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*J Immunol* 2008; 180:2886-2893; doi: 10.4049/jimmunol.180.5.2886
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The Role for Monocyte Chemoattractant Protein-1 in the Generation and Function of Memory CD8⁺ T Cells¹

Tao Wang,² Hehua Dai, Ni Wan, Yolonda Moore, and Zhenhua Dai³

Memory T cells are resistant to the conventional costimulatory blockade and therefore impede tolerance induction. However, their migratory, survival, and functional requirements for chemokines are not well understood. We herein examine the role for MCP-1 or CCL2 in the generation, migration, and function of memory CD8⁺ T cells. We found that overall generation of both central memory (T_{CM}) and effector memory (T_{EM}) CD8⁺ T cells was severely impaired in the absence of MCP-1. Importantly, the survival of T_{EM}, but not T_{CM}, CD8⁺ cells was reduced without MCP-1, whereas the homeostatic proliferation of T_{CM}, but not T_{EM}, CD8⁺ cells was weakened in MCP-1⁻/⁻ mice. However, once they were generated in the absence of MCP-1, in vitro function of both subsets of memory cells remained intact as determined by their proliferation and IFN-γ production. Interestingly, the migration of T_{EM}, but not T_{CM}, CD8⁺ cells to inflammatory sites was significantly delayed without MCP-1, whereas both subsets of memory cells underwent comparable expansion and apoptosis with or without MCP-1 during the effector phase. Moreover, the function to eliminate a graft of TCM, but not TEM, CD8⁺ cells was weakened in MCP-1⁻/⁻ mice. Importantly, the survival of T_{EM}, but not T_{CM}, CD8⁺ cells was severely impaired in the absence of MCP-1. Importantly, the survival of T_{EM}, but not T_{CM}, CD8⁺ cells was reduced without MCP-1, whereas the homeostatic proliferation of T_{EM}, but not T_{CM}, CD8⁺ cells was weakened in MCP-1⁻/⁻ mice. Moreover, the function of donor-specific memory CD8⁺ T cells and demonstrated that MCP-1 plays an important role in the generation and survival of memory CD8⁺ T cells. Interestingly, the survival of effector memory (T_{EM}), but not central memory (T_{CM}), CD8⁺ T cells was reduced without MCP-1, whereas the homeostatic proliferation of T_{CM}, but not T_{EM}, CD8⁺ cells was weakened in MCP-1⁻/⁻ mice. Moreover, the function and migration of T_{CM}, but not T_{EM}, CD8⁺ cells was significantly impaired in the absence of MCP-1.

Materials and Methods

Mice

2C TCR-transgenic C57BL/6 mice on a recombination activating gene-1 knockout (Rag1⁻/⁻) background (2C Rag⁻/⁻) were generated by backcrossing 2C transgenic mice onto Rag1⁻/⁻ mice (The Jackson Laboratory) (6, 12). MCP-1⁻/⁻ mice (B6 background) were ordered from The Jackson Laboratory. Wild-type (WT) BALB/c and C57BL/6 mice were purchased from the National Cancer Institute (National Institutes of Health, Bethesda, MD). All mice were housed in a specific pathogen-free environment, and all animal experiments and protocols were approved by the Animal Care and Use Committee of the University of Texas Health Center.

Pancreatic islet transplantation

Islet donors were 7- to 8-wk-old BALB/c female mice. Islet recipients were 7- to 8-wk-old WT or MCP-1⁻/⁻ (C57BL/6) female mice. Islets were isolated and transplanted into the subcapsular space of the right kidney of recipient mice as described previously (12). Recipient mice were rendered diabetic by a single injection of streptozotocin (Sigma-Aldrich) (180 mg/kg) 10–14 days before islet transplantation (see Fig. 6). For 2C cell migration and apoptosis experiments (see Figs. 3 and 4), recipients were transplanted with allogeneic islets without the prior injection of streptozotocin. Primary graft function was defined as blood glucose <200 mg/dl for 48 h after transplantation. Graft rejection was defined as a rise in blood glucose to >300 mg/dl for 3 consecutive days after primary function.

¹ Abbreviations used in this paper: T_{EM}, effector memory T cells; T_{CM}, central memory T cells; mLN, mesenteric lymph node.

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Received for publication July 11, 2007. Accepted for publication December 21, 2007.

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¹ This work was supported by research grants from the Juvenile Diabetes Research Foundation International and from the American Diabetes Association.

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Memory T cell generation, preparation, and phenotyping

To generate memory CD8+ T cells, naive CD8+ T cells (CD8+CD25−CD44low) from transgenic 2C Rag2−/− mice were isolated by a FACS Aria cell sorter (BD Biosciences). 2C CD8+ T cells that specifically recognize Ld alloantigen on BALB/c cells and can be tracked by a specific Ab, 1B2, are then referred to as CD8+1B2+. Briefly, splenocytes from 2C Rag2−/− mice (B6) were first incubated with anti-CD5-PE, anti-CD25-FTTC, and anti-CD44-PerCP Abs (BD Pharmingen), and CD8+CD25+CD44low+ T cells were then sorted out by FACS Aria. The purity of naive CD8+ T cells was typically >98%. 2C naive CD8+ T cells (5 × 10^5) were then adoptively transferred to B6 mice that were immunized i.p. with irradiated BALB/c splenocytes. 2C CD8+1B2+ memory T cells were finally detected by staining with anti-CD62L-APC or anti-CD44-APC (BD Pharmingen), and CD8+CD25−CD44low+ T cells were then sorted out by FACS Aria. The purity of memory CD8+ T cells was typically >96%.

2C cell proliferation in vitro and supernatant IFN-γ measurement

Naïve or memory 2C cells (1 × 10^5/well), isolated by FACs cell sorting from lymph nodes or livers and kidneys of recipient mice, were cultured with irradiated BALB/c spleen cells (1 × 10^5/well) in 96-well plates (Corning Costar) in complete RPMI 1640 medium (10% FCS, 2 mM of glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin). Cells were cultured for 24 h and pulsed with [3H]thymidine for last 6 h. Cells were then harvested and analyzed by a scintillation counter (PerkinElmer). To measure IFN-γ production by 2C memory cells, the same cells were cultured for up to 24 h, and IFN-γ level in the supernatant was detected by a mouse cytokine ELISA kit according to the manufacturer’s instructions (Invitrogen).

Isolation of graft-infiltrating cells

Graft-infiltrating cells were isolated as described previously (6, 12). Briefly, the kidneys that harbored islet allografts were perfused in situ with heparinized 0.9% saline. They were then minced and digested at 37°C for 30 min in 20 ml RPMI 1640 medium containing 5% FCS, 2 mM of glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were washed for 4 h and pulsed with [3H]thymidine for 6 h. Cells were then harvested by a centrifugation (PerkinElmer). To measure IFN-γ production by 2C memory cells, the same cells were cultured for up to 24 h, and IFN-γ level in the supernatant was detected by a mouse cytokine ELISA kit according to the manufacturer’s instructions (Invitrogen).

Analysis of T cell proliferation in vivo by BrdU labeling and apoptosis

Naive or 2C memory 2C cells (1 × 10^5/well) were isolated by FACs cell sorting from lymph nodes or livers and kidneys of recipient mice that were immunized with irradiated BALB/c spleen cells (1 × 10^5/well) in 96-well plates (Corning Costar) in complete RPMI 1640 medium (10% FCS, 2 mM of glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin). Cells were cultured for 24 h and pulsed with [3H]thymidine for last 6 h. Cells were then harvested and analyzed by a scintillation counter (PerkinElmer). To measure IFN-γ production by 2C memory cells, the same cells were cultured for up to 24 h, and IFN-γ level in the supernatant was detected by a mouse cytokine ELISA kit according to the manufacturer’s instructions (Invitrogen).

T cell proliferation in vivo by BrdU labeling and apoptosis

Analysis of T cell proliferation in vivo by BrdU labeling and apoptosis by a TUNEL method.

Recipient mice were pulsed i.p. with 0.8 mg of BrdU (Sigma-Aldrich) 6 days after islet transplantation. Twenty-four hours later, renal graft-infiltrating cells or renal draining lymph node cells were isolated and stained with anti-CD8-PE and 1B2, followed by rat anti-mouse IgG1-biotin and streptavidin-PerCP. Cells were then fixed in 70% ethanol at room temperature. The pellet was resuspended in 1% Triton X-100 solution, and labeled with fluorescein-tagged dUTP by the TUNEL method according to the manufacturer’s instructions (Roche Applied Science). To detect the homeostatic proliferation and apoptosis of 2C memory cells during maintenance phase (see Fig. 5), immunized mice were pulsed with 0.8 mg of BrdU 4 or 8 wk after the immunization. Cells containing 2C memory cells were then isolated from mesenteric lymph nodes (Tcm) and livers (TEm), stained, and analyzed by the FACs.

Treatment of mice with neutralizing anti-MCP-1 Ab

To neutralize MCP-1 in vivo, WT recipient mice or MCP-1−/− recipients that received islet grafts from WT donors were treated with anti-MCP-1 Ab or isotype control Ab (R&D System) via i.p. injection of 0.2 mg on days 0, 2, 4, 6, and 8 after i.p. immunization for 2C memory cell generation (see Fig. 1), on days 4, 6, 8, and 10 after islet transplantation for allograft rejection by 2C memory cells (see Fig. 6), or on days 0, 2, and 4 after islet transplantation for migration and apoptosis experiments (see Figs. 3 and 4).

Statistical analysis

The analysis of allograft survival data was performed using the Kaplan-Meier log-rank test. Comparison of means was conducted using ANOVA.

Results

Impaired generation of donor-specific memory CD8+ T cells in the absence of MCP-1

It is unknown whether MCP-1 plays a role in the generation of memory T cells. To generate Ag-specific memory CD8+ T cells, we used 2C transgenic CD8+ T cells that specifically recognize the Ld alloantigen on BALB/c cells and can be tracked by a chimeric Ab, 1B2, and are therefore referred to as CD8+1B2+. MCP-1 was backcrossed to Rag1−/− background, and 2C Rag2−/− mice have only a monoclonal T cell population that bears a single transgenic TCR. Hence, all CD8+1B2+ T cells from 2C Rag2−/− mice specifically recognize the Ld alloantigen. Naïve CD8+ T cells (CD8+CD44low) from 2C Rag2−/− mice were purified and transferred to WT or MCP-1−/− B6 mice that were immunized with irradiated BALB/c splenocytes. Ten weeks later, 2C memory CD8+ T cells were isolated and quantitated by FACs analysis.

As shown in Fig. 1A, we found that the percentages of 2C memory CD8+1B2+ T cells in mesenteric lymph nodes (mLN), spleens, and livers from MCP-1−/− mice were much lower than those from WT mice (0.5 vs 1.6% in mLN, 0.4 vs 1.3% in the spleen, and 0.3 vs 0.9% in the liver). Similarly, the percentages of 2C memory CD8+1B2+ T cells from WT mice treated with neutralizing anti-MCP-1 Ab were lower than those from untreated WT mice (0.7 vs 1.6% in mLN, 0.6 vs 1.3% in the spleen, and 0.4 vs 0.9% in the liver). To perform statistical analysis of memory T cell generation, the absolute cell number was calculated on a per-organ basis as shown in Fig. 1C. The absolute numbers of 2C memory cells were significantly decreased in MCP-1−/− mice compared with WT mice (0.7 ± 0.2 vs. 2.7 ± 0.4 in mLN, 3.1 ± 0.7 vs. 10.4 ± 1.5 in the spleen, 1.1 ± 0.4 vs. 3.6 ± 0.7 in the liver, 0.7 ± 0.3 vs. 2.5 ± 0.5 in the kidney, all p < 0.05). Moreover, the absolute numbers of 2C memory cells in WT mice treated with anti-MCP-1 Ab were also reduced compared with untreated WT control group (1.0 ± 0.4 vs. 2.7 ± 0.4 in mLN, 4.7 ± 1.3 vs. 10.4 ± 1.5 in the spleen, 1.5 ± 0.5 vs. 3.6 ± 0.7 in the liver, 1.0 ± 0.3 vs. 2.5 ± 0.5 in the kidney, all p < 0.05). The isotype control Ab did not significantly affect the 2C cell number (data not shown). As a control, 2C cells were also analyzed by the FACs 6 wk after transfer of naive 2C cells to naive WT or MCP-1−/− recipients that were not immunized. There were much fewer 2C cells (<0.3%) detected in both lymphoid and nonlymphoid organs of naive recipients, and there was also no significant difference in the percentage of 2C cells between WT and MCP-1−/− (Fig. 1D). The CD8+1B2+ 2C cells generated in immunized murine recipients were confirmed to be memory T cells because 2C cells derived from lymph nodes were mainly CD44low and CD62Lhigh, a Tcm phenotype, while 2C cells derived from liver tissues were mainly CD44high and CD62Llow, a TEM phenotype (Fig. 1B), as described previously (6, 47, 48). It is noteworthy that very few 2C memory cells were detected in the bone marrow cells of immunized mice in our allogeneic murine system (unpublished observation). Taken together, these data suggest that MCP-1 plays an important role in supporting the generation of both TEM and TCM CD8+ cells.
Memory CD8⁺ T cells, generated in the absence of MCP-1, are functionally intact in vitro

Memory CD8⁺ T cells generated under certain conditions are functionally impaired (18). In addition to their reduced number, memory T cells generated in the absence of MCP-1 also could be functionally impaired. To study whether MCP-1 is required for the generation of functional memory CD8⁺ T cells, 2C naive CD8⁺ cells were transferred to mice that were immunized with irradiated BALB/c splenocytes 1 day later as described in Fig. 1. Ten weeks later, 2C TEM and TCM memory cells, generated in the presence or absence of MCP-1, were isolated and restimulated with irradiated BALB/c splenocytes in vitro. IFN-γ levels in the supernatant were then measured by ELISA, and 2C cell proliferation was measured by [³H]Tdr uptakes. As shown in Fig. 2A, naive 2C cells as a control did not produce IFN-γ within 24 h, whereas 2C TCM cells produced a significant amount of IFN-γ even at the time point of 6 h, confirming that 2C cells generated in our system are true memory T cells. 2C TCM cells did not produce a significant amount of IFN-γ until the time point of 24 h, suggesting that TEM cells respond faster than TCM cells. Interestingly, 2C TCM cells generated in the absence of MCP-1 produced the same amounts of IFN-γ as did those generated in the presence of MCP-1 (6 h: 2.3 ± 0.5 vs 2.6 ± 0.6; 12 h: 4.1 ± 0.6 vs 4.7 ± 0.7; 24 h: 6.0 ± 1.2 vs 5.5 ± 1.1, all p < 0.05). The same basic result occurred with the TCM cells (6 h: 0.3 ± 0.2 vs 0.5 ± 0.3; 12 h: 1.1 ± 0.5 vs 1.5 ± 0.6; 24 h: 2.5 ± 0.4 vs 2.7 ± 0.8, all p > 0.05). Similarly, the absence of MCP-1 impaired 2C TEM cell proliferation by [³H]Tdr uptake. As shown in Fig. 2B, naive 2C cells as a control did not produce IFN-γ within 24 h, whereas 2C TEM or TCM cells generated in the absence of MCP-1 also could be functionally impaired (18). In addition to their reduced number, they were stained and analyzed by FACS. A. The percentage of CD8⁺1B2⁺ 2C memory T cells generated in the absence of MCP-1 were isolated from mLN, spleens, livers/kidneys, and such, and they were stained and analyzed by FACS. B. Phenotypes of 2C TEM (CD44highCD62Lhigh) and TCM (CD44lowCD62Llow) markers. The dotted lines are isotype controls. One representative from three separate experiments is shown.
of MCP-1 during the phase of memory cell generation did not significantly alter 2C TEM or TCM cell proliferation in vitro (TEM: 5.3 ± 1.0 vs 5.8 ± 1.1 and TCM: 2.3 ± 0.7 vs 2.6 ± 0.9 (cpm × 10^9), all p > 0.05) (Fig. 2B). These findings suggest that the absence of MCP-1 impairs the generation of memory CD8⁺ T cell number but not their function. In other words, memory CD8⁺ T cells, although reduced in number in the absence of MCP-1, are functionally intact in vitro once they are already generated. It is unclear whether these memory cells, generated in the absence of MCP-1, can function normally in vivo. Their limited availability due to severely reduced numbers makes it very difficult to perform in vivo experiments.

**Lack of homing of TCM, but not TEM, cells to an inflammatory site in the absence of MCP-1**

To examine whether MCP-1 is required for the migration of memory T cells to an inflammatory site, WT or MCP-1⁻/⁻ mice were transplanted with BALB/c islets under the kidney capsule and received an equal number of 2C memory cells. MCP-1⁻/⁻ recipients were also treated with anti-MCP-1 Ab to neutralize a small amount of MCP-1 released by islet allografts from BALB/c donors, as islet allografts have been shown to be capable of producing the chemokine MCP-1 (43, 44, 49). One and 2 days later, renal graft-infiltrating cells were isolated and 2C cells were quantified by FACS analysis. As shown in Fig. 3A, the percentage of CD8⁺1B2⁺ 2C cells was lower in the kidney of MCP-1⁻/⁻ mice compared with WT recipient mice 24 h after 2C TCM cells were transferred (0.6 vs 1.2%). However, the percentage of CD8⁺1B2⁺ 2C cells in MCP-1⁻/⁻ mice was not significantly decreased when 2C TEM cells were transferred (1.5 vs 1.7%). To perform statistical analyses of memory T cell migration, the absolute cell number per kidney was calculated as shown in Fig. 3B. The 2C cell number per kidney was severely reduced in MCP-1⁻/⁻ mice compared with WT mice when 2C TCM cells were transferred (24 h: 0.8 ± 0.2 vs 1.9 ± 0.3 and 48 h: 1.7 ± 0.5 vs 3.6 ± 0.6, p < 0.05), whereas the 2C cell number was not significantly decreased in MCP-1⁻/⁻ mice when 2C TEM cells were transferred (24 h: 2.9 ± 0.6 vs 3.4 ± 0.7 and 48 h: 4.4 ± 0.7 vs 4.7 ± 0.8, p > 0.05). The isotype control Ab did not significantly affect the 2C cell number (data not shown). Taken together, these data demonstrate that MCP-1 is more important for the migration of TCM cells than for TEM cells to an inflammatory graft site.

**Comparable proliferation and apoptosis of infiltrating memory CD8⁺ T cells in the grafts of WT and MCP-1-deficient mice**

Although our data demonstrate a reduced homing of TCM, but not TEM, cells, it is unclear whether both subsets of memory cells undergo a similar expansion and apoptosis after they migrate to an inflammatory site. To answer this question, 2CTCM cells (CD8⁺1B2⁺ CD44highCD62Lhigh) or TEM cells (CD8⁺1B2⁺ CD44high CD62Llow) were adoptively transferred into WT and MCP-1⁻/⁻ recipient mice that received BALB/c islet allografts under the kidney capsule 1 day later. MCP-1⁻/⁻ recipient mice were also treated with anti-MCP-1 Ab to neutralize a small amount of MCP-1 produced by islet allografts. Seven days later, graft-infiltrating cells were isolated and analyzed for 2C T cell proliferation (BrdU uptake) and for their apoptosis by a TUNEL method (6, 12). As shown in Fig. 4A, the absence of MCP-1 did not suppress 2C memory cell proliferation in vivo compared with the presence of MCP-1 in WT mice (BrdU-positive: TEM: 22 ± 3% vs 23 ± 2%, and TCM: 27 ± 4% vs 30 ± 3%, both p > 0.05). In contrast, as shown in Fig. 4B, the absence of MCP-1 in MCP-1⁻/⁻ recipients also did not significantly alter apoptotic rates of 2C memory cells compared with the presence of MCP-1 in WT mice (TUNEL-positive: TEM: 8 ± 2% vs 7 ± 1%, and TCM: 6 ± 1% vs 5 ± 0%, both p > 0.05). These results suggest that neither TEM nor TCM memory T cells require MCP-1 for their proliferation or apoptosis once they have migrated to an inflammatory site. Moreover, the numbers of 2C memory/effector cells infiltrating grafts were also calculated. We found that 2C cell numbers remained lower in MCP-1⁻/⁻ mice than in WT mice (11.7 ± 1.4 vs 7.1 ± 1.2, p < 0.05) 7 days after 2C TEM cells were transferred (Fig. 4C), although there was no difference in 2C cell numbers between WT and MCP-1⁻/⁻ recipients when 2C TCM cells were transferred (12.5 ± 1.9 vs 11.8 ± 1.7, p > 0.05). These findings indicate that the migration of TCM, but not TEM, cells to inflamed graft sites remains slow in the absence of MCP-1 7 days after transplantation, although both subsets of memory T cells undergo expansion and apoptosis at a similar pace.

**Reduced homeostatic proliferation of TCM CD8⁺ cells and impaired survival of TEM CD8⁺ cells in the absence of MCP-1**

Although our findings suggest that the overall generation of memory CD8⁺ T cells is impaired in the absence of MCP-1 10 wk after immunization, it is unclear whether the reduced number of memory CD8⁺ T cells is also due to their impaired homeostatic proliferation or survival. To answer this question, 2C naïve CD8⁺ T
cells (CD8\(^{+}\)B2\(^{+}\)CD44\(^{\text{low}}\)) were adoptively transferred to either WT or MCP-1\(^{-/-}\) B6 mice that were immunized with irradiated BALB/c splenocytes. Four and 8 wk after the immunization, cells containing 2C memory cells were isolated from mesenteric lymph nodes (T\(_{\text{CM}}\)) and livers (T\(_{\text{EM}}\)) and analyzed for the apoptosis of 2C memory cells by a TUNEL method and for their homeostatic proliferation by BrdU uptakes. As shown in Fig. 5A, 2C T\(_{\text{CM}}\) cells suffered a faster death in MCP-1\(^{-/-}\) than in WT recipients at both 4 and 10 wk after the immunization (% of TUNEL-positive: 4 wk: 7.9 ± 0.8 vs 4.7 ± 0.6, and 8 wk: 5.4 ± 0.6 vs 3.2 ± 0.4, both p < 0.05), whereas 2C T\(_{\text{EM}}\) cells underwent apoptosis at a similar pace in MCP-1\(^{-/-}\) and WT recipients at both time points (% of TUNEL-positive: 4 wk: 4.1 ± 0.7 vs 4.5 ± 0.8, and 8 wk: 3.3 ± 0.7 vs 3.6 ± 0.5, both p > 0.05), suggesting that the survival of T\(_{\text{EM}}\), but not T\(_{\text{CM}}\), CD8\(^{+}\) cells is impaired in the absence of MCP-1. In contrast, as shown in Fig. 5B, 2C T\(_{\text{CM}}\) cells underwent slower homeostatic proliferation in MCP-1\(^{-/-}\) than in WT recipients at both 4 and 10 wk after the immunization (% of BrdU-positive: 4 wk: 1.9 ± 0.5 vs 4.6 ± 0.8, and 8 wk: 1.5 ± 0.4 vs 3.4 ± 0.6, both p < 0.05), whereas 2C T\(_{\text{EM}}\) cells underwent homeostatic proliferation at a similar pace in MCP-1\(^{-/-}\) and WT recipients at both time points (% of BrdU-positive: 4 wk: 3.5 ± 0.6 vs 3.8 ± 0.7, and 8 wk: 2.8 ± 0.8 vs 3.0 ± 0.9, both p > 0.05), indicating that the homeostatic proliferation of T\(_{\text{CM}}\), but not T\(_{\text{EM}}\), CD8\(^{+}\) cells is impaired in the absence of MCP-1.

**Impaired function of T\(_{\text{CM}}\) but not T\(_{\text{EM}}\) CD8\(^{+}\) cells to reject allografts in the absence of MCP-1.**

To further determine whether the ability of T\(_{\text{EM}}\) and T\(_{\text{CM}}\) CD8\(^{+}\) cells to reject an allograft is weakened in the absence of MCP-1, we used a WT, but not lymphocyte-deficient, murine model of allograft rejection mediated by 2C memory T cells. WT B6 mice were transplanted with BALB/c islets and treated with MR1 (anti-CD154 Ab) to prevent islet allograft rejection. They then received 2C T\(_{\text{EM}}\) or T\(_{\text{CM}}\) cells to evoke allograft rejection. As shown in Fig. 6, the recipients did not reject islet allografts within 100 days unless they received either 2C T\(_{\text{EM}}\) or T\(_{\text{CM}}\) cells, because memory T
cells are resistant to the conventional costimulatory blockade (4–7). We were able to observe allograft rejection by memory T cells in this model because primary immune responses to grafts were suppressed while memory T cells were still capable of rejecting an allograft in the face of MR1 treatment. All MCP-1−/− recipients were also treated with anti-MCP-1 Ab to neutralize a small amount of MCP-1 produced by islet allografts. WT (△, n = 6) or MCP-1−/− (○, n = 6) recipients that received 2C TCM cells; WT (●, n = 7) or MCP-1−/− (○, n = 7) recipients that received 2C TEM cells; and WT (●, n = 6) or MCP-1−/− (○, n = 6) recipients, which received or did not receive 2C naive CD8+ cells as controls, were observed for islet allograft rejection.

cells were then adoptively transferred to the recipient mice on day 5 to demonstrate that the in vivo function of TCM but not TEM, CD8+ T cells is significantly delayed in the absence of MCP-1. All WT or MCP-1−/− B6 mice were transplanted with BALB/c islets under the kidney capsule and treated with 0.5 mg of MR1 (anti-CD154 Ab) on days 0, 2, and 4 to prevent islet allograft rejection. 2C TCM or TEM CD8+ cells (2.5 × 107) were then adoptively transferred to the recipient mice on day 5 to observe allograft rejection mediated by memory CD8+ T cells. All MCP-1−/− recipient mice were also treated with anti-MCP-1 Ab on days 4, 6, 8, and 10 after islet transplantation to neutralize a small amount of MCP-1 produced by islet allografts. WT (△, n = 6) or MCP-1−/− (○, n = 6) recipients that received 2C TEM cells; WT (●, n = 7) or MCP-1−/− (○, n = 7) recipients that received 2C TCM cells; and WT (●, n = 6) or MCP-1−/− (○, n = 6) recipients, which received or did not receive 2C naive CD8+ cells as controls, were observed for islet allograft rejection.

Discussion

Using a unique mouse model with transgenic donor-specific CD8+ T cells, we investigated the role for the chemokine MCP-1 in the generation, migration, and function of donor-specific memory CD8+ T cells. We found that the generation of both TCM and TEM CD8+ T cells is severely impaired in the absence of MCP-1. Importantly, the survival of TEM but not TCM, CD8+ cells was reduced without MCP-1, whereas the homeostatic proliferation of TCM, but not TEM, CD8+ cells was weakened in the absence of MCP-1. However, once they were generated in the absence of MCP-1, the in vitro function of both subsets of memory cells remained intact. Interestingly, the migration of TCM, but not TEM, CD8+ T cells to an inflammatory site was significantly delayed in the absence of MCP-1, whereas both subsets of infiltrating memory T cells underwent expansion and apoptosis in vivo in the presence of MCP-1 at a similar pace to those in the absence of MCP-1. Moreover, the function of TCM CD8+ cells to eliminate a graft was impaired in the absence of MCP-1, whereas the TEM CD8+ cell function to reject a graft was not affected in the absence of MCP-1. Thus, this study for the first time demonstrates that MCP-1 plays an important role in not only the migration but also in the generation and function of memory T cells. This finding therefore has important implications for the induction of transplantation tolerance as well as self-tolerance.

Memory T cells are divided into TCM and TEM cells (50–52). TCM cells are CD44highCCR7+CD62Lhigh and home to lymph nodes to generate new waves of effector T cells, while TEM cells are CD44highCCR7−CD62Llow and can rapidly migrate to inflamed peripheral tissues where they perform immediate effector functions. It remains unclear which chemokines are required for memory T cell migration to peripheral tissues. Our data demonstrate that MCP-1 is required for TEM cells to migrate to inflamed grafts, whereas TEM cells do not need MCP-1 for their migration, as determined by the number of 2C memory cells infiltrating grafts 24 and 48 h after islet transplantation. The difference in the number of 2C cells between WT and MCP-1−/− mice remains even 7 days after 2C TCM cell transfer and transplantation, and it is likely due to impaired migration rather than changes in proliferation and death, given that 2C memory/effector cells underwent expansion and apoptosis at a similar pace in MCP-1−/− mice at this time point. Perhaps TCM cells need to be first activated in lymph nodes, and their long-distance trafficking to graft tissues requires the presence of MCP-1, whereas TEM cells can directly migrate to graft tissues in the absence of MCP-1. The chemokines that are essential for TEM cell migration to an inflamed tissue remain to be defined, because MCP-1 is not required for their migration. Therefore, targeting the chemokine MCP-1 alone may not be enough to completely prevent allograft rejection mediated by TEM cells or to induce allograft tolerance. Our data also show that neutralizing MCP-1 during early phase of priming inhibits the generation of both TCM and TEM cells, suggesting that targeting MCP-1 during the early stage of T cell migration and activation is the key to suppressing memory cell-mediated immune responsiveness, because TEM cells are no longer susceptible to MCP-1 blockade once they have been generated.

Although TCM cell migration to inflammatory grafts is significantly delayed, our data have shown that the proliferation and apoptosis of both TCM and TEM cells that are infiltrating grafts are not affected in the absence of MCP-1, indicating that MCP-1 is not required for the expansion and apoptosis of both subsets of memory T cells during the activation/effector phase. This finding also suggests that the delayed islet allograft rejection mediated by TCM cells is likely attributed to their delayed migration to inflamed grafts but not to their impaired effector functions. Importantly, the survival of TEM, but not TCM, CD8+ cells after the effector phase was reduced without MCP-1, suggesting that MCP-1 is important for TEM cell survival during memory cell maintenance phase and that the reduced survival of TEM cells likely contributes to their reduced number or generation in the absence of MCP-1 10 wk after immunization. Interestingly, a new study has recently shown that MCP-1 inhibits T cell apoptosis in vitro induced by growth factor deprivation (53), suggesting that MCP-1 is important for T cell survival postinflammation or right after the effector phase. In contrast, we found that the homeostatic proliferation of TCM, but not TEM, CD8+ cells was weakened in the absence of MCP-1,
suggesting that MCP-1 plays a role in supporting the homeostatic proliferation of TCM cells as well as maintaining sufficient numbers of TCM cells 10 wk after immunization. However, it remains to be elucidated why MCP-1 plays a role in the survival of TEM, but not TCM, CD8+ cells in TCM. This difference between TCM and TEM cells is interesting and warrants further investigation.

We studied CD8+, but not CD4+, memory T cells in this allelogenic setting because: 1) immune regulation in vivo results in a long-lasting CD8+ memory but in a declining CD4+ memory pool over time (54, 55), indicating that the CD8+ memory pool may be more important for long-term immunity than is CD4+ memory; 2) memory CD8+ T cells are resistant to the conventional costimulatory blockade (4, 7, 56) and therefore could function in the absence of chemokines; and 3) memory CD8+ T cells can be generated in response to an alloantigen independently of CD4+ T cell help (16), suggesting that memory CD8+ T cells are independent and may pose a more serious threat to tolerance induction than do their CD4+ counterparts. Our studies demonstrate that the migration and function of TCM, but not TEM, CD8+ cells are impaired in the absence of MCP-1. The role for MCP-1 in the generation, survival, migration, and effector function of memory CD4+ T cells remains to be elucidated.

Disclosures
The authors have no financial conflicts of interest.

References


