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Absence of Recipient CCR5 Promotes Early and Increased Allospecific Antibody Responses to Cardiac Allografts

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Acute rejection is mediated by T cell infiltration of allografts, but mechanisms mediating the delayed rejection of allografts in chemokine receptor-deficient recipients remain unclear. The rejection of vascularized, MHC-mismatched cardiac allografts by CCR5⁻/⁻ recipients was investigated. Heart grafts from A/J (H-2a) donors were rejected by wild-type C57BL/6 (H-2b) recipients on day 8–10 posttransplant vs day 8–11 by CCR5⁻/⁻ recipients. When compared with grafts from wild-type recipients, however, significant decreases in CD4⁺ and CD8⁺ T cells and macrophages were observed in rejecting allografts from CCR5-deficient recipients. These decreases were accompanied by significantly lower numbers of alloreactive T cells developing to IFN-γ, but not IL-4-producing cells in the CCR5⁻/⁻ recipients, suggesting suboptimal priming of T cells in the knockout recipients. CCR5 was more prominently expressed on activated CD4⁺ than CD8⁺ T cells in the spleens of allograft wild-type recipients and on CD4⁺ T cells infiltrating the cardiac allografts. Rejecting cardiac allografts from wild-type recipients had low level deposition of C3d that was restricted to the graft vessels. Rejecting allografts from CCR5⁻/⁻ recipients had intense C3d deposition in the vessels as well as on capillaries throughout the graft parenchyma similar to that observed during rejection in donor-sensitized recipients. Titers of donor-reactive Abs in the serum of CCR5⁻/⁻ recipients were almost 20-fold higher than those induced in wild-type recipients, and the high titers appeared as early as day 6 posttransplant. These results suggest dysregulation of alloreactive Ab responses and Ab-mediated cardiac allograft rejection in the absence of recipient CCR5. The Journal of Immunology, 2005, 174: 6499–6508.

The rejection of allografts when a specific chemokine or chemokine receptor is antagonized has been interpreted to indicate the redundancy in the chemokine system and the ability of other chemokine/receptor interactions to replace the target. Previous studies from this laboratory indicated the prolonged survival of cardiac allografts when recipients were treated with Abs to the T cell chemotactant Mig (9). At the time of rejection, there were many CCR5-expressing cells in the cardiac allografts, suggesting that mononuclear cell expression of CCR5 may substitute for Mig-CXCR3 interactions in directing T cells into the grafts. A recent study reported the prolonged survival of cardiac allografts in CCR5⁻/⁻ recipients (11). In clinical renal transplantation, recipients expressing a homozygous mutation encoding a nonfunctional CCR5 have better graft outcomes than recipients expressing functional receptors (12). These studies suggest that expression of CCR5 may play an important role in directing T cells into allografts to mediate acute rejection. In addition to directing T cell recruitment, in vitro studies have indicated that CCR5 ligands, including MIP-1α, MIP-1β, and RANTES, amplify T cell activation during culture with anti-CD3 mAb or APCs (13–15). In light of these in vitro studies, we have investigated the activation of alloantigen-specific T cells as well as mechanisms of rejection in CCR5-deficient recipients of MHC-mismatched cardiac allografts. The results indicate decreased T cell infiltration into rejecting allografts in CCR5⁻/⁻ recipients that is due to reduced effector T cell priming to alloantigens, and suggest that alternative mechanisms mediate cardiac allograft rejection in CCR5⁻/⁻ recipients.

Materials and Methods

**Animals**

A/J (H-2a), BALB/c (H-2d), C3H/HeN (H-2h), and C57BL/6 (H-2b) mice were obtained through C. Reeder at the National Cancer Institute (Frederick, MD). CCR5⁻/⁻ mice were obtained from The Jackson Laboratory. Adult males of 8–12 wk of age were used throughout this study.
Antibodies

The analyses of Abs were used for immunohistological and flow cytometry analyses: FITC-conjugated goat anti-mouse IgG Ab (Pierce); FITC-conjugated rat anti-mouse IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM Abs, PerCP-conjugated GKL1.5 (rat anti-mouse CD4 mAb) and 53-6.7 (rat anti-mouse CD8 mAb), FITC-conjugated PGp-1, Ly-24 (anti-mouse CD44 mAb), biotinylated and PE-conjugated rat anti-mouse CCR5 mAb, and FITC-conjugated goat anti-mouse IgM and IgG Ab (BD Pharmingen); rat anti-mouse macrophage mAb, F4/80 (Serotec); goat anti-mouse CXCR3 (C20) and goat anti-mouse CCR5 antiserum (Santa Cruz Biotechnology); PE-conjugated donkey anti-goat IgG Ab (Jackson ImmunoResearch Laboratories); and FITC-conjugated rabbit anti-CD3 Ab (DakoCytomation).

Preparation of bone marrow-derived dendritic cells (DC)

Bone marrow cells were flushed from the femurs and tibia of A/J mice and cultured for 7 days in medium containing 10 ng/ml GM-CSF and 10 ng/ml IL-4 to generate mature DC. After the culture, the DC were isolated by positive selection using CD11c+ MACS cell separation magnetic beads (Miltenyi Biotec), and 1.6 × 10⁶ DC were injected i.v. into wild-type C57BL/6 and B6.CCR5−/− mice.

Heterotopic cardiac transplant

Cardiac transplants were performed using the method of Corry et al. (16). Briefly, donor and recipient mice were anesthetized with phenobarbital. Donor hearts were harvested and placed in chilled lactated Ringer’s solution while the recipient mice were prepared. The donor heart was anastomosed to the recipient abdominal aorta and vena cava using microsurgical techniques. Upon completion of the anastomoses and organ perfusion, the transplanted hearts resumed spontaneous contraction. The strength and quality of cardiac impulses were judged by palpation each day, as previously described (9). Rejection of cardiac grafts was considered complete by the cessation of impulse and was confirmed visually for each graft by laparotomy. In C57BL/6 recipients, complete rejection of A/J cardiac grafts occurs between 8 and 10 days after transplantation. Cardiac isografts in C57BL/6 recipients functioned for >100 days. The significance in allograft survival between recipient groups was analyzed by log rank test, and p < 0.01 was considered a significant difference between groups.

Immunohistology

Heart grafts were retrieved from recipients at the time of rejection, embedded in OCT compound (Sakura Finetek), and frozen at −80°C. Sections were cut at 8 μm and mounted onto slides. For immunohistochemistry, sections were fixed in acetone at 0°C for 10 min and air dried. Slides were immersed in PBS for 10 min and then in 0.03% H2O2 for 10 min to eliminate endogenous peroxidase activity. The slides were then stained for 1 h with 5 μg/ml anti-CD4 mAb (GKL1.5), anti-CD8 mAb (53-6.7), or anti-macrophage F4/80 mAb in 0.05 M Tris-HCl with 1% BSA. After 24 h of cell culture at 37°C in 5% CO2, cells were removed from the plate by extensive washing with PBS. Biotinylated anti-IFN-γ mAb (2 μg/ml) or anti-IL-4 (4 μg/ml) was added, and the plate was incubated for 6 h at room temperature. The plate was washed three times with PBS/0.05% Tween 20, and streptavidin-conjugated alkaline phosphatase was added to each well. After 2 h at room temperature, the plates were washed with PBS, and NBT-5-bromo-4-cloro-3-indolyl substrate (Kirkegaard & Perry Laboratories) was added for the detection of IFN-γ or IL-4-producing cells. The resulting spots were counted with an ImmunoSpot Series I analyzer (Cellular Technology) that was designed to detect ELISA spots with predetermined criteria for spot size, shape, and colorimetric density.

Flow cytometry

The expression of CXCR3 and CCR5 on CD4+ and CD8+ T cells in the spleens of naive mice and heart allograft recipients and on graft-infiltrating T cells was tested by flow cytometry. Spleen cell suspensions were prepared, and 10^6 cell aliquots were washed three times with staining buffer (Dulbecco’s PBS with 2% FCS/0.2% NaN3) and stained with PerCP rat anti-mouse CD4 or anti-mouse CD8α mAb, FITC anti-mouse CD44 mAb, and goat anti-mouse CXCR3 or biotinylated anti-mouse CCR5 Ab for 25 min on ice. The cells were washed twice, and the cells were stained with PE-streptavidin to detect CCR5+ cells or PE anti-goat IgG to detect CXCR3+ cells. After washing five times, the cells were analyzed by flow cytometry using a FACScan (BD Biosciences). The CD4+ or CD8+ T cells were gated and analyzed for expression of CD44 and CXCR3 or CCR5. To isolate allograft-infiltrating cells, the allograft was retrieved on day 7 post-transplant, cut into small pieces, and incubated at 37°C in 5% CO2, cells were fixed in acetone for 10 min and air dried. Slides were dehydrated and viewed under light microscopy, and the images were captured using ImagePro Plus (Media Cybernetics). Numbers of cells staining positive were counted in eight fields in three different tissue sections from three different grafts, and the significance between mean numbers of positive cells per field in different treatment groups was tested using Mann-Whitney U test. To investigate colocalization of CCR5 with graft-infiltrating CD4 and CD8 T cells and macrophages, adjacent sections were cut and placed on separate slides as mirror images (17). One slide was stained with goat anti-mouse CCR5 antiserum and the other with Ab to detect infiltrating CD4 or CD8 T cells or macrophages, as above.

For immunofluorescent staining of C3d, 8-μm frozen sections were fixed in acetone for 10 min and air dried. Slides were immersed in PBS for 10 min, and FITC-conjugated rabbit anti-CD3 Ab diluted 1/100 in 1% BSA/PBS was applied for 30 min in a humid chamber. After three washes with PBS, the slides were mounted with VECTASHIELD with 4’,6’-diamino-2-phenylindole (Vector Laboratories) and viewed under a fluorescent microscope.

ELISPOT assay

Priming of alloantigen-specific T cells from heart allograft recipients was investigated by enumerating IFN-γ-producing and IL-4+ T cells using ELISPOT assays, as previously described (18, 19). Briefly, ELISA spot plates (UniFilter 250; Polylittronics) were coated with 2 μg/ml IFN-γ- or IL-4-specific mAb and incubated overnight at 4°C. After removal of the plate by extensive washing with PBS. Biotinylated anti-IFN-γ mAb (2 μg/ml) or anti-IL-4 (4 μg/ml) was added, and the plate was incubated for 6 h at room temperature. The plate was washed three times with PBS/0.05% Tween 20, and streptavidin-conjugated alkaline phosphatase was added to each well. After 2 h at room temperature, the plates were washed with PBS, and NBT-5-bromo-4-cloro-3-indolyl substrate (Kirkegaard & Perry Laboratories) was added for the detection of IFN-γ or IL-4-producing cells. The resulting spots were counted with an ImmunoSpot Series I analyzer (Cellular Technology) that was designed to detect ELISA spots with predetermined criteria for spot size, shape, and colorimetric density.
Flow cytometry to detect and measure donor-specific Abs in cardiac allograft recipient serum was performed using a modification of the method described by Wasowska et al. (20). Thymocyte suspensions from naive A/J, P/J, and C57/BL6 mice were prepared in HBSS, and 50-μl aliquots containing 1.5 × 10^5 thymocytes were incubated with 50 μl of 4-fold dilutions of sera from naive wild-type C57BL/6 and wild-type and CCR5-deficient heart allograft recipients for 1 h on ice. The cells were washed three times and suspended in 50 μl of staining buffer (Dulbecco’s PBS with 2% FCS/0.2% NaN₃) containing a mixture of FITC-conjugated goat Abs specific for mice IgG and IgM (BD Pharmingen) for 30 min on ice. The cells were washed twice, fixed, and analyzed by flow cytometry. The mean channel fluorescence of each dilution of each serum sample was determined, and the dilution that returned the mean channel fluorescence to the level observed when A/J thymocytes were stained with a 1/4 dilution of normal wild-type serum was divided by two and reported as the titer. Serum from groups of five cardiac allograft recipients was tested, and differences in mean titer between wild-type and CCR5-deficient recipients were determined using Mann-Whitney U test.

To test for the presence of donor-reactive IgM and IgG isotypes in naive and cardiac allograft recipient serum, 10^6 A/J thymocytes were incubated with a 1/20 dilution of serum. The cells were washed three times and stained with FITC-conjugated Abs to mouse IgM and IgG isotypes for 30 min on ice. The cells were washed three times and analyzed by flow cytometry. Results shown are the percentage of A/J thymocytes that bound detectable naive and allograft recipient Ab.

**Results**

**Delayed rejection, but decreased T cell infiltration into heart allografts in CCR5−/− recipients**

To begin to investigate the potential role of CCR5 in cardiac allograft rejection, the rejection of complete MHC-mismatched A/J heart grafts by wild-type C57BL/6 and CCR5−/− recipients was compared. Wild-type recipients rejected the allografts at day 8–10 posttransplant (Fig. 1). A modest prolongation to day 8–11 was observed in CCR5-deficient recipients, but this prolongation was not statistically significant. Tissue sections prepared from rejecting allografts retrieved from wild-type recipients indicated the intense mononuclear cell infiltration typical of acute rejection (Fig. 2). In contrast, rejecting allografts retrieved from CCR5−/− recipients had a clear decrease in mononuclear cell infiltration. When frozen sections were stained to assess infiltration by CD4 and CD8 T cells and macrophages, there was a striking decrease in infiltration by all three leukocyte populations into the rejecting allografts retrieved from CCR5-deficient recipients (Fig. 3). When random sections of slides were counted, a 4-fold decrease in infiltrating CD4⁺ and CD8⁺ T cells (p < 0.01) was observed in the

**FIGURE 2.** Histological analysis of rejecting allografts from wild-type C57BL/6 vs CCR5−/− recipients. At the time of rejection, A/J cardiac allografts from wild-type C57BL/6 (a) and CCR5−/− (b) recipients were retrieved, and formalin-fixed sections were prepared and stained with H&E and viewed under light microscopy. Magnification, ×200.

**FIGURE 3.** Immunohistological analysis of rejecting allografts from wild-type C57BL/6 and CCR5−/− recipients. At the time of rejection, A/J cardiac allografts were retrieved from wild-type C57BL/6 (d–f) and CCR5−/− (a–c) recipients, and frozen sections were prepared and stained with Abs to CD4 (a and d), CD8 (b and e), and F4/80 to detect macrophages (c and f). Magnification, ×200.
CCR5-deficient recipients when compared with infiltration by these T cells in rejecting grafts from wild-type recipients at the time of rejection (Fig. 4). The number of infiltrating macrophages was ~2-fold less in the CCR5$^{-/-}$ recipient ($p < 0.05$).

**Expression of CCR5 by heart allograft-infiltrating cells**

A potential mechanism underlying the decreased infiltration of mononuclear cells into heart allografts in CCR5$^{-/-}$ recipients was...
the need for CCR5 expression to direct this infiltration. To investigate this, the colocalization of CCR5 on infiltrating CD4\(^+\) and CD8\(^+\) T cells and macrophages in rejecting heart allografts retrieved from wild-type recipients was analyzed. Adjacent, mirror-image sections were prepared from the heart allografts at the time of rejection, and one was stained to detect CCR5 expression and the other stained with Abs to detect the phenotype of the infiltrating cell populations. Infiltrating CD4\(^+\) T cells clearly expressed
CCR5 (Fig. 5a). In contrast, graft-infiltrating CD8− T cells and macrophages did not colocalize with CCR5 staining, whereas vascular smooth muscle cells known to constitutively express CCR5 (21) stained positively (Fig. 5, b and c).

The expression of CCR5 on allograft-infiltrating CD4+ and CD8+ T cell populations was further investigated using another approach. The allografts were retrieved on day 7 posttransplant, the allograft tissue was digested, and aliquots of the cells were stained with Abs and analyzed by flow cytometry. Although allograft infiltration by CD4+ T cells was much less intense than infiltration by CD8+ T cells (Figs. 4 and 5), 50% of the infiltrating CD4+ T cells expressed CCR5, whereas only 31% of the infiltrating CD8+ T cells expressed CCR5 (Fig. 6).

Temporal expression of CXCR3 and CCR5 on T cells from recipients of heart allografts

The expression of CCR5 on allograft-infiltrating CD8+ T cells and macrophages suggested that suboptimal trafficking into the allograft was unlikely to account for the reduced cellular infiltration observed in rejecting heart allografts in CCR5−/− recipients. The expression of chemokine receptors on recipient T cells during priming for rejection of heart allografts has remained undefined. To investigate this aspect of the T cell response, the expression of the chemokine receptors CXCR3 and CCR5 was compared on CD4+ and CD8+ T cells in the spleen of allograft recipients. In addition, the expression of CD44 as an indicator of cellular activation was used to test chemokine receptor expression on activated T cell populations in the allograft recipients. Low numbers of CXCR3-expressing CD4+ T cells were observed in naive mice, and this expression was restricted to the CD44high population (Fig. 7a). In heart allograft recipients, these numbers increased slightly on day 4 posttransplant and then increased much more on days 6 and 8, with the expression again restricted to the CD44high cell population. Although more CXCR3+ CD8+ than CD4+ T cells were observed in the spleens of naive mice, similar results were observed with CD8+ T cell expression of CXCR3 during progression to allograft rejection (Fig. 7b). CD4+ T cell expression of CCR5 followed a similar pattern as CXCR3 expression, with slight increases at day 4 posttransplant and further increases afterward (Fig. 7c). In contrast, expression of CCR5 was not as prominent on CD8+ T cells in allograft recipients and was expressed at lower levels than observed on CD4+ T cells (Fig. 7d). At day 4 posttransplant, CCR5 expression was observed only on CD44high cells for each T cell population, but was observed on populations of CD44low and CD44high CD4+ and CD8+ T cells at days 6 and 8 posttransplant.

**Decreased alloreactive T cell priming in CCR5−/− allograft recipients**

The role of CCR5 in alloreactive T cell priming was investigated by comparing the development of alloreactive T cells from wild-type and CCR5−/− heart allograft recipients with IFN-γ and IL-4-producing cells using ELISPOT assays. The number of IFN-γ-producing cells in the CCR5−/− recipient spleen was less than half that observed in recipients of wild-type grafts at day 7 posttransplant (Fig. 8). The number of alloantigen-specific T cells producing IL-4 was similar in each set of recipients, suggesting that immune deviation did not account for the decreased priming of alloreactive T cells to IFN-γ-producing cells observed in CCR5−/− recipients.

The possibilities that the decreased priming of alloreactive T cells in CCR5-deficient recipients of heart allografts was due to a direct requirement for CCR5 for T cell priming vs an inhibitory effect of passenger leukocytes in the allograft on the priming of the CCR5-deficient T cells were addressed. First, purified A/J DC were administered to wild-type and CCR5−/− recipients, and ELISPOT assays were performed 4 days later (Fig. 9a). The number of alloreactive T cells producing IFN-γ in CCR5-deficient recipients of the DC was 50% that induced in wild-type recipients. Second, heart allograft donor A/J mice were subjected to 1100 rad whole body irradiation to deplete the donors of radiation-sensitive passenger leukocytes, and 2 days later the hearts were transplanted to wild-type and CCR5−/− recipients. On day 7 posttransplant, graft recipient spleen cells were tested for levels of alloreactive T cell priming using IFN-γ ELISPOT assays (Fig. 9b). Again, the...
number of alloreactive T cells producing IFN-γ in the CCR5-deficient recipients was almost 50% that observed in wild-type recipients of the heart allografts from the irradiated donors. Collectively, the results in Figs. 8 and 9 indicate the reduced priming of T cells to alloantigen in the absence of CCR5.

**Increased alloantigen-specific Ab production in CCR5−/− allograft recipients**

Because CCR5-deficient recipients had decreased alloreactive T cell priming and infiltration into the allograft at rejection, the potential role of alloantigen-specific Ab in the rejection of cardiac allografts in these recipients was investigated. First, sections of rejecting allografts were prepared and stained to detect deposition of C3d as an indication of Ab reactivity in the allograft. As a positive control, wild-type C57BL/6 mice that had previously rejected an A/J skin allograft and had also been primed with two injections of A/J spleen cells to induce high levels of donor-reactive Abs were transplanted with A/J cardiac allografts that were rejected on day 5 and were stained for C3d deposition. Whereas isografts had no detectable C3d staining, allografts from donor-sensitized recipients had intense C3d staining around all large vessels as well as on capillaries throughout the heart allograft parenchyma (Fig. 10, a vs b). Low, but detectable C3d deposition was observed in allografts retrieved from wild-type recipients, and this staining was restricted to the large vessels (Fig. 10c). In contrast, C3d deposition in rejecting allografts retrieved from CCR5−/− recipients was much more intense around the large vessels and included C3d-positive capillaries throughout the graft parenchyma (Fig. 10d) similar to staining observed in the allografts retrieved from donor-sensitized wild-type recipients.

The levels of donor-reactive Ab in the serum of groups of five wild-type vs CCR5−/− recipients were compared at days 6 and 8.
the day of allograft rejection (Fig. 11, a transplant and were slightly increased by day 8 (435.2 and promote long-term graft survival. Studies from several labo-

ratory sets of molecules to inhibit cellular infiltration into allografts

infiltration is largely mediated by the coordinated functions of ad-

cellular infiltration into the allograft. This

function and promote leukocyte adhesion to the vascular endothe-

lum (24, 25). In vitro studies have indicated that RANTES aug-

mation of T cells by anti-CD3 Ab to produce IL-2 and proliferate (13, 14). The role of chemokines in the activation of T

cells during the initiation of in vivo responses remains poorly understood.

The key characteristic of acute allograft rejection is alloantigen-

primed T cell and macrophage infiltration into the allograft. This

infiltration is largely mediated by the coordinated functions of ad-

hesion molecules and chemokines. A great deal of interest has

centered on the ability of strategies neutralizing the function of

these sets of molecules to inhibit cellular infiltration into allografts

and promote long-term graft survival. Studies from several labo-

ratories have indicated that antagonism of specific chemokines or

their receptors delays or inhibits rejection of skin, cardiac, islet, and small bowel allografts in rodent models (6, 26–28). Much of

the early work was focused on CXCR3 and its ligands IFN-γ-inducible protein-10 and Mig, potent chemokactants for Ag-

primed T cells. Studies from Hancock et al. (7) indicated pro-

longed survival of MHC-mismatched cardiac allografts from 7 to

8 days in wild-type recipients to beyond 60 days posttransplant in

CXR3−/− recipients. Previous studies from this laboratory re-

ported the absence of class II MHC disparate skin allograft rejec-

tion in recipients treated with Abs to Mig (26). Treatment with

Mig-specific Abs also significantly prolongs the survival of MHC-

mismatched cardiac allografts to day 18–25 posttransplant (9). At

the time of cardiac allograft rejection in Mig-Ab-treated recipients,

high expression of CR5 was observed in the grafts, suggesting a

potential compensation for Mig antagonism by CXCR5-binding che-

mokines in directing T cell graft infiltration.

The role of CR5 and its primary ligands, MIP-1α, MIP-1β, and RANTES, in allograft rejection has generated a considerable amount of recent interest. In human renal transplant patients, expression of a nonfunctional CR5, CR5Δ32, is associated with decreases in acute rejection and better graft outcomes (12). Improved lung allograft survival in rat recipients is observed by treat-

ment with anti-RANTES Abs (5). In a mouse model, allogeneic islets in wild-type recipients are rejected by day 10 posttransplant, whereas 80% of islet allografts are rejected by day 30 in CR5−/− recipients and the remainder survive beyond day 100 (27).

A recent study indicated prolongation of BALB/c cardiac allograft survival from day 7–8 posttransplant in C57BL/6 wild-type recipients to day 20 in CR5−/− recipients (11). Comparison of allograft histology at day 7 posttransplant indicated significant re-

ductions in T cell and macrophage infiltration into BALB/c al-

lografts in CR5-deficient recipients when compared with rejecting allografts in wild-type recipients. The data in the current study indicate a more modest prolongation of A/J cardiac allograft sur-

vival in CR5−/− recipients. This difference in survival is not

apparently due to the use of A/J mice as the allograft donor, as we

have also observed rejection of BALB/c cardiac allografts at day

10/11 posttransplant in CR5−/− recipients. Although survival of

A/J cardiac allografts is only extended 2–3 days in CR5-deficient recipients, there are striking decreases in T cell and macrophage infiltration at the time of rejection when compared with rejecting allografts in wild-type recipients that raised questions regarding mechanisms underlying this rejection in CR5−/− recipients.

Rejecting cardiac allografts in CR5−/− recipients stained inten-

sely with Abs to C3d, an indication of potential Ab deposition in

the grafts. The histology of the allografts appeared identical with

rejecting allografts from donor-sensitized wild-type recipients with

heavy deposition of C3d around the large vessels and capillaries of

the allograft. Consistent with this staining, CR5−/− allograft re-

cipients had high serum levels of donor-specific Ab, with titers

>20-fold that observed in wild-type recipients. The presence of

this Ab response appeared within 6 days after transplantation at a

time when CR5 expression was detectable on activated CD4+ T

cells in wild-type recipients of the cardiac allografts. These results

are strongly suggestive of an Ab-mediated rejection mechanism in

the absence of recipient CR5.

Several recent studies are supportive of a potential role of CR5 in

the regulation of Ab responses. The addition of RANTES and

MIP-1α to human B cell cultures has been shown to up-regulate

IgG4 and IgE production (29), although the requirement for CR1 or

CR5 for this effect remains unclear. Immunization of

CR5−/− mice with T-dependent Ags also enhances Ig responses

(30), but the consequence of this increase on an ongoing immune

response such as during an infection or to an allograft has not been
previously addressed. In conjunction with these studies, the current results suggest dysregulation of Ab responses in the absence of CCR5. The mechanism underlying this dysregulation remains unclear. In contrast to activated CD4+ T cells, we could not detect expression of CCR5 on B cells from C57BL/6 recipients of cardiac allografts, suggesting that CCR5-mediated regulation of Ig responses is mediated through T cells. The absence of CCR5 on B cells has also been observed in BALB/c mice, whereas B cells from NOD mice experiencing active diabetes expressed CCR5 (31). The alloreactive CD4+ T cell response in CCR5-deficient recipients did not deviate to an IL-4-producing phenotype, suggesting that another CCR5-expressing T cell population may regulate this Ab response to the cardiac allograft. An intriguing possibility arises from studies indicating that a population of γδ T cells restricts the magnitude of Ab responses in lpr recipients. Nevertheless, the results suggest dysregulation of Ab responses in the absence of CCR5.

In the current study, CCR5 expression is a dominant factor directing T cells into murine cardiac allografts during acute rejection. J. Immunol. 169: 1556–1560.


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**Disclosures**

The authors have no financial conflict of interest.