

# CCR5 Signaling Suppresses Inflammation and Reduces Adverse Remodeling of the Infarcted Heart, Mediating Recruitment of Regulatory T Cells

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**Myocardial infarction triggers an inflammatory reaction that is involved in cardiac remodeling. Cardiac repair is dependent on regulatory mechanisms that suppress inflammation and prevent excessive matrix degradation. Chemokine induction in the infarcted heart mediates recruitment of leukocyte subsets with distinct properties. We demonstrate that signaling through the CC chemokine receptor 5 (CCR5) prevents uncontrolled postinfarction inflammation and protects from adverse remodeling by recruiting suppressive mononuclear cells. CCR5 and its ligands macrophage inflammatory protein (MIP)-1 $\alpha$  and MIP-1 $\beta$  were markedly induced in the infarcted mouse myocardium. In addition, almost 40% of the mononuclear cells infiltrating the infarct expressed CCR5. CCR5<sup>-/-</sup> mice exhibited marked upregulation of proinflammatory cytokine and chemokine expression in the infarct. In wild-type infarcts CCR5<sup>+</sup> mononuclear cells had anti-inflammatory properties, expressing higher levels of IL-10 than CCR5<sup>-</sup> cells. In contrast, mononuclear cells isolated from CCR5<sup>-/-</sup> infarcts had reduced IL-10 expression. Moreover, enhanced inflammation in the absence of CCR5 was associated with impaired recruitment of CD4<sup>+</sup>/foxp3<sup>+</sup> regulatory T cells (Tregs). The CCR5<sup>+</sup> Treg subset exhibited increased IL-10 expression, reflecting potent anti-inflammatory activity. Accentuated inflammation in CCR5<sup>-/-</sup> infarcts was associated with increased matrix metalloproteinase (MMP) expression, reduced TIMP levels, and enhanced MMP-2 and MMP-9 activity, resulting in worse cardiac dilation. These results suggest that CCR5-mediated Treg recruitment may restrain postinfarction inflammation, preventing excessive matrix degradation and attenuating**

**adverse remodeling.** (*Am J Pathol* 2010, 176:2177–2187; DOI: 10.2353/ajpath.2010.090759)

Leukocyte trafficking in sites of inflammation is governed by chemokines. Most chemokines bind to extracellular matrix molecules or are immobilized in the luminal endothelial cell surface,<sup>1</sup> which mediates inflammatory cell transmigration through ligation of G protein-coupled chemokine receptors expressed on leukocytes. Differential expression of chemokine receptors by monocyte and lymphocyte subsets determines their homing in inflamed tissues<sup>2,3</sup> resulting in infiltration with subpopulations with distinct functional properties. Emerging evidence suggests important roles for various mononuclear cell subsets, recruited through distinct chemokine-mediated pathways in tissue inflammation and repair.<sup>4,5</sup>

Healing of myocardial infarction is dependent on a chemokine-driven inflammatory response that ultimately results in replacement of dead cardiomyocytes with collagen-based scar.<sup>6</sup> Infiltration of the infarcted myocardium with leukocytes is associated with activation of proteases and extensive degradation of the cardiac extracellular matrix, resulting in chamber dilation and adverse ventricular remodeling. Dilative remodeling is a poor prognostic indicator in patients with myocardial infarction and is associated with ventricular arrhythmias, the development of heart failure, and increased mortality. Chemokine signaling through the receptors CCR1 and CCR2 appears to promote injury and chamber dilation after myocardial infarction.<sup>7</sup> Disruption of the CCL2/CCR2 axis, or CCR1 deficiency, results in reduced inflammation and attenuated protease activity, protecting the infarcted heart from the development of adverse re-

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modeling.<sup>8–10</sup> The effects of CCR2 signaling are mediated through the selective recruitment of the Ly-6Chi subset of monocytes that exhibit enhanced phagocytic, proteolytic, and proinflammatory properties.<sup>5</sup>

We have previously demonstrated that CCR5 and its ligands macrophage inflammatory protein (MIP)–1 $\alpha$ /CCL3 and MIP-1 $\beta$ /CCL4 are markedly induced in murine myocardial infarcts.<sup>9,11</sup> Because CCR5 signaling may be responsible for recruitment of distinct mononuclear cell subsets in the infarcted heart, we examined the effects of CCR5 deficiency on infarct healing and cardiac remodeling. Surprisingly, in contrast to CCR1 and CCR2 mutants, CCR5-null mice exhibited enhanced inflammation, accentuated matrix metalloproteinase (MMP) expression, and increased dilative remodeling after reperfused myocardial infarction. Defective control of the postinfarction inflammatory response in CCR5<sup>-/-</sup> animals was associated with impaired recruitment of CCR5<sup>+</sup> foxp3<sup>+</sup> regulatory T cells (Tregs), a CD4<sup>+</sup> lymphocyte subset with potent anti-inflammatory properties. Our findings suggest that chemokine signaling through CCR5 prevents uncontrolled inflammation in the infarcted myocardium attenuating matrix degradation and adverse cardiac remodeling.

## Materials and Methods

### Murine Model of Reperfused Myocardial Infarction

CCR5-null mice (KO, B6;129P2-Ccr5<sup>tm1Kuz</sup>/J) and their corresponding wild-type B6129PF2/J littermates, purchased from Jackson Laboratories, were used for reperfused myocardial infarction experiments. Female and male mice, 2 to 4 months of age (18.0 to 22.0 g body weight), were anesthetized by an intraperitoneal injection of sodium pentobarbital (60  $\mu$ g/g). A closed-chest mouse model of reperfused myocardial infarction was used as previously described,<sup>11</sup> to avoid the confounding effects of surgical trauma and inflammation, which may influence the baseline levels of chemokines and cytokines. The left anterior descending coronary artery was occluded for 1 hour then reperfused for 6 hours to 7 days. At the end of the experiment, the chest was opened and the heart was immediately excised, fixed in zinc-formalin, and embedded in paraffin for histological studies, or snap frozen and stored at  $-80^{\circ}\text{C}$  for RNA isolation. Animals used for histology underwent 24-hour and 72-hour reperfusion protocols (nine animals per group). Mice used for RNA extraction underwent six hours (wild-type,  $n = 8$ ; KO,  $n = 8$ ), or 24 hours of reperfusion (wild-type,  $n = 9$ ; KO,  $n = 10$ ), whereas mice used for assessment of MMP activity were killed after 72 hours of reperfusion (wild-type,  $n = 8$ ; KO,  $n = 8$ ). CCR5 KO and wild-type mice used for flow cytometric analysis of the mononuclear cell infiltrate ( $n = 6$  per group) underwent 24 hours of reperfusion. Additional animals were used for perfusion-fixation after 7 days of reperfusion ( $n = 12$  per group) to assess remodeling-associated parameters.

### Immunohistochemistry and Quantitative Histology

Murine hearts were fixed in zinc-formalin (Z-fix; Anatech, Battle Creek, MI), and embedded in paraffin. The hearts were sectioned from base to apex at 200- $\mu$ m intervals. Ten serial sections were cut at each interval; to identify the area of infarction the first section from each interval was stained for hematoxylin/eosin. To assess infiltration with inflammatory leukocytes, sections from the infarcted heart were stained immunohistochemically with a rat anti-neutrophil antibody (Serotec, Raleigh NC) and with the rat anti-mouse macrophage antibody Mac-2 (Cedarlane) as previously described.<sup>11,12</sup> Staining was performed using a peroxidase-based technique with the Vectastain ELITE rat, or rabbit kit (Vector Labs, Burlingame, CA) and developed with diaminobenzidine + nickel (Vector). Stained sections were scanned using a Zeiss Axioskop microscope equipped with a Zeiss digital camera. Three sections from each heart and five fields from each section were used for quantitative analysis. Assessment of neutrophil and macrophage density was performed by counting the number of neutrophils and of Mac-2-immunoreactive cells respectively in the infarcted area using ImagePro image analysis software.<sup>9</sup> Neutrophil and macrophage density was expressed as cells/mm<sup>2</sup>.

### Echocardiography

Echocardiographic studies were performed before instrumentation and after 7 days of reperfusion (wild-type,  $n = 12$ ; CCR5<sup>-/-</sup>,  $n = 13$ ) using an 8-MHz probe (Sequoia C256; Acuson, Mountain View, CA). Short-axis M-mode was used for measurement of systolic and diastolic ventricular and anterior wall diameters. The left ventricular end-diastolic diameter (LVEDD) was measured as an indicator of remodeling and the fractional shortening (FS = [LVEDD - LVESD]  $\times$  100/LVEDD) was calculated for assessment of systolic ventricular function as previously described.<sup>13</sup>

### Perfusion Fixation and Assessment of Ventricular Volumes

For assessment of postinfarction remodeling, infarcted hearts after 7 days of reperfusion were used for perfusion-fixation ( $n = 12$  per group) as previously described.<sup>9</sup> Briefly, a cardioplegic solution was perfused through the jugular vein to promote relaxation. After excision and rinsing in cold cardioplegic solution, the aorta was cannulated, a PE-50 catheter was pushed into the left ventricle and secured in place. Hearts were fixed for 10 minutes with 10% zinc-buffered formalin by aortic perfusion. After paraffin embedding, the entire heart was cross-sectioned from base to apex at 250- $\mu$  intervals. Ten serial 5- $\mu$  sections were obtained at each interval. The left ventricular end-diastolic volume (LVEDV) and the left ventricular mass were assessed with ImagePro software using methods developed in our laboratory.<sup>9</sup> The size of the infarct was expressed as a percentage of the left ventricular volume.

### RNA Extraction and Ribonuclease Protection Assay

RNA was extracted from whole hearts using established protocols.<sup>13</sup> Inflammatory gene expression in murine hearts was assessed using ribonuclease protection assay as previously described.<sup>11</sup> The mRNA expression level of MMP-2, -3, -8, -9, and TIMP-1, the chemokines MIP-1 $\alpha$ , MIP-1 $\beta$ , MIP-2, monocyte chemoattractant protein (MCP)-1, and IP-10, and the cytokines TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, TGF- $\beta$ 1 was determined using a ribonuclease protection assay (RiboQuant; Pharmingen) according to the manufacturer's protocol. Phosphorimaging of the gels was performed (Storm 860; Molecular Dynamics, Sunnyvale, CA) and signals were quantified using Image QuANT software and normalized to the ribosomal protein L32 mRNA.

### Real-Time PCR

To assess *foxp3* mRNA expression in the heart, real-time PCR was performed. Primers were synthesized in the Baylor College of Medicine Child Health Research Center core facility. The sequence for the *foxp3* was: forward 5'-CACCTATGCCACCTTATCC-3' and reverse 3'-CGAATCATGCGAGTAAACCAA-5'. The housekeeping gene cyclophilin was used as a control (forward, 5'-GCGTCTCCTTCGAGCTGT-3'; reverse, 3'-GGAACCCTTATAGCAAATCC-5'). Isolated total RNA from the heart was reverse transcribed to cDNA using the iScript cDNA synthesis kit (Bio-Rad) following the manufacturer's guidelines. Quantitative PCR was performed using the SYBR green (Bio-Rad) method on the iQ5 Real-Time PCR Detection System (Bio-Rad) for 40 cycles at an annealing temperature of 53.9°C. Each sample was run in triplicate. The  $\Delta C_T$  method using cyclophilin as the reference gene was used for relative quantification of *foxp3* expression.

### Assessment of Gelatinolytic Activity in the Infarcted Myocardium

MMP activity in infarcted myocardium was examined by gelatin zymography. The samples of infarcted myocardium were homogenized in 300  $\mu$ l of an ice-cold extraction buffer containing cacodylic acid (10 mmol/L), NaCl (150 mmol/L), ZnCl<sub>2</sub> (20 mmol/L), NaN<sub>3</sub> (1.5 mmol/L), and 0.01% Triton X-100 (pH 5.0). Subsequently, the homogenate was centrifuged (4°C, 10 minutes, 10,000g), and the supernatant decanted and saved on ice. The protein concentration in tissue extracts was measured using BCA Protein Assay Kit (Thermo Scientific). The myocardial extracts at final protein concentration of 20  $\mu$ g were mixed in a ratio 1:2 with zymogram sample buffer (Bio Rad) and loaded onto 10% polyacrylamide electrophoretic precast gels (Bio Rad) containing 1 mg/ml of gelatin under nonreducing conditions. The gels were run at 50 V/gel through stacking phase and 100 V/gel for the separating phase, maintaining a running buffer temperature of 4°C. Subsequently, the gels were renatured in 2.5% Triton X-100 for 30 minutes, rinsed in water, and incubated for 48 hours in Zymogram Development Buffer

(Bio Rad) at 37°C. After incubation the gels were stained with Coomassie brilliant blue R-250 (Bio Rad) and subsequently destained until clear bands appeared against blue background. Digital images were scanned and the optical density of the bands was measured using ImageJ software.

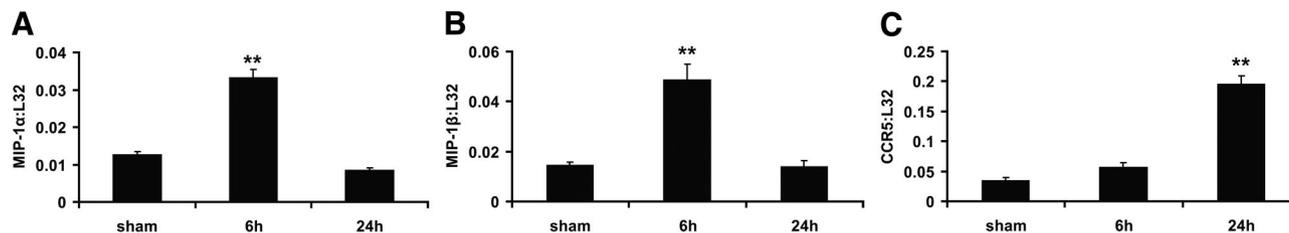
### Mononuclear Cell Isolation

Mononuclear cells were isolated from infarcted wild-type ( $n = 6$ ) and KO ( $n = 6$ ) hearts after 24 hours of reperfusion. Briefly, hearts were rapidly excised and placed in ice-cold Krebs-Henseleit (KH) buffer containing (in g/L) 2 glucose, 0.141 MgSO<sub>4</sub>, 0.16 NaH<sub>2</sub>PO<sub>4</sub>, 0.35 KCl, 6.9 NaCl, 2.1 NaHCO<sub>3</sub>, 0.373 CaCl<sub>2</sub>, 1 NaN<sub>3</sub> at pH 7.4. Subsequently, the aorta was cannulated with a 22-gauge tubing adaptor and flushed with ice-cold KH buffer to remove residual cells in the coronary vasculature. Two hearts per experiment were minced with fine scissors and placed into a cocktail of 0.25 mg/ml Liberase Blendzyme 3 (Roche Applied Science), 20 U/ml DNase I (Sigma Aldrich), 10 mmol/L HEPES (Invitrogen), 0.1% Sodium Azide in HBSS with Ca<sup>2+</sup> and Mg<sup>2+</sup> (Invitrogen) and shaken at 37°C for 40 minutes. Cells were then passed through a 40- $\mu$ m nylon mesh (BD Falcon), centrifuged (10 minutes, 500g, 4°C), and resuspended in 0.1% sodium azide solution in HBSS without Ca<sup>2+</sup> and Mg<sup>2+</sup>. Consecutively, two volumes of the cell suspension were layered on top of one volume of 15% w/v iodixanol Opti-Prep (Axis-Shield) at density of 1.084 g/ml and centrifuged (20 minutes, 750g, 20°C). Mononuclear cells were harvested from interphase, diluted with HBSS with 0.1% sodium azide (1:3 v/v), and centrifuged (10 minutes, 500g, 4°C) to remove iodixanol. Finally, cells were reconstituted with staining buffer (dPBS without Ca<sup>2+</sup> and Mg<sup>2+</sup>, 2% FBS, 0.1% sodium azide) and counted using Trypan blue (Sigma Aldrich). The mean and SEM for live cell recoveries were  $2.15 \pm 0.3 \times 10^6$ /heart versus  $2.2 \pm 0.5 \times 10^6$ /heart in wild-type and KO infarcts respectively (pNS).

In additional experiments, spleens from control wild-type mice were removed, pressed through stainless sieve, and filtered using 40- $\mu$ m nylon mesh (BD Falcon). The cell suspension was centrifuged at 500g for 10 minutes at 4°C and resuspended with HBSS without Ca<sup>2+</sup> and Mg<sup>2+</sup> with 0.1% sodium azide. Splenic mononuclear cells were purified using density barrier according to protocol described above. Finally, cells were reconstituted with staining buffer (dPBS without Ca<sup>2+</sup> and Mg<sup>2+</sup>, 2% FBS, 0.1% sodium azide) and counted using Trypan blue (Sigma Aldrich).

### Flow Cytometry

$5 \times 10^5$  to  $10^6$  cells were incubated with either anti-Fc $\gamma$ III/II (clone 2.4G2) antibody (BD Pharmingen) or purified mouse IgG (Sigma Aldrich) for 5 minutes and labeled at 4°C for 30 minutes simultaneously with two of the following antibodies purchased from BD Pharmingen: FITC-labeled anti-CD45 (clone 30-F11), anti-CD14 (rmC5-3); PE-labeled anti-CD3 (clone 145-2C11), anti-CD195/CCR5 (clone C34-3448); PE-Cy5 labeled anti-CD45 (clone 30-F11), anti-CD4 (clone



**Figure 1.** Induction of the CC chemokines MIP-1 $\alpha$  and MIP-1 $\beta$  and their receptor CCR5 in reperused mouse infarcts. MIP-1 $\alpha$  (A) and MIP-1 $\beta$  mRNA (B) expression peaked after 6 hours of reperfusion and was followed by CCR5 upregulation (C) after 24 hours of reperfusion (\* $P < 0.05$ , \*\* $P < 0.01$  versus sham). Statistical analysis was performed using eight animals per group.

H129.19). For intracellular staining, cells were fixed and permeabilized for 45 minutes at 4°C with fixation/permeabilization kit (eBioscience). Subsequently, cells were incubated with one or two colors using the following antibodies from eBioscience: FITC-labeled anti-foxp3 (clone FJk-16s), anti-IL-1 $\beta$  (polyclonal); PerCP-Cy5.5 labeled anti-IL-10 (Clone JES5-16ES). Finally, cells were washed twice, re-suspended in staining buffer, and immediately analyzed with a Cell Lab Quanta SC flow cytometer (Beckman Coulter). Data analysis was performed using FlowJo (Tree Star, Inc.).

### Statistical Analysis

Statistical analysis was performed using analysis of variance followed by *t* test corrected for multiple comparisons (Student–Newman–Keuls). Paired *t* test was used to compare echocardiographic parameters before myocardial infarction and after 7 days of reperfusion. Mortality was compared using the log rank test. Data were expressed as mean  $\pm$  SEM. Statistical significance was set at 0.05.

## Results

### Induction of the Chemokine Receptor CCR5 and its Ligands MIP-1 $\alpha$ and MIP-1 $\beta$ in Reperused Mouse Infarcts

Using a ribonuclease protection assay we found that the CCR5 ligands MIP-1 $\alpha$  and MIP-1 $\beta$  were markedly induced in the infarcted myocardium after 6 hours of reperfusion (Figure 1, A and B). In contrast, regulated on activation, normal T cell expressed and secreted (RANTES)/CCL5 remained below the level of detection of the assay (<0.5% of the housekeeping gene L32). MIP-1 $\alpha$  and MIP-1 $\beta$  upregulation was followed by marked induction of their receptor CCR5 after 24 hours of reperfusion (Figure 1C).

### CCR5-Null and Wild-Type Animals Exhibited Comparable Mortality after Myocardial Infarction

Mortality during the initial instrumentation surgery was comparable between wild-type and CCR5 KO mice (wild-type 10.2% versus KO 8.8%, pNS). Mortality during coronary occlusion was not significantly different between wild-type and CCR5 KO mice (mortality during coronary

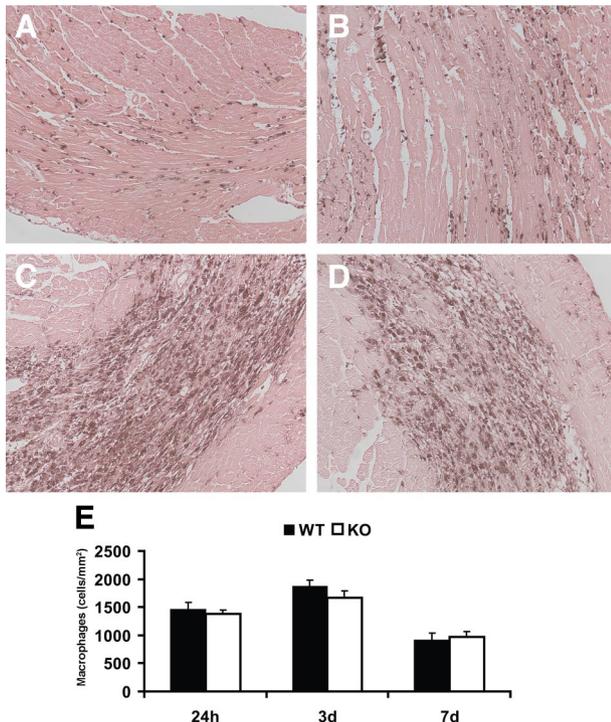
occlusion, wild-type 5.66% versus KO 10.84%, pNS). Only one wild-type mouse, and no KO mice, died during reperfusion (mortality during reperfusion, wild-type 1.1% versus KO 0).

### CCR5-Null and Wild-Type Mouse Infarcts Exhibited Comparable Infiltration with Neutrophils and Macrophages

Leukocytes rapidly infiltrate the infarcted myocardium. Neutrophil density peaks after 24 hours of reperfusion,<sup>11</sup> whereas macrophage density remains high after 3 to 7 days of reperfusion. The absence of CCR5 did not affect recruitment of inflammatory leukocytes in the infarcted heart. Peak neutrophil density was comparable in CCR5<sup>-/-</sup> and wild-type infarcts after 24 hours of reperfusion (KO 853 + 119.2 cells/mm<sup>2</sup> versus wild-type 921 + 41.9 cells/mm<sup>2</sup>, pNS). In addition, CCR5-null and wild-type animals had comparable macrophage density in the infarcted heart at all timepoints examined (Figure 2, A–E).

### CCR5-Null Mice Had Markedly Enhanced Inflammatory Cytokine and Chemokine Expression in the Infarcted Heart

Reperused murine myocardial infarction induces intense upregulation of cytokines and chemokines that peaks after 3 to 6 hours of reperfusion.<sup>11</sup> CCR5<sup>-/-</sup> infarcts exhibited markedly increased peak mRNA expression of the proinflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 after 6 hours of reperfusion (Figure 3A). CCR5<sup>-/-</sup> mice also demonstrated higher expression of the inhibitory cytokines IL-10 and TGF- $\beta$ 1 in the infarcted heart when compared with wild-type animals (Figure 3B). However, the ratio of expression of IL-1 $\beta$  to IL-10 and TGF- $\beta$ 1 was significantly higher in CCR5-null infarcts in comparison with wild-type animals (Figure 3C), indicating that CCR5 absence resulted in a shift toward enhanced proinflammatory cytokine production. CCR5-null infarcts exhibited increased peak expression of the CC chemokines MIP-1 $\alpha$  and MIP-1 $\beta$  and of the CXC chemokines MIP-2/CXCL2 and interferon- $\gamma$ -inducible protein (IP)-10/CXCL10 after 6 hours of reperfusion (Figure 3D). In contrast, wild-type and CCR5-null infarcts had comparable MCP-1/CCL2 mRNA expression levels in the infarcted heart (Figure 3E).



**Figure 2.** CCR5-null and wild-type (WT) infarcts exhibit comparable infiltration with macrophages. **A–D:** Mac2 immunohistochemistry identified macrophages in wild-type (**A, C**) and CCR5 KO infarcts (**B, D**) after 24 hours (**A, B**) and 72 hours of reperfusion (**C, D**). **E:** Quantitative analysis showed no significant differences in macrophage density between CCR5<sup>-/-</sup> and wild-type infarcts at all time points examined ( $n = 9$  mice/group).

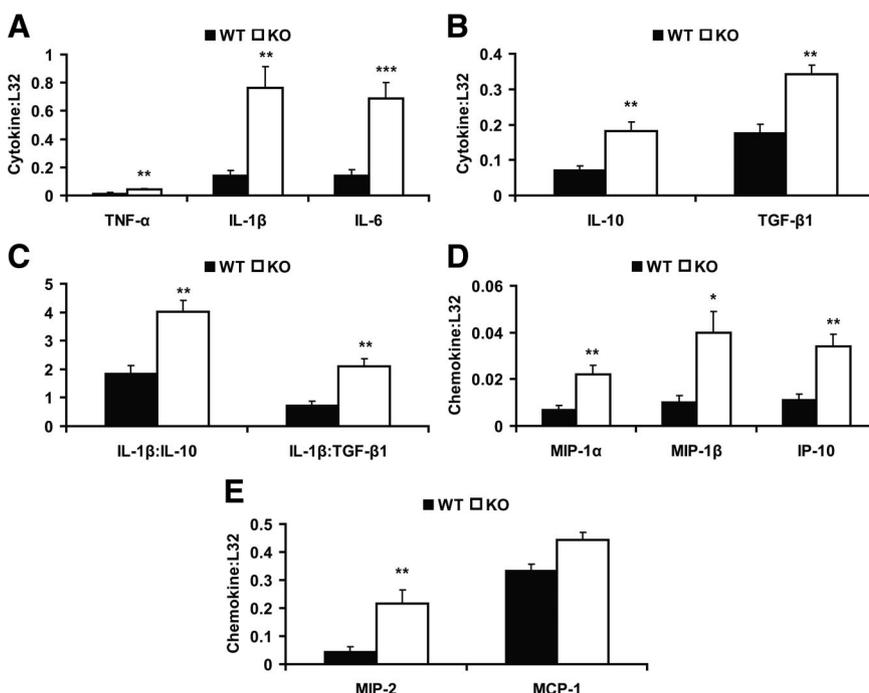
### Identification of CCR5<sup>+</sup> Mononuclear Cells in the Infarcted Heart

The striking augmentation of inflammatory cytokine synthesis in CCR5-null infarcts, in the absence of significant dif-

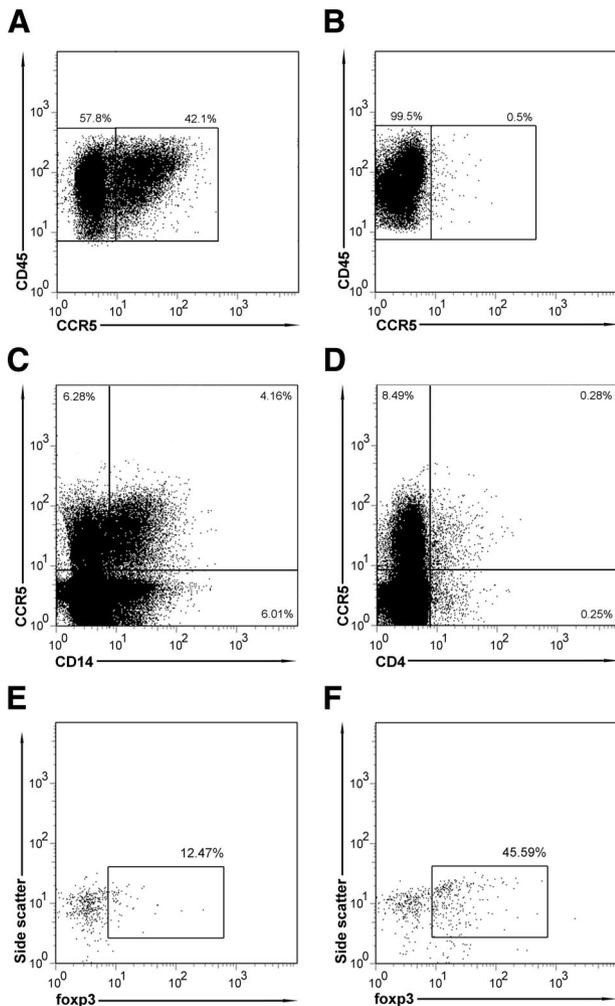
ferences in macrophage and neutrophil density, suggested that CCR5 deficiency may result in selectively impaired recruitment of mononuclear cell subsets with anti-inflammatory properties. To test this hypothesis we isolated mononuclear cells from infarcted mouse hearts after 24 hours of reperfusion and used the cells for flow cytometry. Flow cytometric analysis demonstrated that CCR5<sup>-/-</sup> and wild-type infarcts had comparable numbers of hematopoietic CD45<sup>+</sup> cells (wild-type 38.11% ± 2.00 versus KO 42.13% ± 1.07 of total events, pNS). In wild-type infarcts, 38.3% ± 3.75 of the CD45<sup>+</sup> hematopoietic cells expressed CCR5; in contrast, as expected, CCR5-null infarcts had no CCR5-positive cells (Figure 4, A and B). In wild-type infarcts a significant number of the CCR5<sup>+</sup> cells (38.25% ± 3.3) were identified as CD14<sup>+</sup> monocytes, whereas 3.73 ± 0.37% were CD4<sup>+</sup> T cells (Figure 4, C and D). A large percentage of the CD4<sup>+</sup> CCR5<sup>+</sup> cells (45.59% ± 4.38) expressed the Treg-specific marker foxp3. In contrast, only 12.47% ± 1.45 of the CCR5-negative CD4<sup>+</sup> cells were foxp3 + Tregs (Figure 4, E and F).

### The CCR5-Positive Subpopulation of Mononuclear Cells Showed Increased Expression of the Inhibitory Cytokine IL-10

To dissect the mechanisms responsible for enhanced inflammation in CCR5-null mice we tested the hypothesis that CCR5<sup>+</sup> mononuclear cells exhibit anti-inflammatory properties. Expression of the proinflammatory cytokine IL-1β and the inhibitory cytokine IL-10 was assessed in mononuclear cells isolated from wild-type infarcts using flow cytometry. The CCR5<sup>+</sup> subpopulation of infarct mononuclear cells exhibited increased expression of the inhibitory cytokine IL-10 (22.48% ± 1.5 of CCR5<sup>+</sup> cells expressed IL-10 versus 10.34% ± 1.54 of the CCR5<sup>-</sup> cells,  $P < 0.05$ ; Figure 5, A–E).



**Figure 3.** CCR5 deficiency is associated with markedly increased expression of inflammatory cytokines and chemokines in the infarcted myocardium. **A:** Ribonuclease protection assay analysis demonstrated that CCR5-null mice show significantly higher peak mRNA expression of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 (\*\* $P < 0.01$ , \*\*\* $P < 0.001$  versus wild-type [WT]). **B:** Expression of the inhibitory cytokines IL-10 and TGF-β1 was also higher in CCR5-null infarcts when compared with wild-type animals (\*\* $P < 0.01$  versus wild-type). **C:** The IL-1β:IL-10 and IL-1β:TGF-β1 expression ratio was significantly higher in CCR5-null infarcts (\*\* $P < 0.01$  versus wild-type) indicating that CCR5 absence shifted the balance of cytokine expression toward increased proinflammatory activity. **D:** Expression of the chemokines MIP-1α, MIP-1β, and IP-10 was also significantly higher in CCR5-null infarcts (\* $P < 0.05$ , \*\* $P < 0.01$  versus wild-type) after 6 hours of reperfusion. **E:** MIP-2 expression was markedly higher in CCR5<sup>-/-</sup> infarcts; however, MCP-1 levels were comparable between groups ( $n = 8$  mice/group).

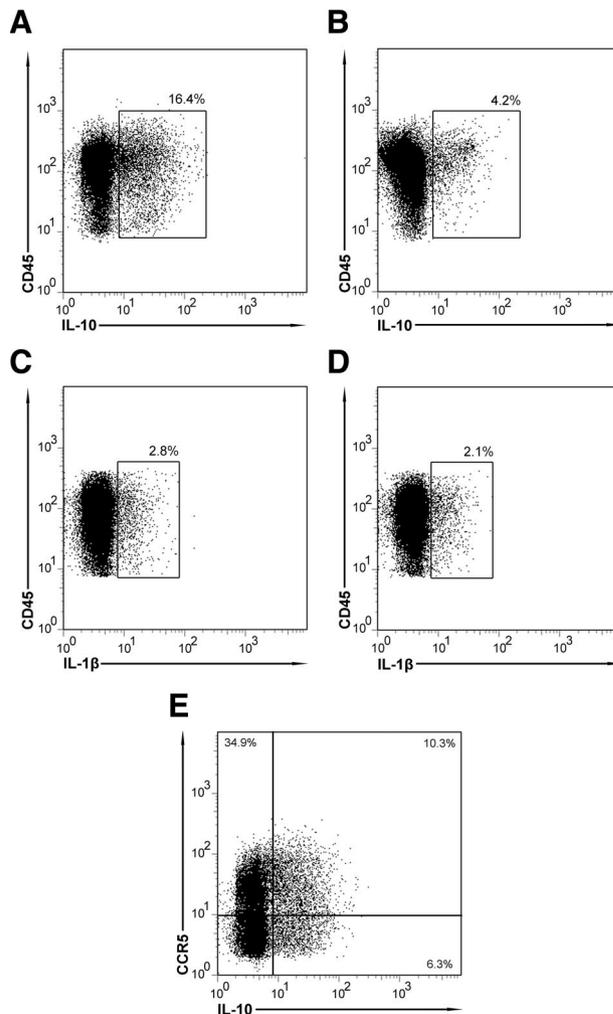


**Figure 4.** The infarcted myocardium mobilizes CCR5<sup>+</sup> mononuclear cells. **A:** Flow cytometry analysis of the infarct infiltrate revealed that a significant percentage of mononuclear cells infiltrating the infarcted heart after 24 hours of reperfusion are CCR5<sup>+</sup> (38.3%±3.7 of the CD45<sup>+</sup> cells isolated from the infarct are CCR5<sup>+</sup>). **B:** As expected, CCR5 KO infarcts had no CCR5<sup>+</sup> cells. **C:** In wild-type mouse infarcts a large percentage of the CCR5<sup>+</sup> cells (38.25 ± 3.3) were identified as CD14<sup>+</sup> monocytes. **D:** A significant number (3.73% ± 0.37) of the CCR5<sup>+</sup> cells were CD4<sup>+</sup> T cells. **E–F:** CCR5 expression in CD4<sup>+</sup> T cells was associated with expression of the Treg-specific marker foxp3. The CD4<sup>+</sup>/CCR5<sup>+</sup> subset (**F**) had a higher percentage of foxp3<sup>+</sup> Tregs than the CD4<sup>+</sup>/CCR5<sup>-</sup> population (**E**) (percentage of foxp3<sup>+</sup> Tregs, CD4<sup>+</sup>/CCR5<sup>+</sup> subpopulation: 45.49 ± 4.38 versus CD4<sup>+</sup>/CCR5<sup>-</sup> subpopulation: 12.47% ± 1.45, *P* < 0.01). The dot plots and percentages reflect a representative experiment pooling 2 infarcted hearts. Statistical analysis was based on three independent experiments using 6 infarcted hearts.

IL-1β expression was found in a relatively low percentage of the mononuclear cells isolated from the infarct. There was a trend toward increased IL-1β expression in the CCR5<sup>+</sup> subset (6.56% ± 1.06 of CCR5<sup>+</sup> cells expressed IL-1β versus 1.82% ± 0.4 of the CCR5<sup>-</sup> cells; *P* = 0.08).

#### *In CCR5-Deficient Animals, Infarct Mononuclear Cells Exhibited Reduced IL-10 Expression*

Flow cytometry demonstrated that CD45<sup>+</sup> hematopoietic cells isolated from CCR5-null infarcts had significantly reduced IL-10 expression in comparison with cells from wild-type infarcts (percentage of IL-10–positive cells, wild-type

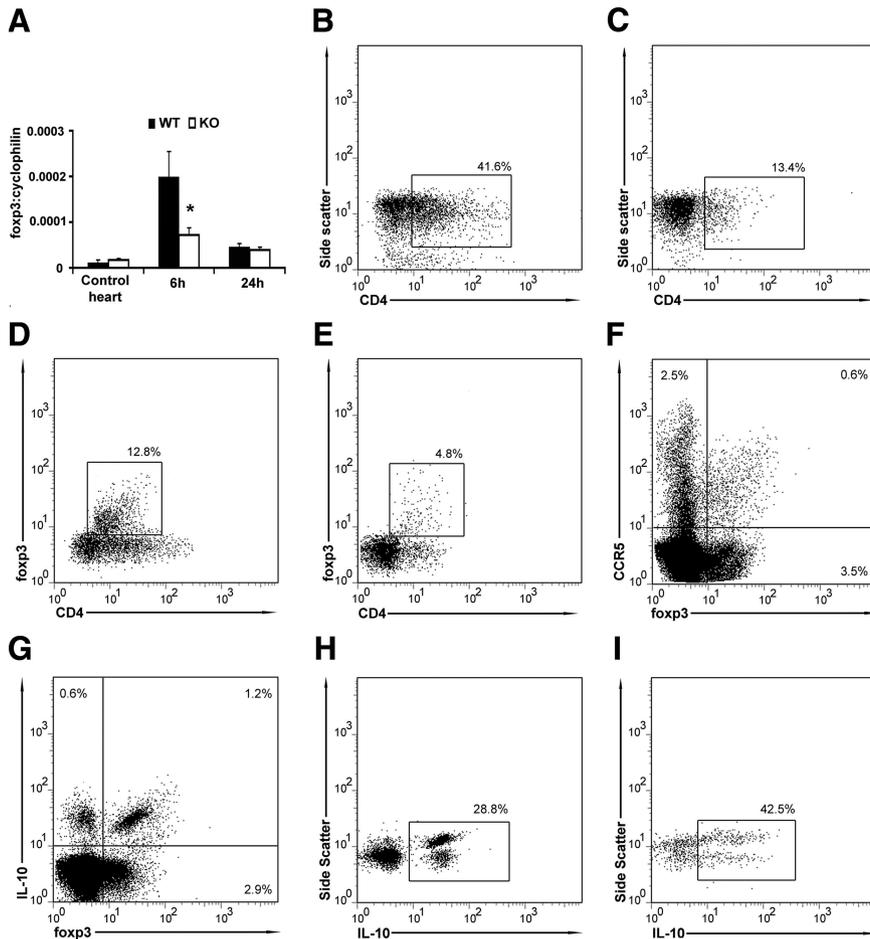


**Figure 5.** Mononuclear cells infiltrating CCR5<sup>-/-</sup> infarcts show decreased expression of IL-10. Representative flow cytometric analysis of CD45 and IL-10 expression in wild-type (**A**) and CCR5 KO (**B**) infarct mononuclear cells. Similarly, CD45 and IL-1β expression was assessed in mononuclear cell suspensions from wild-type (**C**) and CCR5 KO (**D**) infarcts. Cells isolated from CCR5 KO infarcts had reduced IL-10 expression when compared with cells from wild-type infarcts (percentage of IL-10<sup>+</sup> cells, wild-type 19.1% ± 2.08 versus KO 5.36% ± 1.12, *P* < 0.01). In contrast, the percentage of IL-1β–positive cells was comparable between groups. Representative percentages indicate the fractions of CD45<sup>+</sup> cells positive for IL-1β or IL-10. In the wild-type (**E**) infarcts CCR5<sup>+</sup> subset of infarct mononuclear cells exhibited increased expression of the inhibitory cytokine IL-10 in comparison with the CCR5<sup>-</sup> subpopulation (percentage of CCR5<sup>+</sup> cells expressing IL-10 22.48% ± 1.5 versus percentage of CCR5<sup>-</sup> cells expressing IL-10 10.34 ± 1.54, *P* < 0.05). Representative dot plots show expression of IL-10 in CCR5<sup>+</sup> and CCR5<sup>-</sup> cells gated for CD45. The dot plot and percentages reflect the representative experiment pooling two hearts. Statistical analysis was based on three independent experiments using six infarcted hearts.

19.1% ± 2.08 versus KO 5.36% ± 1.12, *P* < 0.01). In contrast, the percentage of IL-1β–positive cells was comparable between wild-type and CCR5-null infarcts (wild-type: 2.55 ± 0.34 versus KO: 2.4 ± 0.057, *p*NS; Figure 5).

#### *CCR5 Deficiency Was Associated with Markedly Reduced Infiltration of the Infarcted Myocardium with CD4<sup>+</sup> Regulatory T Cells*

Because emerging evidence suggests that CCR5 may play a role in trafficking of regulatory T cells,<sup>14,15</sup> we



**Figure 6.** CCR5 deficiency results in impaired recruitment of regulatory T cells (Tregs) to infarcted myocardium. **A:** Real-time PCR showed that CCR5-null infarcts had markedly reduced mRNA expression of foxp3, a specific marker of Tregs, in comparison with WT (wild-type) infarcts after 6 hours of reperfusion ( $*P < 0.05$  versus wild-type). **B** and **C:** Flow cytometric analysis of the CD4<sup>+</sup> subset as a percentage of CD3<sup>+</sup> T cells in wild-type (**B**) and CCR5 KO (**C**) infarcts. CCR5-null infarcts had a markedly lower number of CD4<sup>+</sup> T cells when compared with wild-type animals (wild-type 45%  $\pm$  3.3 versus KO 13.3%  $\pm$  1.2,  $P < 0.01$ ). **D** and **E:** Expression of CD4/foxp3 by CD3-gated mononuclear cells from wild-type (**D**) and CCR5 KO (**E**) infarcts. CCR5<sup>-/-</sup> mice had a significantly lower percentage of CD4<sup>+</sup> foxp3<sup>+</sup> Tregs in the infarcted heart than wild-type animals (foxp3<sup>+</sup> CD4<sup>+</sup> cells as a percentage of total CD3<sup>+</sup> cells, wild-type: 13.3%  $\pm$  1.1 versus KO: 7.2%  $\pm$  1.1,  $P < 0.05$ ). A representative experiment derived from isolation of mononuclear cells from two infarcted hearts for each group is shown. For statistical analysis three independent experiments (each one using pooled cells from two infarcted hearts) were performed. Flow cytometry analysis of CCR5/foxp3 (**F**) and IL-10/foxp3 (**G**) expression in wild-type splenocytes showed that foxp3<sup>+</sup> Tregs were the predominant IL-10-producing cells (IL-10 expression was found in 27.99%  $\pm$  3.63 of foxp3<sup>+</sup> T cells versus 0.61  $\pm$  0.32 of foxp3<sup>-</sup> cells,  $P < 0.01$ ). **H** and **I:** CCR5<sup>+</sup> Tregs had significantly higher IL-10 expression than CCR5<sup>-</sup> Tregs (IL-10-positive cells, CCR5<sup>+</sup>/foxp3<sup>+</sup> 42.73%  $\pm$  4.07 versus CCR5<sup>-</sup>/foxp3<sup>+</sup> 28.8  $\pm$  4.25,  $P < 0.05$ ) suggesting potent anti-inflammatory properties of the CCR5-expressing Treg subpopulation. A single representative dot plot derived from experiment using one spleen. Three independent experiments (three wild-type and three KO spleen) were used for statistical analysis.

examined whether enhanced inflammation in CCR5-null infarcts is associated with decreased recruitment of Tregs. Two distinct techniques suggested that CCR5-null mice have impaired recruitment of Tregs in the infarcted myocardium. qPCR demonstrated that expression of the Treg-specific transcript foxp3 was markedly upregulated in the infarcted myocardium after 6 hours of reperfusion, reflecting infiltration with regulatory T cells. Infarcted CCR5<sup>-/-</sup> hearts had significantly lower Foxp3 mRNA expression than wild-type infarcts (Figure 6A). In addition, flow cytometric analysis showed that CCR5-null infarcts had a markedly lower density of CD4<sup>+</sup> T cells in the mononuclear infiltrate when compared with wild-type infarcts (CD4<sup>+</sup> cells as a percentage of total CD3<sup>+</sup> T cells, wild-type: 45%  $\pm$  3.3 versus KO: 13.3%  $\pm$  1.2;  $P < 0.01$ ). CCR5 absence resulted in significantly reduced infiltration of the infarct with CD4<sup>+</sup>/foxp3<sup>+</sup> Tregs (foxp3<sup>+</sup> CD4<sup>+</sup> cells as a percentage of total CD3<sup>+</sup> T cells, wild-type 13.3  $\pm$  1.1 versus KO 7.2  $\pm$  1.1,  $P < 0.05$ ; Figure 6, B–I).

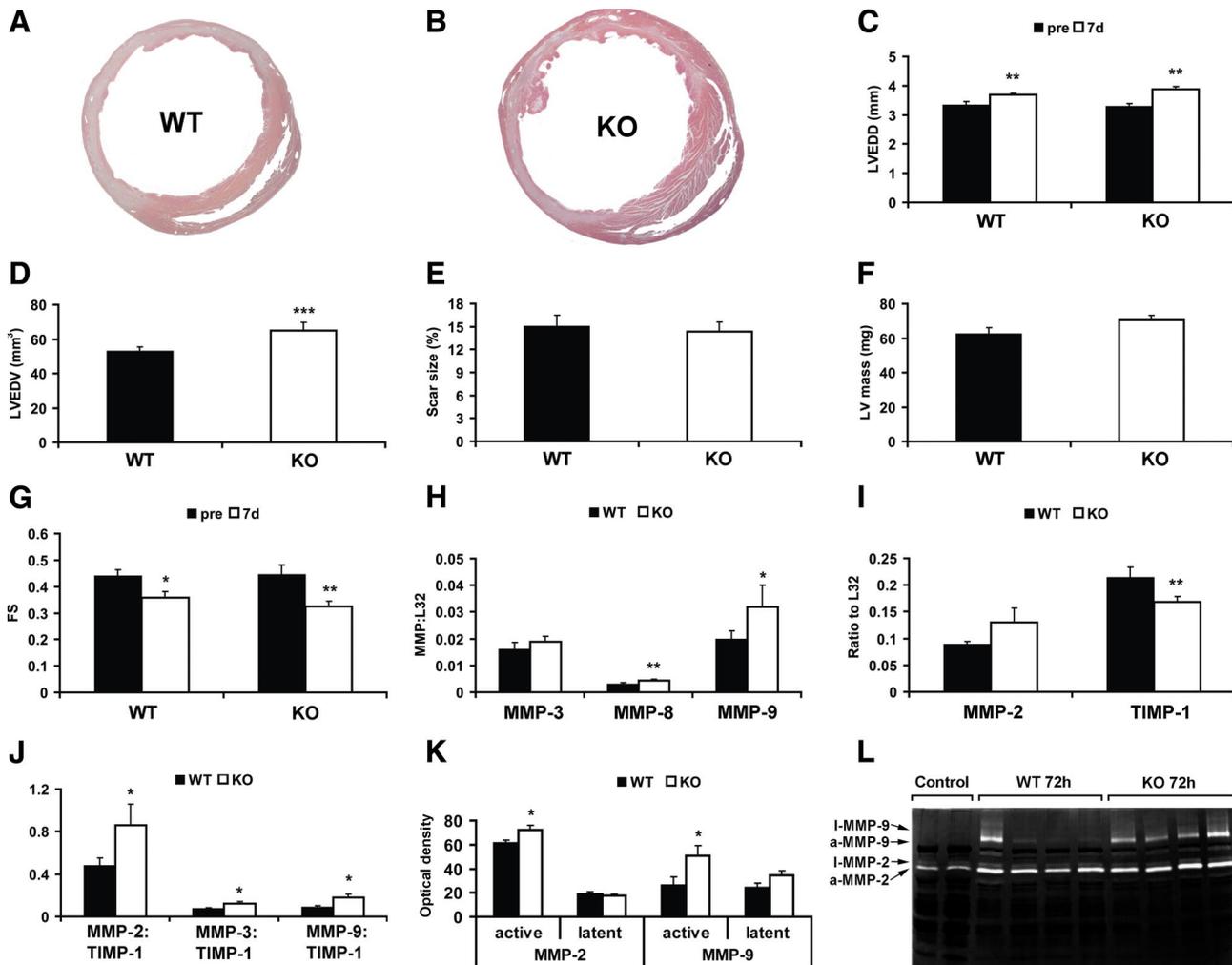
### CCR5<sup>+</sup> Regulatory T Cells Exhibited Increased Expression of Anti-Inflammatory Cytokines

To examine whether CCR5<sup>+</sup> Tregs exhibit enhanced anti-inflammatory properties we compared IL-10 expression between CCR5<sup>+</sup> and CCR5<sup>-</sup> foxp3<sup>+</sup> Tregs. Because of its high content in Tregs, the mouse spleen was used as

a source of mononuclear cells. Among splenocytes, Tregs were the main IL-10-producing cells (27.99%  $\pm$  3.63 of foxp3<sup>+</sup> cells expressed IL-10 versus 0.61%  $\pm$  0.32 of the foxp3<sup>-</sup> cells,  $P = 0.02$ ). CCR5<sup>+</sup> Tregs exhibited significantly higher expression of IL-10 than CCR5<sup>-</sup> Tregs (percentage of IL-10-positive cells: CCR5<sup>+</sup> foxp3<sup>+</sup>, 42.73%  $\pm$  4.07 versus CCR5<sup>-</sup> foxp3<sup>+</sup> 28.8%  $\pm$  4.25,  $P < 0.05$ ) indicating the more potent anti-inflammatory properties of the CCR5<sup>+</sup> Treg subpopulation (Figure 6).

### CCR5 Deficiency Resulted in Adverse Postinfarction Remodeling

Enhanced inflammation in CCR5-null infarcts was associated with accentuated adverse remodeling. Both morphometric (Figure 7, A, B, D–F) and echocardiographic studies (Figure 7, C and G) demonstrated that CCR5<sup>-/-</sup> animals had increased ventricular dilation after infarction when compared with wild-type mice. In the absence of injury, wild-type and CCR5-null hearts had comparable baseline LVEDD. In both wild-type and CCR5-null animals, LVEDD significantly increased after 7 days of reperfusion in comparison with preinfarction values, reflecting the development of dilative remodeling (Figure 7C). However, postinfarction LVEDD was significantly higher in CCR5<sup>-/-</sup> hearts (Figure 7C), indicating worse dilative remodeling. Morphometric analysis confirmed that CCR5 absence was associ-



**Figure 7.** CCR5 absence is associated with increased MMP expression in the infarcted myocardium accentuated postinfarction dilative remodeling. Compared with wild-type (WT) (A), CCR5-null animals (B) exhibited accentuated dilative remodeling after 7 days of reperfusion. Quantitative morphometry (A, B, D, E, F) and echocardiography (C, G) were used to assess remodeling-associated parameters. Echocardiographic analysis demonstrated that LVEDD increased significantly after 7 days of reperfusion in both wild-type and CCR5-null mice (\*\* $P < 0.01$  versus corresponding preinfarction values). CCR5-null hearts showed increased LVEDD (C) and LVEDV (D) after 7 days of reperfusion, suggesting accentuated dilative remodeling (\*\* $P < 0.05$  versus corresponding wild-type). E: Increased dilative remodeling in CCR5-null infarcts was not attributable to a difference in the size of the infarct; scar size was comparable between wild-type and CCR5-null mice after 7 days of reperfusion. F: A trend toward increased LV mass was noted in CCR5 KO mice after 7 days of reperfusion ( $P = 0.08$ ), suggesting enhanced hypertrophic remodeling. G: FS significantly decreased in both wild-type and CCR5<sup>-/-</sup> mice after 7 days of reperfusion (\*\* $P < 0.01$  versus corresponding preinfarction values), indicating the development of systolic dysfunction following myocardial infarction. However, the difference in fractional shortening between wild-type and CCR5 KO animals did not reach statistical significance ( $n = 12$  mice/group). H: CCR5 KO mice had significantly higher MMP-8 and MMP-9 mRNA levels than wild-type animals after 24 hours of reperfusion (\* $P < 0.05$ , \*\* $P < 0.01$  versus wild-type). In contrast, MMP-3 mRNA expression was comparable between groups. I: There was a trend toward increased MMP-2 mRNA expression in infarcted CCR5 KO hearts ( $P = 0.20$ ). TIMP-1 mRNA levels, on the other hand, were significantly lower in wild-type infarcts. J: The ratios MMP-2:TIMP-1, MMP-3:TIMP-1, and MMP-9:TIMP-1 were significantly higher in CCR5 KO infarcts (\*\* $P < 0.01$  versus corresponding wild-type), indicating that CCR5 absence was associated with increased expression of matrix-degrading proteases in the infarcted heart (wild-type  $n = 9$  mice, KO  $n = 10$  mice). K: MMP-2 and MMP-9 activity were assessed in the infarcted heart using gelatin zymography. Quantitative analysis demonstrated that CCR5<sup>-/-</sup> infarcts had markedly higher active MMP-9 levels after 72 hours of reperfusion (\* $P < 0.05$ ) in comparison with wild-type infarcts. In addition, active MMP-2 levels were modestly, but significantly, higher in infarcted CCR5-null hearts (\* $P < 0.05$ ). L: Representative gelatin zymography experiments illustrate the increased MMP-2 and MMP-9 activity in CCR5 KO infarcts. Two control wild-type hearts, four wild-type infarcts, and four CCR5-null infarcts are shown for comparison. The arrows show the bands corresponding to active MMP-2 (a-MMP-2), latent MMP-2 (I-MMP-2), active MMP-9 (a-MMP-9), and latent MMP-9 (I-MMP-9).

ated with increased chamber dilation after infarction, demonstrating significantly higher LVEDV in infarcted CCR5-null hearts (Figure 7D). Accentuated adverse remodeling in the absence of CCR5 was not attributable to an increase in the size of the infarct; the size of the scar was comparable between wild-type and CCR5<sup>-/-</sup> hearts after 7 days of reperfusion (Figure 7E). Infarcted CCR5-null hearts also demonstrated a trend toward an increased left ventricular (LV) mass ( $P = 0.08$ ; Figure 7F), indicating increased hypertrophic remodeling. Both wild-type and CCR5-null mice exhibited a significant deterioration in systolic function after

myocardial infarction, evidenced by a significant reduction in FS. There was no statistically significant difference in postinfarction FS between CCR5-null and wild-type infarcted hearts (Figure 7G).

#### CCR5-Null Mice Exhibited Enhanced MMP Expression and Activity in the Infarcted Heart

Because MMPs are critically involved in the pathogenesis of dilative cardiac remodeling,<sup>16-18</sup> we hypothesized that

enhanced inflammation in CCR5-null mice may have resulted in increased chamber enlargement through accentuation of MMP synthesis. CCR5-null infarcts had significantly higher MMP-8 and MMP-9 mRNA expression after 24 hours of reperfusion; in contrast, there was only a trend for increased MMP-2 levels ( $P = 0.20$ ), and MMP-3 expression was comparable between groups (Figure 7, H and I). TIMP-1 mRNA expression, on the other hand, was significantly lower in CCR5<sup>-/-</sup> infarcts (Figure 7I). The ratio of MMP:TIMP mRNA expression was significantly higher in CCR5-null animals, reflecting increased synthesis of matrix-degrading enzymes (Figure 7J). In addition, gelatinase activity in the infarcted heart was compared between wild-type and CCR5-null animals. When compared with wild-type animals, CCR5-null infarcts had markedly higher MMP-9 activity in the infarcted myocardium and exhibited modestly, but significantly, higher MMP-2 activity (Figure 7, K and L).

## Discussion

Chemokines are markedly and consistently induced in experimental models of myocardial ischemia<sup>7</sup> and are rapidly released in the serum of patients with myocardial infarction.<sup>19</sup> Extensive evidence suggests that chemokine signaling mediates proinflammatory actions in the infarcted myocardium through the recruitment of activated leukocytes. CXC chemokines containing the ELR motif play an important role in recruitment of neutrophils,<sup>20</sup> whereas the CC chemokine MCP-1/CCL2 is critically involved in infiltration of the infarct with monocytes.<sup>9</sup> Disruption of the MCP-1/CCR2 axis results in attenuated inflammation and reduced adverse remodeling,<sup>9</sup> possibly by preventing infiltration with proteolytic and proinflammatory CCR2<sup>+</sup> monocytes.<sup>5</sup> We present the first evidence suggesting that a chemokine-mediated pathway may play a role in suppression of the postinfarction inflammatory response. Our study demonstrates that, in contrast to animals with CCR1 or CCR2 disruption, CCR5-null mice exhibit worse adverse remodeling associated with enhanced inflammatory activity after myocardial infarction. CCR5 absence resulted in impairment of the endogenous mechanisms involved in suppression of postinfarction inflammation, markedly reducing infiltration of the infarct with suppressive Tregs. Defective control of inflammation was associated with increased proinflammatory cytokine expression (Figure 3), shifting the balance of MMP:TIMP expression toward enhanced matrix degradation, increasing MMP activity, and leading to accentuated adverse remodeling (Figure 7).

Evidence on the role of CCR5 signaling in immune responses is contradictory. Several studies have demonstrated a role for CCR5 in suppression of immunoinflammatory processes. CCR5-null mice displayed accentuated pulmonary inflammation and increased mortality on infection with influenza A virus<sup>21</sup> and had an enhanced delayed type hypersensitivity reaction suggesting a role for CCR5 in downmodulating T cell-dependent immune responses.<sup>22</sup> In addition, CCR5<sup>-/-</sup> animals developed an exacerbated form of crescentic glomerulonephritis

associated with increased mortality and enhanced recruitment of T cells and monocytes.<sup>23</sup> Absence of CCR5 was also associated with the induction of CD8<sup>+</sup> T cell-mediated immunopathology leading to marked hepatic steatosis in viral hepatic inflammation.<sup>24</sup> In contrast, other investigations suggested that CCR5 signaling may enhance inflammatory activity. CCR5-deficient animals exhibited reduced macrophage infiltration in the brain after infection with neurotropic coronavirus.<sup>25</sup> Huffnagle et al showed that CCR5<sup>-/-</sup> mice have defects in macrophage infiltration in the brain, but not in the lung, on infection with *Cryptococcus neoformans*,<sup>26</sup> suggesting that CCR5 may have organ-specific defects in leukocyte trafficking. Our study demonstrates that although CCR5 does not play a crucial role in monocyte recruitment in the infarcted myocardium, its absence is associated with markedly enhanced proinflammatory cytokine expression through impaired recruitment of leukocyte subsets with suppressive properties.

What are the mechanisms responsible for enhanced inflammation in CCR5<sup>-/-</sup> infarcts? To address this question, we first examined whether CCR5 mediates infiltration of the infarct with monocyte subsets that possess anti-inflammatory properties. Nahrendorf and coworkers have recently demonstrated that mouse myocardial infarcts are sequentially infiltrated by two distinct monocyte subpopulations.<sup>5</sup> Ly-6C hi monocytes are recruited during the inflammatory phase of healing; these cells express CCR2 and exert potent phagocytic and proteolytic effects secreting large amounts of cytokines. Ly-6C lo/CCR2-negative monocytes, on the other hand, infiltrate the myocardium during the reparative phase and promote healing by stimulating myofibroblast accumulation and angiogenesis.<sup>5</sup> In wild-type mice we identified a sizeable population of CCR5-expressing cells that infiltrate the infarcted myocardium accounting for almost 40% of the CD45<sup>+</sup> hematopoietic cells in the infarct (Figure 4). This CCR5<sup>+</sup> subpopulation of mononuclear cells exhibited increased expression of the anti-inflammatory cytokine IL-10 in comparison with the CCR5<sup>-</sup> subset (Figure 5). In addition, CCR5 gene disruption resulted in markedly enhanced upregulation of proinflammatory cytokines in the infarcted myocardium (Figure 3) associated with reduced expression of inhibitory cytokines in mononuclear cells isolated from the infarct (Figure 5). Thus, CCR5 expression appears to identify mononuclear cell subsets with anti-inflammatory properties.

Because inflammatory responses are often orchestrated by migration of T cell subsets,<sup>4</sup> we focused our attention on the potential effects of CCR5 deficiency on infiltration of the infarcted myocardium with T lymphocytes. Several lines of evidence suggest that CCR5 may regulate immune responses by mediating recruitment of Tregs, a CD4<sup>+</sup> subpopulation of T cells with an essential role in immunological homeostasis and self-tolerance.<sup>27</sup> CCR5 signaling was essential for homing of tumor-associated Tregs in a model of pancreatic cancer<sup>14</sup> and was a key regulatory signal in migration of Tregs to the site of *Paracoccidioides* infection.<sup>28</sup> In addition, the CCR5 axis is critically involved in Treg recruitment in chronic intestinal inflammation<sup>15</sup> and directs homing of Tregs to *Leishma-*

nia-infected dermal sites promoting long-term survival of the parasite in the immune host.<sup>29</sup> Our experiments suggest that the anti-inflammatory actions of CCR5 signaling may be attributable to impaired recruitment of Tregs in the infarcted myocardium. Although the role of Tregs in restraining autoimmunity and in providing protection from the immune response to infection is well-documented, their involvement in tissue repair after ischemic injury has not been investigated. We identified a sizeable population of Tregs in the infarcted myocardium (Figure 4), as CD4<sup>+</sup> cells expressing foxp3, a transcription factor restricted to Tregs and indispensable for their differentiation.<sup>30</sup> Almost half of the CCR5<sup>+</sup>/CD4<sup>+</sup> cells infiltrating the infarct were identified as foxp3-expressing Tregs, whereas the majority of CCR5<sup>-</sup>/CD4<sup>+</sup> T cells were foxp3-negative (Figure 4). Thus, in the population of CD4<sup>+</sup> T cells infiltrating the infarct, CCR5 expression was preferentially found in Tregs. Two lines of evidence suggested a crucial role for CCR5 in recruitment of Tregs in the infarcted heart. First, foxp3 mRNA expression levels were markedly reduced in CCR5-null infarcts (Figure 6A). Second, CCR5 absence was associated with significantly lower numbers of foxp3<sup>+</sup> T cells in the mononuclear infiltrate of the infarct (Figure 6). Reduced infiltration with Tregs appears to abrogate regulatory signals that suppress inflammation in the infarcted heart. The molecular pathways responsible for Treg-mediated suppression of the immunoinflammatory response remain unknown. Although *in vitro* studies have demonstrated that Treg-mediated suppression requires direct cell contact, several *in vivo* investigations have suggested an essential role for secreted mediators, such as IL-10 and TGF- $\beta$ .<sup>31,32</sup> Rubtsov and coworkers<sup>33</sup> have recently demonstrated that, although Treg-derived IL-10 synthesis was not required to prevent systemic autoimmunity, it was essential for protection from localized immune responses at environmental interfaces. Although the mechanisms involved in mediating the anti-inflammatory effects of Tregs in the healing infarct remain unknown, these cells may use multiple suppression mechanisms to control various aspects of inflammation.<sup>33</sup>

Timely resolution of inflammation appears to be essential for prevention of adverse remodeling after myocardial infarction.<sup>34,35</sup> Uncontrolled postinfarction inflammation may promote adverse remodeling through several distinct mechanisms. First, proinflammatory cytokines may enhance MMP synthesis,<sup>36,37</sup> increasing matrix degradation in the infarcted heart. Second, inflammatory mediators, such as TNF- $\alpha$  and IL-1 $\beta$ , may enhance cardiomyocyte apoptosis in the remodeling myocardium.<sup>38</sup> Third, proinflammatory cytokine signaling may suppress contractility accentuating ventricular systolic dysfunction.<sup>39</sup> Our findings demonstrated that although accentuated inflammation in CCR5-null mice did not affect the size of the infarct (Figure 7E), it was associated with increased MMP expression and activity (Figure 7, H-L). Thus, in the absence of CCR5 enhanced matrix degradation may result in adverse dilative remodeling of the infarcted ventricle.

Our study identifies a chemokine-mediated mechanism responsible for suppression of the postinfarction

inflammatory response and for protection from adverse remodeling after myocardial infarction. CCR5 signaling is an important molecular pathway controlling the postinfarction inflammatory response through the recruitment of Tregs. Infiltration of the infarct with regulatory T cells appears to provide essential inhibitory signals that prevent excessive inflammatory activity in the infarcted heart. Thus, pharmacological interventions or cell therapy enhancing the suppressive actions of Tregs after infarction may be promising new therapeutic strategies.

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