−/− DC was reduced by ~50% compared with transfer of both wild-type populations (Fig. 4B). Activation of CTL in both situations was comparable, as determined by the expression of the activation markers CD44 and CD62L (Fig. 4C). Surprisingly, the

FIGURE 2. Effect of CCR7 deficiency on CTL clonal burst after LCMV infection. A and B, Eight days after LCMV infection, lymphocytes were isolated from spleen, MLN, liver, lung, and blood and analyzed for expression of CD8 and for reactivity with gp33-tetramer. Values in A represent the percentage of CD8+ T cells staining positively for the gp33 tetramer; values in B represent the absolute numbers of CD8+ tet-gp33+ cells ± SD in the indicated organ (n = 6). Pooled data from two separate experiments are shown. C, Absolute numbers of CD8+ T cells staining with the gp33 tetramer at various time points after infection (n = 3). D, Footpad swelling reaction after infection with 50 PPU of LCMV-WE into the hind footpads (n = 2). E, The expression of different activation markers on CD8+ tet-gp33+ cells from acutely LCMV infected CCR7−/− (line plot) and CCR7+/− (shaded plot) mice is shown.
Pooled data from three separate experiments are shown and are representative of two separate experiments. Indicate the mean from the indicated organs of CCR7 51Cr-labeled EL4 cells pulsed with gp33 or np396. E:T cell ratios were and analyzed for cytolytic activity in a 5-h 51Cr release assay on day 8 postinfection. Ex vivo CTL activity of lymphocytes from spleen and MLN from CCR7 postinfection. Ex vivo CTL activity of lymphocytes from spleen and MLN of CCR7 IN ANTIVIRAL CYTOTOXIC T CELL RESPONSES.

Induction of functional LCMV-specific effector CTL in the absence of CCR7. A, Lymphocytes were isolated from the indicated organs and analyzed for cytolytic activity in a 5-h 51Cr release assay on day 8 postinfection. Ex vivo CTL activity of lymphocytes from spleen and MLN from CCR7−/− (□ and △) and CCR7+/− (■ and ▲) mice was tested on 51Cr-labeled EL4 cells pulsed with gp33 or np396. E:T cell ratios were corrected for the number of tetramer-positive CD8+ cells (see Fig. 2A) and indicate the mean ± SD of three or four mice per group. B, CD8+ T cells from the indicated organs of CCR7−/− (black line) and CCR7+/− mice (gray area) were gated by flow cytometry and analyzed for LCMV-gp33-specific IFN-γ production on day 14 postinfection. Values indicate the mean ± SD of three mice. Data shown in A and B are from one experiment and are representative of two separate experiments. C, LCMV titers were determined in different organs on days 4, 8, and 13 after infection with 200 PFU of LCMV-WE. Values represent organ titers of individual mice. Pooled data from three separate experiments are shown.

Kinetics of CTL expansion in the absence of CCR7

We next assessed, using adoptive transfer of CFSE-labeled P14 × CCR7−/− CD8+ T cells into CCR7−/− mice and of P14 CD8+ T cells into C57BL/6 mice, whether the reduced magnitude in the LCMV-induced CTL response in CCR7−/− mice (Fig. 2) is due to an impaired Ag-specific proliferation. On day 2 after infection with 200 PFU of LCMV-WE, neither CCR7-positive nor -negative P14 CD8 T cells had entered the proliferation phase (Fig. 5A). However, P14 T cells were present at higher frequencies in spleens and lymph nodes of C57BL/6 recipients, most likely reflecting the more efficient recruitment of CCR7-competent T cells to secondary lymphoid organs. On day 4 after infection, P14 CD8+ T cells had already undergone at least five rounds of cell divisions, in contrast to P14 × CCR7−/− cells that had not yet started the proliferation program (Fig. 5B). Nevertheless, on day 7 postinfection, both P14 and P14 × CCR7−/− cells had efficiently proliferated (Fig. 5C). These data support the idea that the altered kinetics of CTL expansion in CCR7-deficient mice after LCMV infection are due to a weaker recruitment of CD8+ T cells to secondary lymphoid organs and a delayed onset of Ag-specific proliferation.

Antiviral memory CTL responses in the absence of CCR7

We next set out to examine the role of CCR7 in the distribution of memory CTL after viral infection. For this purpose, we determined the absolute numbers of gp33-specific CTL in different lymphoid and nonlymphoid organs during the memory phase of the LCMV infection. Absolute numbers of gp33-specific memory CTL on day 80 postinfection were lower in all organs of CCR7−/− mice compared with CCR7+/− mice (Fig. 6, first data point). Comparison of these values with the expansion of CTL on day 40 postinfection (Fig. 2C) indicated that the decay of memory CTL in CCR7−/− mice did not differ from that observed in CCR7+/− mice. However, it is noteworthy that memory CTL were efficiently excluded from lymph nodes, indicating that the absence of CCR7 impacts mainly memory CTL distribution.

To further confirm this interpretation, we tested whether CCR7−/− mice were able to mount a protective response against a second viral infection. For this purpose, LCMV-immune CCR7−/− and CCR7+/− mice were i.v. challenged with 2 × 106 PFU VV-gp33C on day 80 after primary infection. The second data point in Fig. 6 indicates the expansion of CCR7−/− and CCR7+/− memory CTL after challenge infection. In all organs priming efficacy was significantly reduced when CCR7 expression on the transferred cell populations was disparate, i.e., CCR7-negative H8 DC failed to prime CCR7-positive P14 CD8+ T cells and vice versa (Fig. 4, B and C). These results strongly suggest that productive cognate interaction between Ag-bearing DC and responding CTL requires homing of both cell populations to the same compartment, be it the T cell zone or the red pulp/marginal zone. Indeed, visualization of the intrasplenic positioning of CCR7-competent DC and CD8+ T cells revealed that DC and T cells homed mainly to the T cell zone (Fig. 4D), whereas CCR7-deficient DC and T cells migrated exclusively to the red pulp or the marginal zone (Fig. 4G). Importantly, DC and CD8+ T cells from CCR7-disparate donors were usually not able to establish productive contacts, because CCR7-positive cells homed to the T cell zone, whereas CCR7-negative cells were sequestered in the red pulp and the marginal zone (Fig. 4, E and F). Taken together, these data show that DC-CTL contact in the T cell zone is not an absolute requirement for efficient CTL priming. Nevertheless, a microenvironment that facilitates concentration of both cell populations and increases the likelihood of their cognate interaction appears to be important for maximal amplification of the immune response.

FIGURE 3. Induction of functional LCMV-specific effector CTL in the absence of CCR7. A, Lymphocytes were isolated from the indicated organs and analyzed for cytolytic activity in a 5-h 51Cr release assay on day 8 postinfection. Ex vivo CTL activity of lymphocytes from spleen and MLN from CCR7−/− (□ and △) and CCR7+/− (■ and ▲) mice was tested on 51Cr-labeled EL4 cells pulsed with gp33 or np396. E:T cell ratios were corrected for the number of tetramer-positive CD8+ cells (see Fig. 2A) and indicate the mean ± SD of three or four mice per group. B, CD8+ T cells from the indicated organs of CCR7−/− (black line) and CCR7+/− mice (gray area) were gated by flow cytometry and analyzed for LCMV-gp33-specific IFN-γ production on day 14 postinfection. Values indicate the mean ± SD of three mice. Data shown in A and B are from one experiment and are representative of two separate experiments. C, LCMV titers were determined in different organs on days 4, 8, and 13 after infection with 200 PFU of LCMV-WE. Values represent organ titers of individual mice. Pooled data from three separate experiments are shown.
FIGURE 4. Relevance of CCR7 on DC vs CD8⁺ T cells during priming. A, Adoptive transfer of ⁵¹Cr-loaded, bone marrow-derived DC from C57BL/6 mice and CCR7⁻/⁻ mice into C57BL/6 and analysis of perfused organs 24 h post-transfer. B, Expansion of CD8⁺ tetgp33⁺ CTL after priming of 3 × 10⁵ P14 or P14 × CCR7⁻/⁻ MACS-purified CD8⁺ T cells with 2 × 10⁶ H8-DC or H8-CCR7⁻/⁻ bone marrow-derived DC on day 5 after adoptive transfer into B6PL.Thy1.1 recipients. Bars represent the mean of three mice. One representative of two experiments is shown. C, Expression profile of CD44 and CD62L on P14/P14 × CCR7⁻/⁻ CTL in spleen (upper panel) and liver (lower panel) 5 days after adoptive transfer. D–G, Relative distribution of intrasplenically injected P14 MACS-purified CD8⁺ T cells (labeled with CFSE) and bone marrow-derived H8-DC (labeled with 5-(and-6)-(((4-chloromethyl)benzoyl)amino)tetramethyl-rhodamine) in spleen in the absence or the presence of CCR7, 5 h after adoptive transfer.
except lymph nodes, gp33-specific CTL lacking CCR7 expanded to identical or even increased numbers compared with CCR7-competent gp33-specific CTL (Fig. 6, second data point), and both groups of mice had completely eliminated the VV by day 3 post-challenge (data not shown). Thus, memory CTL from CCR7-/- mice demonstrated a high protective antiviral capacity. Interestingly, CCR7-deficient memory CTL were still excluded from lymph nodes (Fig. 6B) after secondary infection with VV-gp33C.

**CCR7 expression does not correlate with the activity of antiviral memory CTL from lymphoid vs nonlymphoid organs**

Memory CTL from peripheral nonlymphoid organs exert rapid effector function (28) and are thought to provide a mechanism for rapid pathogen containment. It has been suggested that the presence of such effector memory T cells in peripheral organs is associated with loss of CCR7 expression and gain of effector function (12). We therefore assessed the immediate IFN-γ production of CCR7-deficient or -competent memory CTL from different lymphoid and nonlymphoid organs on day 80 after infection with LCMV-WE after a short term in vitro stimulation with gp33. Both CCR7-/- and CCR7+/+ gp33-specific memory CTL from all organs tested readily produced IFN-γ (Fig. 7A). These data indicate that memory CTL from both lymphoid and nonlymphoid organs can mount immediate effector function, and that this function is not dependent on CCR7 expression. Although the absolute numbers of gp33-specific memory CD8+ T cells producing IFN-γ after short term peptide stimulation was decreased in CCR7-/- mice compared with CCR7+/+ mice (Fig. 7A), these numbers did not significantly differ from the values obtained for the respective groups by tetramer analysis (compare Fig. 6). When memory CTL from lymphoid vs peripheral organs were tested in a 5-h 51Cr release assay, both memory CTL from spleens and lungs of CCR7-/- mice and CCR7+/+ controls showed efficient target lysis (Fig. 7B). Furthermore, the expression of the cell surface markers CD44, CD62L, CCR5, and the activation-associated isoform of CD43 (1B11) was similar on gp33-specific memory CTL from lymphoid and nonlymphoid organs of CCR7-/- mice and CCR7+/+ (Fig. 7C). Taken together, these data further support our conclusion that CCR7 is important for the distribution of memory CTL in lymphoid and nonlymphoid organs, but that CCR7 expression does not correlate to their functional differentiation.

**Discussion**

The role of CCR7 in the induction and maintenance of antiviral effector and memory CTL responses was examined in CCR7-/- mice using LCMV-specific tetramers and analysis of effector function on the single cell level. The presented data indicate that CCR7 deficiency mainly impacts on the magnitude of virus-specific CTL responses and the distribution of these cells between lymphoid and nonlymphoid tissues. On the single cell level, however, the function of CCR7-deficient CTL was not impaired. Taken together, these data indicate that CCR7 is crucial for the coordinate migration and expansion of antiviral effector and memory CTL. However, the CCR7-dependent structural differentiation of the lymphoid T cell zone is not essential for the generation and maintenance of antiviral CTL responses.

In vitro studies using polarized T cells or T cell lines and in vivo studies (3, 11) have shown a correlation between effector function and low CCR7 expression. In accordance with these findings, we found that CCR7 is highly expressed on naive CTL in lymphoid and nonlymphoid organs, and that virus-specific CTL in blood and peripheral organs of mice had down-regulated CCR7 during acute virus infection. However, it is interesting to note that a significant proportion of LCMV-specific CTL in spleen and lymph nodes had not lost CCR7 and CD62L expression during the effector phase. In contrast to previous studies describing antiviral effector CTL to be CCR7low in LCMV infections (11, 14), we have not exclusively used transgenic CD8+ T cells, but also followed the complete gp33-specific effector CTL population by tetramer analysis. This population is polyclonal (29) and likely to be composed of T cells with different TCR affinities and activation requirements. Therefore, the expression levels of CCR7 and CD62L may vary at a given time point of effector differentiation. Moreover, our data corroborate a recent study by Unsoeld et al. (30), suggesting that during the acute phase of an antiviral immune response both CCR7-positive and -negative CTL from secondary lymphoid organs can exert full effector function. It appears therefore that the CCR7low phenotype of CTL is associated with acute effector function in peripheral nonlymphoid organs, but not in secondary lymphoid organs.

It has been shown that CCR7 deficiency results in impaired T cell responses, such as the complete abolishment of DTH reactions.
against FITC or keyhole limpet hemocyanin after a short (4-day) priming period (8). Similarly, we have demonstrated reduced LCMV-mediated footpad swelling in CCR7−/− mice. DTH reactions depend strongly on DC-mediated Ag transport from the site of inoculation to secondary lymphoid organs (31). We consider it likely that severely impaired DTH reactions in CCR7−/− mice are the result of a nearly complete blockade of DC migration from skin to local lymph nodes, and that only limited amounts of DC are available for T cell priming (8, 32). However, the induction of CTL responses during LCMV infection is probably less dependent on DC-mediated Ag influx to secondary lymphoid organs, because this rapidly disseminating virus can spread independently of cell trafficking. Therefore, the reduced CTL expansion in CCR7−/− mice after LCMV infection is presumably accentuated by ectopic DC and CTL positioning in secondary lymphoid organs, because this rapidly disseminating virus can spread independently of cell trafficking. Therefore, the reduced CTL expansion in CCR7−/− mice after LCMV infection is presumably accentuated by ectopic DC and CTL positioning in secondary lymphoid organs. The presented adoptive transfer experiments using CCR7-deficient DC and CTL support this interpretation because ectopic CTL priming in the red pulp and/or the marginal zone resulted in reduced priming efficacy.

CCR7-deficient mice and plt/plt mice, which lack the CCR7 ligand chemokines CCL19 and CCL21-Ser within secondary lymphoid organs (9), exhibit comparable morphology in lymph nodes and spleens. However, unlike CCR7−/− mice, plt/plt mice are able to mount nearly unimpaired DTH reactions (33) and antiviral CTL responses (15). The plt/plt mice express the leucine isoform of CCL21 (CCL21b) outside lymphoid tissues in lymphatic vessels. In addition, CCL21b is expressed constitutively in nonlymphoid organs (10, 34) and may therefore improve the recruitment of antiviral CCR7-positive effector CTL to peripheral tissues. Furthermore, a recent study reported that peripheral CCL21 caused lymph node congestion and augmented the initiation of T cell responses during inflammation (32). The expression of CCL21b in lymph vessels and peripheral tissues in plt/plt mice thus most likely contributes to the preserved antiviral CTL responses after LCMV infection, as observed in our previous study (15), whereas DC and

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**FIGURE 6.** Distribution of LCMV-specific memory CTL in CCR7-deficient mice. The first data point in A–E indicates the number of gp33-specific CTL ± SD for spleen (A), MLN (B), liver (C), lung (D), and blood (E) on day 80 after infection of CCR7+/− (●) and CCR7−/− mice (□) with 200 PFU of LCMV-WE. The second data point in A–E (day 85 after primary LCMV infection) indicates the expansion of CD8+ tet-gp33+ memory cells ± SD on day 5 after i.v. challenge infection with 2 × 106 PFU VV-gp33C. Data shown are from one experiment and are representative of two separate experiments using three mice per group.

**FIGURE 7.** Analysis of LCMV-specific memory responses in CCR7−/− mice. A, Absolute numbers of CD8+ T cells producing IFN-γ after short term in vitro restimulation with gp33 were assessed for spleen, MLN, liver, and lung on day 80 after i.v. infection of CCR7−/− (●) and CCR7+/− mice (□) with 200 PFU of LCMV-WE. B, Ex vivo CTL activity of lymphocytes isolated from spleen and lung of CCR7−/− (●) and CCR7+/− (□) mice, as assessed by lysis of 51Cr-labeled EL4 cells pulsed with gp33 (● and □) or unpulsed control cells (□ and □). Data indicate values derived from pooled samples of three to six mice. E:T cell ratios were adjusted for the percentage of CD8+ tet-gp33+ cells to compensate for the reduced numbers of gp33-specific CTL in CCR7−/− mice. Data from one experiment are shown and are representative of two similar experiments. C, T cell activation markers on CD8+ tet-gp33+ cells from LCMV memory mice were assessed by flow cytometry as described (line plot, CCR7−/−; shaded plot, CCR7+/−).
T cells in CCR7\(^{-/-}\) mice remain unresponsive to the peripheral effect of CCL21b.

It is noteworthy that the alternative priming sites for antiviral CTL in the absence of CCR7 or its ligand chemokines, e.g., the marginal zone or the red pulp in the spleen, still facilitate efficient DC-CTL interaction. CTL priming outside the T cell zone resulted in appropriate differentiation of CTL, which were able to produce effector cytokines, exhibited significant cytolytic activity, and mediated antiviral protection in both CCR7\(^{-/-}\) mice as well as \(p/t\)/pl mice (this study and Ref. 15). Therefore, it is reasonable to assume that CCR7-mediated organization of the lymphoid T cell zone increases the likelihood of productive DC-T cell contacts and is thus necessary for the maximal amplification of virus-induced CTL responses.

During the memory phase of an anti-LCMV immune response, a significant proportion of specific CTL retained in peripheral organs had down-regulated both CCR7 and CD62L, whereas those found in lymph nodes and splenic white pulp exhibited mainly the central memory-like CD62L\(^{high}\)CCR7\(^{low}\) phenotype. Our data are therefore compatible with the concept that CD62L\(^{low}\)CCR7\(^{low}\) effector memory CTL reside primarily in the periphery, and that CD62L\(^{high}\)CCR7\(^{high}\) central memory CTL localize mainly to secondary lymphoid organs. However, the observed functional characteristics of those cell subsets differ from those proposed in the original model of central vs effector memory (12). Antiviral CTL from both peripheral nonlymphoid and secondary lymphoid organs rapidly produced IFN-\(\gamma\) after specific restimulation and displayed significant cytolytic activity. This confirms previous studies showing that immediate effector function of memory T cells is not strictly associated with down-regulation of CCR7 (14, 30, 35, 36). In particular, our data are consistent with a recent study demonstrating significant ex vivo lytic activity of memory CTL from both spleen and liver (14). The somewhat longer incubation time for the direct CTL assay after infection with LCMV-Armstrong was probably necessary in that study, because the clonal burst size after LCMV-Armstrong infection is weaker than that after LCMV-WE infection (37). Regarding the functional activity of lymphoid vs nonlymphoid memory CTL, recent data suggest that the memory T cell pool of most nonlymphoid organs is mobile, and thus in constant exchange with the lymphoid memory pool (38, 39), and that both pools can exocytose IFN-\(\gamma\) and lytic granules to the same extent in response to specific Ag (14, 39). Therefore, a strict functional distinction of peripheral vs lymphoid memory CTL is not supported in several systems and readouts.

One study demonstrating stronger direct CTL activity from peripheral organs than from spleen (28) used vesicular stomatitis virus, a virus that does not rely on CTL for antiviral protection because it is cleared from the host within hours via neutralizing Abs (40). The maintenance of memory CTL activity in such an infection may rely on mechanisms differing from those that mediate the maintenance of memory CTL against persistent viruses such as LCMV.

Taken together, we have shown in this study that CCR7 plays a key role in the homeostatic recirculation of antiviral memory CTL. However, the strict differentiation between resting central vs active effector memory T cells based on their CCR7 expression probably does not reflect the complex in vivo situation as seen, for example, during a viral infection. Furthermore, our data underscore the importance of organized lymphoid structures for the generation of primary immune responses and delineate the role of CCR7-mediated lymphocyte and DC migration in the induction and maintenance of antiviral immunity.

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References


