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Differential Role of CCR2 in Islet and Heart Allograft Rejection: Tissue Specificity of Chemokine/Chemokine Receptor Function In Vivo

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Chemokines have a pivotal role in the mobilization and activation of specific leukocyte subsets in acute allograft rejection. However, the role of specific chemokines and chemokine receptors in islet allograft rejection has not been fully elucidated. We now show that islet allograft rejection is associated with a steady increase in intragraft expression of the chemokines CCL8 (monocyte chemoattractant protein-2), CCL9 (monocyte chemoattractant protein-5), CCL5 (RANTES), CXCL-10 (IFN-γ-inducible protein-10), and CXCL9 (monokine induced by IFN-γ) and their corresponding chemokine receptors CCR2, CCR5, CCR1, and CXCR3. Because CCR2 was found to be highly induced, we tested the specific role of CCR2 in islet allograft rejection by transplanting fully MHC mismatched islets from BALB/c mice into C57BL/6 wild-type (WT) and CCR2-deficient mice (CCR2<sup>−/−</sup>). A significant prolongation of islet allograft survival was noted in CCR2<sup>−/−</sup> recipients, with median survival time of 24 and 12 days for CCR2<sup>−/−</sup> and WT recipients, respectively (p < 0.0001). This was associated with reduction in the generation of CD8<sup>+</sup>, but not CD4<sup>+</sup> effector alloreactive T cells (CD62L<sub>low</sub>CD44<sub>high</sub>) in CCR2<sup>−/−</sup> compared with WT recipients. In addition, CCR2<sup>−/−</sup> recipients had a reduced Th1 and increased Th2 alloresponse in the periphery (by ELISPOT analysis) as well as in the grafts (by RT-PCR). However, these changes were only transient in CCR2<sup>−/−</sup> recipients that ultimately rejected their grafts. Furthermore, in contrast to the islet transplants, CCR2 deficiency offered only marginal prolongation of heart allograft survival. This study demonstrates the important role for CCR2 in early islet allograft rejection and highlights the tissue specificity of the chemokine/chemokine receptor system in vivo in regulating allograft rejection. The Journal of Immunology, 2004, 172: 767–775.

Islet transplantation holds great promise for the treatment of type 1 diabetes. Allograft rejection, however, remains a major barrier to long-term islet graft survival, revealing the need for new strategies to prevent islet rejection. Inhibiting the chemokine system presents a potentially new therapeutic strategy for preventing transplant rejection.

Chemokines are chemotactic cytokines that induce the directed migration and activation of cells by binding to and activating specific G protein-coupled receptors expressed on target cells. The chemokine system has been demonstrated to play important roles in guiding the migration of leukocytes necessary to generate an immune response as well as to deliver this response to a particular tissue site. Data in animal models suggest that chemokines play important roles in regulating transplant rejection (1).

Chemokines were first implicated in the pathogenesis of allograft rejection from studies that demonstrated the induction of chemokine gene expression in rejecting allografts. In murine studies, using skin allografts, the sequential induction of CCL2 (monocyte chemoattractant protein-1 (MCP-1)), CCL3 (macrophage-inflammatory protein-1α), CXCL10 (IFN-γ-inducible protein-10), CCL10 (IFN-γ-inducible protein-10 (IP-10)), monokine induced by IFN-γ (Mig) (CXCL9), and CCL5 (RANTES), ligands for CCR2, CCR1, CCR5, and CXCR3, has been described (2, 3). In rejecting murine heart allografts, a similar pattern of chemokine induction has been elucidated that includes prominent induction of CCL5 (RANTES), CXCL10 (IP-10), and CXCL9 (Mig) (4, 5). Targeting both CXCR3 and its ligands has been shown to prolong heart allograft survival (4–6). Other studies have delineated a more limited role for CCR1 and CX3CR1 in cardiac allograft rejection (7, 8). Data in humans have suggested that chemokines and their receptors participate in pathological rejection by describing that the susceptibility of human renal allograft recipients to acute rejection episodes is influenced by their CCR5 and CCR2 receptor genotypes (9, 10).

Islet transplantation into CCR5-deficient mice has revealed a role for CCR5 in islet rejection (11). However, the majority of these mice did eventually reject the islet allograft, suggesting that other chemokine systems eventually compensated for the loss of CCR5. To better understand the roles of the relevant chemokine

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4 Abbreviations used in this paper: MCP, monocyte chemoattractant protein; IP-10, IFN-γ-inducible protein-10; Mig, monokine induced by IFN-γ; MST, median survival time; QPCR, quantitative PCR; WT, wild type.
systems involved in the process of islet allograft rejection, we have analyzed the pattern of chemokine/receptor expression over time. CCR2 was found to be distinctly overexpressed, and its expression increased steadily over time.

CCR2 is a specific receptor for CCL2, 7, 13 (MCP-1, -3, -4) (human), and CCL12 (MCP-5) (mouse), which are potent chemotacticants for monocytes, macrophages, immature dendritic cells (dendritic cells), NK cells, and activated T cells (12). Mice deficient in CCR2 had diminished monocyte recruitment and lesion formation in murine models of atherosclerosis (13), experimental autoimmune encephalomyelitis (14), and pulmonary tuberculosis (15). In addition, T cell priming was delayed in CCR2−/− mice, and fewer CD4+ and CD8+ T cells primed to produce IFN-γ accumulated in the lungs of the CCR2−/− mice that were infected with *Mycobacterium tuberculosis* (15). CCR2−/− mice also appear to have a propensity to develop a diminished Th1-type and enhanced Th2-type immune response compared with wild-type (WT) controls, suggesting a role for CCR2 in T cell differentiation (16).

These data indicate that CCR2 is important for monocyte, macrophage, and dendritic cell trafficking as well as Th1/Th2 differentiation, all of which are important in the pathogenesis of graft rejection. Even though CCR2 is one of the better-studied chemokine systems, little has been published about its role in allograft rejection. In the present study, we have examined the dynamic changes in chemokine/chemokine receptor expression during the process of islet allograft rejection and examined the functionality of CCR2 in this process.

**Materials and Methods**

**Mice**

CCR2−/− mice (17) in the C57BL/6 (H-2b) background were maintained as a breeding colony at the Massachusetts General Hospital barrier facility (Boston, MA). C57BL/6 (H-2b) and BALB/c (H-2a) mice aged 6–8 wk were purchased from The Jackson Laboratory (Bar Harbor, ME).

**Islet isolation, transplantation, and graft removal**

Islets isolated from the MHC-mismatched male BALB/c (H-2b) donors were transplanted in the CCR2−/− and WT C57BL/6 mice. The recipients were rendered diabetic by streptozotocin. The details on islet isolation, transplantation, and glucose monitoring have been previously published (11, 18).

**Heart transplantation**

Hearts from BALB/c (H-2b) donors were transplanted in the CCR2−/− and C57BL/6 mice. The vascularized cardiac allografts were placed in an intra-abdominal location using microsurgical techniques, as previously described (19). Graft function was assessed daily by palpation.

**ELISPOT assay**

The ELISPOT assay was performed, as previously described (11). Briefly, Immunospot plates (Cellular Technology, Cleveland, OH) were coated with capture Abs against IL-4 or IFN-γ (BD PharMingen, San Diego, CA). Splenocytes in complete HL-1 medium (BioWhittaker, Walkersville, MD) were then added to each well with an equal number of irradiated syngeneic or alloimmune splenocytes. The resulting spots, or cytokine-producing cells per million splenocytes, were counted on a computer-assisted ELISAspot image analyzer (Cellular Technology). The number of spots in the wells with medium alone or syngeneic cells was subtracted from alloresponses to take into account the background when analyzing the data.

**Immunohistology**

Indirect frozen section immunohistology was performed using primary Abs for CD3, CD4, CD8+ T cells, and macrophages using the markers, F4/80, MOMA2, MOMA1, and CD169 (BD PharMingen). Islets were stained for insulin and glucagon using guinea pig anti-insulin (1/10 dilution; DAKO, Carpinteria, CA); biotin-labeled goat anti-guinea pig Ig (1/200; Vector Laboratories, Burlingame, CA), and developed with Vector ABC (11, 18).

**RNA extraction and real-time PCR**

Total RNA was extracted from the islet grafts using TRIzol reagent, according to the manufacturer’s protocol. After DNase I treatment (Invitrogen, San Diego, CA) treatment, the extracted RNA was reverse transcribed to synthesize 100 μl of cDNA. The following 25 μl quantitative PCR (QPCR) contained 2 μl of cDNA, 12.5 μl of 2× SYBR Green master mix (Stratagene, La Jolla, CA), and 250 nmol of sense and antisense primers. Primers were designed, as previously described (20). The reaction conditions were as follows: 50°C for 2 min, 95°C for 10 min, then 40 cycles of 95°C for 15 s and 60°C for 1 min. Emitted fluorescence for each reaction was measured during the annealing/extension phase. The calculated number of copies was divided by the number of copies of the housekeeping gene GAPDH. No significant changes in the QPCR results were noted when the data were normalized using another constitutively active gene such as β2-microglobulin.

**Cytofluorimetry**

Splenocytes recovered from the recipients were resuspended in FACS buffer (PBS containing 1% FCS/0.1% sodium azide) at a concentration of 2 × 10⁶/ml. Cells were incubated at 4°C for 10 min with 2.4G2 anti-Flk-1/III/II (BD PharMingen). Cells were stained with anti-CD3 FITC, anti-CD8 FITC, anti-CD4 FITC, anti-CD11c FITC, anti-CD11b allophycocyanin, anti-CD62L allophycocyanin, and anti-CD44 PE, and isotype controls (all BD PharMingen). Cytofluorimetry was performed using a FACSCaliber cytometer (BD Biosciences, San Jose, CA) and analyzed using CellQuest software. CD8+ effector cells expressing CD44high and CD62Llow were enumerated, as previously described (21, 22).

**Statistical analyses**

Data were analyzed by Instat software (GraphPad, San Diego, CA), using the Mann-Whitney test for data ELISPOT and expression studies, and the Kaplan-Meier for differences in graft survival. All the data are presented as means ± SD. Values of ρ less than 5% were considered statistically significant.

**Results**

**Temporal chemokine gene expression levels**

Islets were transplanted into two groups of recipients: allografts (BALB/c→C57BL/6) and isografts (C57BL/6→C57BL/6). RNA recovered from islet grafts removed at day 3, 6, 8, and 12 posttransplant was used for real-time QPCR analysis. In isografts, the expression of CCL5 (RANTES); CCL3 (macrophage-inflammatory protein-1β); CCL2, 8 (MCP-1, -2), -5; CXCL10 (IP-10); and CXCL9 (Mig) was induced to low levels as early as day 3 posttransplant, and peaked at day 6 or 8 (Fig. 1A). The expression of the corresponding chemokine receptors, CCR1, CCR5, CCR2, and CXCR3, was also weakly induced at day 3 posttransplant and peaked at day 6 (Fig. 1A). In allografts, the expression of these chemokines and their receptors was induced to higher levels than in isografts, and their expression was more sustained over time (Fig. 1C). Among different ligands for CCR2, the expression of CCL2 (MCP-1) was induced early (peaked at day 3) and then diminished over time. In contrast, the expression of CCL8 and CCL12 (MCP-2 and MCP-5) was also seen at day 3, but their levels increased over time to higher levels than CCL2 (MCP-1). The expression of CCL5 (RANTES) was also detected at day 3 posttransplant in allografts and increased over time until the time of rejection at ~day 12. Expression of CXCL10 (IP-10) and CXCL9 (Mig) was also first evident in allografts at day 3 posttransplant and peaked at day 6 or 8 (Fig. 1C). In the allografts, the expression of the corresponding chemokine receptors was noted at day 3 posttransplant; however, their level of expression was found to be higher than those in the isografts, and continued to increase over time until rejection (Fig. 1C). At day 3 posttransplant, IFN-γ and IL-12 transcripts were detected at comparable levels in isografts and allografts. After that time, however, expression of IFN-γ and IL-12 decreased in isografts, but increased in allografts (Fig. 1, B and D).
Prolongation of islet allograft survival in CCR2−/− recipients

All transplants were performed using islets isolated from the MHC-mismatched male BALB/c (H-2d) donors into WT and CCR2−/− C57BL/6 mice. The median survival times for CCR2−/− and WT recipients were 24 and 12 days, respectively (p < 0.0001) (Fig. 2). Interestingly, ~25% of recipients exhibited long-term graft survival (>100 days). However, when those recipients were excluded from the analysis, graft survival remained statistically significantly longer in CCR2−/− as compared with WT recipients (p < 0.0001). Because a small percentage of streptozotocin-induced diabetics sometimes recover from diabetes, the functionality of long-term grafts was tested by reappearance of hyperglycemia following graft removal.

Histology

A large number of grafts recovered from CCR2−/− and WT recipients was examined for the presence of inflammatory cell posttransplant (n = 30 grafts). All samples showed comparable amounts of inflammatory cell infiltrates. However, the infiltrating cells in WT recipients invaded the islets, causing insulitis, while in CCR2−/− recipients, grafts were well preserved and only surrounded by inflammatory cells without apparent insulitis (Fig. 3, A and B). Interestingly, the surviving grafts in CCR2−/− recipients contained less mononuclear cell infiltrates over time (Fig. 3, C–E).

These data were confirmed in the same recipient by implanting two grafts adjacent to each other under the same kidney capsule, and performing serial graft biopsies at different times posttransplant.

FIGURE 1. Dynamics of chemokine, chemokine receptor, and cytokine gene expression in islet isografts and allografts over time. QPCR analysis on RNA extracted from the isografts (A and B) and allografts (C and D) recovered at days 3, 6, 8, and 12 posttransplantation as well as from the islets before transplantation. Data are presented as the number of mRNA copies normalized to the RNA control GAPDH averaged for six independent islet transplants per data point.

FIGURE 2. Islet allograft survival. BALB/c islets were transplanted into WT and CCR2−/− recipients. Rejection was defined as the return of hyperglycemia (blood glucose level >250 mg/dl on two consecutive measurements). WT and CCR2−/− recipients rejected islet grafts with the MSTs of 12 (n = 20) and 24 (n = 35) days, respectively (p < 0.0001).
FIGURE 3. Figure legend continues.
The frequency of IFN-\( \gamma \)/H9253 aortic lymph nodes recovered at day 8 posttransplantation by and composition of immune cells in the islet allografts and para-
percentage of CD4\( ^{+} \)/H11001 fi 4-producing cells was signi-
cantly higher in splenocytes isolated
At the time of rejection, however, CCR2\( ^{+} \)/H11002 4
expression of IL-4, IL-10, and IL-5 was higher in
Intragraft responses
At day 8 posttransplant, grafts from WT and CCR2\(^{+/-}\) recipients were harvested for QPCR analysis. Islet grafts transplanted into WT recipients displayed significantly higher levels of IFN-\( \gamma \) and IL-12 transcripts than those transplanted into CCR2\(^{+/-}\) recipients. In contrast, the expression of IL-4, IL-10, and IL-5 was higher in islets transplanted into CCR2\(^{+/-}\) recipients (Fig. 5A). These data are similar to our findings using ELISPOT analysis of splenocytes and indicate that CCR2\(^{+/-}\) mice developed a Th2-type alloresponse in contrast to the Th1-type alloresponse seen in WT recipients. Analysis of rejecting grafts from CCR2\(^{+/-}\) and WT recipients showed that the expression of IFN-\( \gamma \), IL-4, IL-5, and IL-10 was similar in both groups. The expression of IL-12, however, was significantly higher in the grafts of WT recipients. By contrast, the long-term CCR2\(^{+/-}\) recipients maintained low expression levels for IFN-\( \gamma \). These data are consistent with the ELISPOT data described above.

Immunohistopathology
Immunophenotyping of day 8 islet grafts revealed equivalent numbers of CD4\(^{+}\) and CD8\(^{+}\) T cells and F480\(^{+}\) macrophages in WT and CCR2\(^{+/-}\) recipients (Fig. 3, F–K). However, consistent with the histology revealing more severe insulitis in WT recipients, grafts in WT mice were more heavily invaded by CD8\(^{+}\) T cells than grafts in CCR2\(^{+/-}\) mice. We have also analyzed the number and composition of immune cells in the islet allografts and para-

**FIGURE 3.** Histopathology and immunohistology. A–E, H&E and insulin staining of islet allografts isolated from WT C57BL/6 (B/6) (A) and CCR2\(^{+/-}\) recipients (B–E). Islet grafts from both WT and CCR2\(^{+/-}\) recipients contained comparable inflammatory cell infiltrates; however, grafts recovered from WT recipients revealed a greater degree of insulitis compared with CCR2\(^{+/-}\) recipients (A and B are representative of 15–16 grafts in each group). Surviving grafts from CCR2\(^{+/-}\) have fewer inflammatory cells over time (C and D are representative of 4–5 grafts). Immunohistology of islet grafts from WT (F, H, and J) and CCR2\(^{+/-}\) (G, I, and K) recipients and stained with CD4 (F and G), F480 (H and I), and CD8 (J and K) at day 8 posttransplant. Grafts from both groups exhibited a comparable degree of CD4, CD8, and F480 staining; however, CD8\(^{+}\) T cells do not appear to invade the islets in CCR2\(^{+/-}\) recipients to the same extent as islets in WT recipients (F–K are representative of 4–5 grafts in each group).
Decreased generation of CD62L<sup>low</sup>CD44<sup>high</sup> effector CD8<sup>+</sup> T cells in CCR2<sup>+/−</sup> recipients

To determine the effect of CCR2 signaling on the generation and function of alloreactive CD8<sup>+</sup> T cells, we measured the number of CD62L<sup>low</sup>CD44<sup>high</sup> found in the spleens of CCR2<sup>+/−</sup> and WT recipients before (naive) and 8 days following transplantation (Fig. 6). CD8 effector cells have been shown to be reliably identified as CD62L<sup>low</sup>CD44<sup>high</sup> CD8<sup>+</sup> (21–23). The percentage of CD62L<sup>low</sup>CD44<sup>high</sup> cells increased significantly in WT transplant recipients compared with the naive WT controls (44 ± 4% vs 26 ± 3%, respectively, p < 0.003, n = 8). However, the percentage of effector cells found in CCR2<sup>+/−</sup> transplant recipients did not increase over that of naive CCR2<sup>+/−</sup> mice. The percentage of effector CD8<sup>+</sup> cells was significantly higher in the WT recipients than those in the CCR2<sup>+/−</sup> recipients (44 ± 4% vs 24 ± 5%, respectively, p < 0.007). No difference was noted in the percentage of resident CD62L<sup>low</sup>CD44<sup>high</sup> CD8 cells in naive WT and CCR2<sup>+/−</sup> mice. Flow cytometry analysis of splenocytes recovered at the time of rejection indicated that CCR2<sup>+/−</sup> recipients were able to generate effector CD8<sup>+</sup> cells in a similar fashion as WT recipients (Fig. 6). Both CCR2<sup>+/−</sup> and WT recipients displayed a significant increase in the CD4<sup>+</sup> effector cells when compared with CCR2<sup>+/−</sup> and WT naives. In addition, no difference was observed in the percentage of CD4<sup>+</sup> CD44<sup>high</sup> between CCR2<sup>+/−</sup> and WT recipients (data not shown). These data indicate that CCR2 signaling plays a role in the generation and/or maintenance of alloreactive CD8<sup>+</sup> effector T cells in vivo following islet transplantation. However, similar to the Th2 switch, this appears to be a transient effect in CCR2<sup>+/−</sup> animals that ultimately reject their grafts.

Other chemokine pathways in the CCR2<sup>+/−</sup> recipients

Most CCR2<sup>+/−</sup> recipients eventually reject islet allografts, albeit in a delayed fashion. Furthermore, given the findings that

**FIGURE 5.** Intragraft expression of Th1- and Th2-type cytokines in WT and CCR2<sup>+/−</sup> recipients. QPCR analysis on RNA isolated from islet grafts recovered from WT and CCR2<sup>+/−</sup> recipients at day 8 posttransplantation (A) and at the time of rejection (B). Data are presented as the number of mRNA copies normalized to the RNA control GAPDH averaged for six to eight independent islet transplants per data point.

**FIGURE 6.** CD8<sup>+</sup> effector cells in WT and CCR2<sup>+/−</sup> recipients. CD8<sup>+</sup> splenocytes recovered from WT and CCR2<sup>+/−</sup> recipients on day 8 postallogeneic islet transplantation were analyzed by flow cytometry using CD62L and CD44 as markers for effector CD8 cells. The percentage of effector CD8<sup>+</sup> cells (CD62L<sup>low</sup>CD44<sup>high</sup>) in WT recipients increased markedly following islet transplantation, while this increase was not seen in CCR2<sup>+/−</sup> recipients (A–D). Both CCR2<sup>+/−</sup> and WT recipients were able to generate effector CD8<sup>+</sup> cells in a similar fashion at the time of rejection (E and F). The data are representative from six different experiments and are expressed as the mean percentage ± SD (n = 8).
CCR2−/− rejectors switch their immune response back to a Th1 phenotype and also are able to generate effector CD8+ alloreactive cells, we hypothesized that other chemokine/chemokine receptor pairs must take over and generate a Th1 and effector alloimmune response resulting in allograft rejection. Therefore, we explored the expression of chemokines and chemokine receptors in islet allografts harvested from CCR2−/− recipients. In CCR2−/− recipients, CCR5 was still highly induced in the allografts (Fig. 7A). Consistent with CCR5 playing a compensatory role in CCR2−/− mice, CCL5 (RANTES), a CCR5 ligand, was also expressed at highest levels in islets recovered from CCR2−/− recipients. In CCR2−/− recipients, CCR5 was still highly induced in the allografts (Fig. 7A). Consistent with CCR5 playing a compensatory role in CCR2−/− mice, CCL5 (RANTES), a CCR5 ligand, was also expressed at highest levels in islets recovered from CCR2−/− mice (Fig. 7B). In addition to CCL5 (RANTES), the CXCR3 ligand CXCL9 (Mig), CCL8 (MCP-2), and in particular CCL12 (MCP-5) were also overexpressed in islets recovered from CCR2−/− mice. These results suggest that, indeed, there are compensatory chemokine pathways that are capable of ultimately initiating a destructive alloimmune response in CCR2-deficient recipients. It is possible that when these compensatory mechanisms are not induced, the grafts do not get rejected and exhibit long-term survival, as observed in some of the CCR2−/− recipients.

**CCR2 plays a less important role in heart allograft rejection**

To begin to determine the organ specificity of the CCR2 chemokine system in allograft rejection, we performed heterotopic vascular cardiac allograft across MHC class I and II (BALB/c heart transplanted into CCR2−/− or WT C57BL/6 mice). In contrast to islet transplantation, CCR2−/− recipients had only marginal protection for heart allografts. The median survival time (MST) for CCR2−/− and WT was 12 ± 1 vs 8 ± 1 days, respectively (p < 0.001) (Fig. 8). To test whether the long-term islet allografts induced tolerance in the CCR2−/− recipients, we transplanted BALB/c heart allografts into the long-term functioning CCR2−/− C57BL/6 islet allograft recipients (>100 days). Interestingly, these

**FIGURE 7.** A and B, Chemokine and chemokine receptor gene expression in WT and CCR2−/− islet allografts recipients. QPCR analysis on RNA isolated from islet grafts recovered at day 8 posttransplantation from syngeneics and WT and CCR2−/− allogeneic recipients. Data are presented as the number of mRNA copies normalized to the RNA control GAPDH averaged for five to six independent islet transplants per data point.

**FIGURE 8.** CCR2−/− and heart allograft survival. BALB/c hearts were heterotopically transplanted into C57BL/6 WT and CCR2−/− recipients. WT and CCR2−/− recipients rejected islet grafts with the MSTs of 8 ± 1 (n = 7) and 12 ± 1 (n = 7) days, respectively (p < 0.001).
recipients rejected the heart allografts with the MSTs of 18 days compared with 8 days for naive CCR2−/− recipients \((n = 4, p < 0.002)\). These long-term CCR2−/− islet recipients that rejected heart allografts then went on to subsequently reject their previously functioning islet grafts within 1 wk, indicating that the heart allografts sensitized the CCR2−/− recipients to overcome local tolerance mechanisms. These data indicate that there is organ specificity for chemokine function in regulating allograft rejection and that CCR2 plays a more important role in inducing local islet cell tolerance than in inducing systemic tolerance.

We have also performed ELISPOT and flow cytometry analyses on splenocytes recovered form the CCR2−/− and WT heart allograft recipients at day 8 posttransplantation. Although the number of IL-4-producing cells was significantly higher in the CCR2−/− (603 ± 26) than in the WT recipients (200 ± 5) \((p < 0.003)\), the number of IFN-γ-producing cells (1220 ± 86) was significantly higher in the WT recipients than those in the CCR2−/− (690 ± 30) \((p < 0.001)\). These data are consistent with the data from islet transplant recipients described above. The flow cytometry analysis data showed that similar to the islet recipients, the CCR2−/− recipients of the heart allografts failed to generate effector CD8+ cells following transplantation, while both groups effectively generated effector CD4+ cells in a similar fashion (data not shown).

Discussion

In this study, we have demonstrated that in both isografts and allografts the expression of chemokinases and chemokine receptors was similarly detected as early as day 3 posttransplantation. However, only in the allografts was their expression sustained and increased over time. The levels of expression of CCR2 were found to be higher than those of CCR1, CXCR3, and CCR5. Of the CCR2 ligands, CCL12 (MCP-5) was induced to the highest and to be higher than those of CCR1, CXCR3, and CCR5. Of the increased over time. The levels of expression of CCR2 were found to be significantly higher in the CCR2−/− recipients than in the CCR2+/+ recipients. Delayed rejection in the periphery and in the graft and with the presence of fewer CD8+ cells being differentiated into CD44+ effector cells. The presence of decreased pathogenic CD8+ cells may be related to a shift toward a Th2 response as activated CD8+ cells produce large amounts of Th1 cytokine IFN-γ \((24, 25)\). Although the role of Th1 or Th2 response in the process of rejection remains controversial, the association of the Th2 response with the prolongation of islet allograft is similar to our previous observation in the CCR5−/− mice \((11)\). In support of this suggestion are data showing that lymphocytes isolated from a destructive peri-insulitis of nonobese diabetic mice were found to produce a large amount of IFN-γ and little IL-4, resembling a Th1 response. Conversely, lymphocytes isolated from a nondestructive autoimmune response produce more IL-4 and little IFN-γ, thus indicating a Th2-like response \((26)\). We have examined a large number of grafts recovered from CCR2−/− and WT recipients for the presence of inflammatory cells posttransplant. All samples showed comparable amounts of inflammatory cell infiltrates, including similar numbers of CD3+, CD4+, CD8+, and F4/80 immunoreactive cells. Therefore, the prolongation of islet allograft survival was not necessarily due to a deficiency in mononuclear cell recruitment into the grafts. Interestingly, long-term surviving grafts had minimal inflammatory infiltrates, suggesting that the recruited cells eventually leave the grafts or that inflammatory cells that die in the graft are not replaced. These findings may explain the observed islet-specific tolerance that appears in some CCR2−/− recipients.

The majority of CCR2−/− rejected the islet allografts with a delay. Although CCR2−/− recipients displayed a Th2 response early after the transplant, a Th1 phenotype with a capacity to generate effector CD8+ allosreactive cells was noted in the CCR2−/− recipients at the time of rejection. The intragraft expression data suggest that other chemokine/chemokine receptor pairs are functional in CCR2−/− recipients in promoting a destructive Th1 alloimmune response in vivo, albeit delayed in tempo as compared with WT controls. Rejection caused by compensatory chemokine pathways also highlights the important message that to achieve long-term allograft survival, it is likely that multiple chemokine/chemokine receptors will need to be targeted in vivo. The exact mechanisms by which some CCR2−/− recipients exhibit long-term survival and do not develop a Th1 alloimmune response requires further investigation.

In contrast to islet allografts, cardiac allografts had only marginal survival benefit in CCR2−/− recipients. In addition, even long-term islet allograft CCR2−/− recipients (>100 days) rejected heart allografts with the MST of 18 days, indicating that CCR2−/− long-term islet recipients were not tolerant, per se, due to clonal deletion or suppression. This difference in outcome indicates that each organ or tissue may require a unique set of chemokines to generate a destructive alloimmune response leading to acute rejection. We and others have previously shown that CD4+ T cell depletion leads to significantly greater prolongation of heart allograft survival than depletion of CD8+ T cells \((27, 28)\). Conversely, CD8+ T cells play a central role in islet allograft rejection \((29, 30)\). Compared with WT recipients, CCR2−/− recipients of heart allograft not only displayed a Th2 response, but also failed to generate CD8+ effector cells following heart transplantation. Because the generation of CD8+ effector cells was left intact, only marginal prolongation of heart allograft survival was observed. These data are consistent with the differential roles of CD4+ vs CD8+ T cells in islet and heart allograft rejection.

We conclude that CCR2 deficiency offered a significant prolongation of islet allograft survival. This prolongation was associated with a Th2 alloresponse as well as a failure to generate CD8+ effector cells. Interfering with CCR2 function offers a novel approach for the management of islet allograft recipients.

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References


