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Role of CXCR3 and CCR5 in Allograft Rejection

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ABSTRACT

Chemokines are known to participate in allograft rejection by mediating leukocyte trafficking. Despite redundancy in chemokine family, several chemokine-chemokine receptor interactions have proven critical in alloimmune responses. We sought to determine the effect of combined blockade of CXCR3 and CCR5, two critical chemokine receptors, in acute rejection.

Methods: Heterotopic heart transplantation was performed using BALB/c to B6/129 mice deficient in CCR5. Following transplantation these mice were treated with goat anti-CXCR3 serum every other day. In the control group, BALB/c hearts were transplanted in wild type B6/129 recipients and treated with goat serum alone. No immunosuppression was given to either group. Recipient mice were then assessed daily for allograft function by abdominal palpation, and graft survival was confirmed by laparotomy.

Results: The donor hearts in the control group were rejected at 6 ± 1 days posttransplantation. Combined blockade of CXCR3 and CCR5 prolonged allograft survival versus control; all allografts survived to 24 days. In addition, there was a decrease in graft infiltrating CD4 and CD8 lymphocytes in the experimental group at 24 days.

Conclusion: Combined CXCR3 and CCR5 blockade is effective in prolonging allograft survival in a fully MHC mismatched murine model. Combined chemokine blockade holds promise in control of acute rejection in organ transplantation.

ACUTE REJECTION continues to be a significant problem in solid organ transplantation despite advances in immunosuppressive therapies. Episodes of acute rejection or untreated, low-grade rejection may be a major determinant to long-term allograft survival. Recruitment and activation of mononuclear cells are key features of acute allograft rejection that are mediated through the interaction of chemokines and their respective chemokine receptors.

Chemokines are a large family of secretory proteins expressed on various inflammatory cells that act on responsive leukocytes via their corresponding G-protein coupled receptor.[1] Chief among these chemokine receptors are CCR5 and CXCR3. Studies have demonstrated that elevated expression of CCR5, CXCR3, and their corresponding ligands are present in both murine and human cardiac allograft rejection.[2–4]

In experimental models, due to the redundancy of receptor-ligand interaction, the deficiency or blockade of a single chemokine does not protect the allograft from acute rejection.[5] However, recent studies have demonstrated that the blockade or absence of a single chemokine receptor does prolong allograft survival in a fully MHC mismatched model.[6] Targeting of both CCR5 and CXCR3 individually has proven effective in prolonging allograft survival, but all fully mismatched allografts are eventually rejected.[3,6,7]

In this study we investigated the effect of combined blockade of both CXCR3 and CCR5 in a fully mismatched murine model. Our data demonstrate the effectiveness of

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targeting two chemokine receptors in attenuating acute allograft rejection and prolonging allograft survival.

MATERIALS AND METHODS

Animals

Adult female CCR5−/− (B6;129P-Cmkbr5^{tmlKuz}), wild-type B6; 129PF2/J, and BALB/C mice, 6 to 12 weeks old, were purchased from the Jackson Laboratories (Bar Harbor, Maine). Anti-CXCR3 serum was prepared a previously described.[8]

Experimental Groups

BALB/C strain donor hearts were transplanted into CCR5−/− (B6;129P-Cmkbr5^{tmlKuz}) and wild-type B6;129PF2/J recipient mice. Intraabdominal heterotopic heart transplantation was performed using a modification of the method outlined by Corry et al.[9] Recipient mice in the experimental groups received 0.5 mL polyclonal goat antimouse CXCR3 antibody intraperitoneally every other day, beginning on postoperative day 0 ($n = 5$). In control groups, the recipient mice received 0.5 mL goat serum intraperitoneally using a similar protocol ($n = 5$). No immunosuppression was given. Function of the allografts was assessed by abdominal palpation and confirmed by laporatomy. Allografts were harvested on cessation of contraction or at 24 days.

Morphometric Analyses

The explanted hearts underwent serial sectioning (5-μm thick) from the midventricular level to the base. Verhoeff elastic staining was performed for morphometric analyses of arterial intimal lesions. Three cross sections of each mouse heart were evaluated. The number of analyzed vessels per heart was 9 ± 2. Luminal and intimal and luminal areas (I + L) areas were traced, and the areas quantitated with Optimas software (Optimas 6.0, Media Cybernetics, Silver Spring, Md). Intimal thickening was calculated according to the formula (I/I + L) and expressed as a percentage.

Graft-Infiltrating Cell Isolation and FACS Analysis

Hearts were digested in collagenases-D solution. Isolated cells were counted after lysis of erythrocytes. Surface labeling of cells was performed by FITC- and PE-labeled CD4 and CD8 antibodies (BD PharMingen, San Diego, Calif). Rabbit antimouse CXCR3 labeling was followed with FITC-labeled goat antirabbit secondary Ab (Zymed, San Francisco, Calif). FACS analysis of labeled cells was conducted on an EPICS XL-MCL flow cytometer (Coulter, Miami, Fla).

Statistical Analyses

All results were expressed as mean ± SEM. Data were analyzed with a paired Student's t test.

RESULTS

Control mice receiving a full MHC mismatched allograft and treated with goat serum alone rejected their cardiac allografts at 6 ± 1 days, while all experimental mice had surviving allografts at 24 days posttransplantation. As expected, histologic examination of the control allografts revealed marked infiltration with mononuclear cells, myocyte necrosis, and swelling characteristic of acute cellular rejection. In contrast, examination of donor hearts in the experimental group revealed intact myocardial architecture, with minimal mononuclear cell infiltration (Fig 1).

Work by our group and others has previously demonstrated that surviving allografts are characterized by a decrease in the number of graft infiltrating mononuclear cells. Analysis of graft infiltrating cells was performed by flow cytometry. As expected, acutely rejecting grafts were infiltrated with large number of CD4 and CD8 lymphocytes. There was a reduction in the number of CD4 and CD8

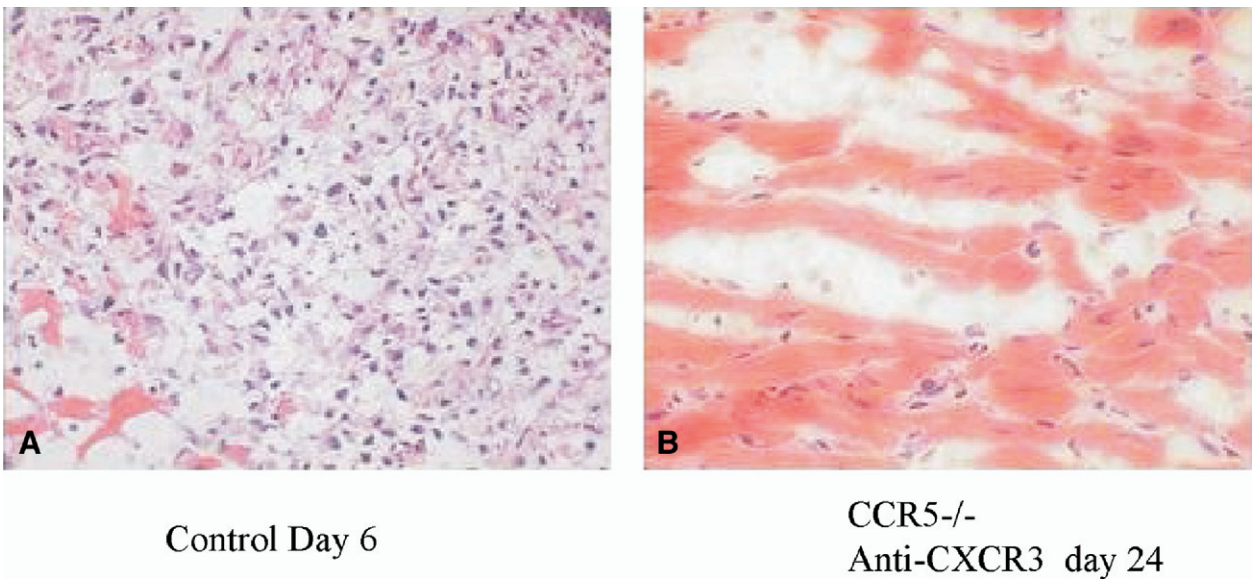

Fig 1. **(A)** Histologic analyses of control allografts revealed intense infiltration of myocardium with mononuclear cells and myocyte necrosis. **(B)** Examination of the donor hearts in experimental groups demonstrated intact myocardial architecture with minimal mononuclear cell infiltration.

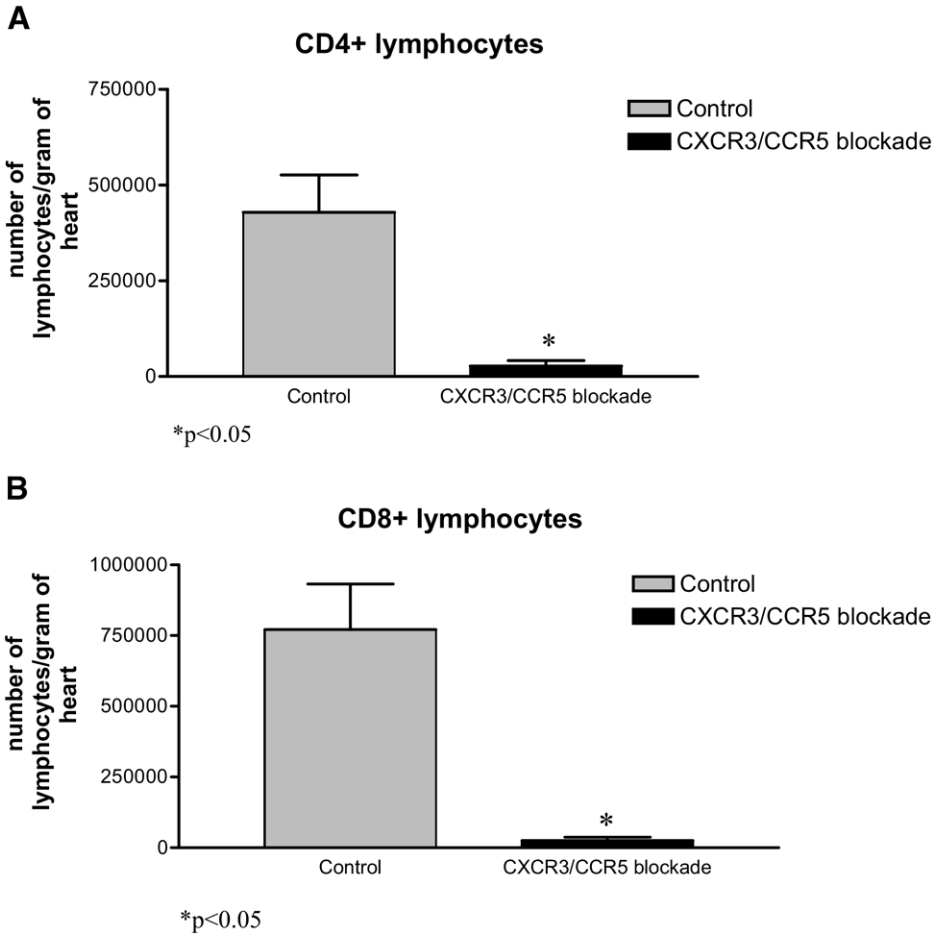

Fig 2. The acutely rejecting donor hearts in the control group were infiltrated with a large number of **(A)** CD4 and **(B)** CD8 lymphocytes vs experimental group at 24 days.

lymphocytes in the experimental group (combined CXCR3/CCR5 blockade group) at 24 days (Fig 2).

DISCUSSION

This study demonstrated that CCR5 and CXCR3 combined chemokine blockade is effective in prolonging allograft survival and limiting acute rejection. The mechanisms of this finding appear to be, in part, a decrease in T-lymphocyte mediated injury.

A large body of work has accumulated evaluating the importance of CXCR3, CCR5, and their respective chemokine ligands in the development of acute and chronic rejection in human transplantation. Increased numbers of both CXCR3 and CCR5+ cells are found in acutely rejecting human renal allografts.[10] Elevated levels of the CXCR3 ligand ITAC are present in the serum of heart transplant recipients with chronic rejection, and CXCR3+ mononuclear cells are seen within the vascular lesions.[11] In experimental studies, the blockade of individual chemokine receptors has been shown to prolong allograft survival modestly; yet all of the allografts were eventually lost to rejection.[6,7,12] These studies also demonstrated that CXCR3 plays a more important role in mediating experimental acute rejection than CCR5. The findings of the current study demonstrated that the combined blockade of the two chemokine receptors previously implicated in the development of acute rejection prolongs graft survival by at least fourfold.

Given the potent chemotactic effects of CXCR3 and CCR5 on activated T-lymphocytes, it is not surprising that the dual chemokine receptor blockade strategy decreased the number of graft infiltrating CD4 and CD8 lymphocytes and consequently resulted in attenuation of graft injury. In addition to chemokines' role in leukocyte trafficking, recent data have shown that chemokines play other roles in modulating leukocyte function such as proliferation and effector cytokine production.[13,14] The contribution of the extrachemotactic properties of chemokine receptor blockade in this model is unknown.

In summary, the findings of this study demonstrated that blockade of CXCR3 and CCR5 in a murine heart transplant model decreases the infiltration of CD4 and CD8 lymphocytes into the graft and leads to prolongation of allograft survival. Despite the known redundancy in the chemokine/chemokine receptor family, the strategy of com-

bining critical receptor blockade holds promise in controlling alloimmune responses in organ transplantation.

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