Anti-CCL21 Antibody Attenuates Infarct Size and Improves Cardiac Remodeling After Myocardial Infarction

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Key Words
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Abstract
Background/Aims: Over-activation of cellular inflammatory effectors adversely affects myocardial function after acute myocardial infarction (AMI). The CC-chemokine CCL21 is, via its receptor CCR7, one of the key regulators of inflammation and immune cell recruitment, participates in various inflammatory disorders, including cardiovascular ones. This study explored the therapeutic effect of an anti-CCL21 antibody in cardiac remodeling after myocardial infarction. Methods and Results: An animal model of AMI generated by left anterior descending coronary artery ligation in C57BL/6 mice resulted in higher levels of circulating CCL21 and cardiac CCR7. Neutralization of CCL21 by intravenous injection of anti-CCL21 monoclonal antibody reduced infarct size after AMI, decreased serum levels of neutrophil and monocyte chemo attractants post AMI, diminished neutrophil and macrophage recruitment in infarcted myocardium, and suppressed MMP-9 and total collagen content in myocardium. Anti-CCL21 treatment also limited cardiac enlargement and improved left ventricular function. Conclusions: Our study indicated that CCL21 was involved in cardiac remodeling post infarction and anti-CCL21 strategies might be useful in the treatment of AMI.

Introduction
Acute myocardial infarction (AMI) is usually complicated by pathological cardiac remodeling potentially underlying ventricular arrhythmias and heart failure, and ultimately increasing cardiovascular mortality [1, 2]. In the infarcted myocardium, sudden necrosis of
a large number of cardiomyocytes results in release of their intracellular contents which triggers an intense inflammatory reaction, characterized by the release of cytokines and chemokines and recruitment of neutrophils and mononuclear cells, leading to ventricular dilatation and adverse remodeling [3]. Therefore, chemokine-induced leucocyte recruitment during myocardial repair might be an attractive therapeutic target to improve cardiac function following AMI [4-6].

The homeostatic chemokine CCL21 is, via its receptor CCR7, a potent regulator of T-cell migration into non-lymphoid tissue [7, 8]. CCR7 and CCL21 are widely expressed in non-lymphoid cells such as fibroblasts, vascular smooth muscle cells (SMCs), and endothelial cells, and may also regulate vascular inflammation, SMCs proliferation, and matrix remodeling [9, 10]. CCR7 is involved in directing the migration, positioning, and interaction between dendritic cells (DCs) and naive T cells in secondary lymphoid organs to activate the adaptive immune response [11]. Chemokine CCL21 could stimulate migration of DCs in Akt2-dependent manner by regulating Ca\(^{2+}\) signal [12]. Both CCL21/CCR7 levels significantly increased in skin tissue after whole body irradiation, which might contribute to enhanced DCs migration [13]. Moreover, CCL21 plays vital role in lymphocyte trafficking and the pathogenesis of various inflammatory disorders [14-16]. For instance, a recent study found markedly raised serum levels of CCL21 in patients with chronic heart failure (HF) [17]. In addition, Finsen et al. found CCL21/CCR7 interactions might be involved in the response to pressure overload secondary to symptomatic aortic stenosis and modulates left ventricular remodeling [18].

Because unrestrained inflammation in the infarcted myocardium induces matrix degradation and cardiomyocyte apoptosis, timely and effective suppression of the post-infarction inflammatory reaction is of great importance to protect the myocardium from dilative remodeling and progressive cardiac dysfunction [19]. This study therefore investigated whether treatment with a neutralizing monoclonal antibody (mAb) to mouse CCL21 would be of therapeutic value in a mouse model of AMI, potentially providing a new means to prevent post-infarction heart failure and further insight into its molecular pathogenesis.

**Materials and Methods**

**Animal care**

Animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no.85-23, revised 1996). The study protocol was approved by the Institutional Animal Care and Use Committee of Tongji Medical College.

**Mice and Antibodies**

Male C57BL/6 mice (8-12 weeks of age with 23-25 g body weight) were obtained from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology, China. Goat anti-mouse CCL21 affinity purified polyclonal antibody (Ab) AF457 and goat IgG isotype control were purchased from R&D Systems Inc. (Minneapolis, MN, USA) and administered at a dose of 1 mg/mouse.

**Induction of AMI**

A large non-reperfused infarction was induced by permanent coronary artery ligation as described [20, 21]. Briefly, after being anaesthetized with urethane (1.0 - 1.2 g/kg), male C57BL/6 mice were intubated, placed in the right lateral decubitus position, subjected to left thoracotomy, pericardiectomy and ligation of the left anterior descending coronary artery, and after closure of the thorax placed in the prone position until spontaneous breathing occurred. Either goat anti-mouse CCL21 mAb (1.0 mg/mouse) or isotype-IgG control (1.0 mg/mouse) was administered intravenously to neutralize serum and cardiac CCL21 bioactivity in vivo at prespecified time points post myocardium infarction. Sham-operated mice were subjected to the same surgical protocol except for arterial occlusion (Fig. 1A).
Assessment of area at risk (AAR) and infarct size (IS)

Myocardial infarct size was determined by Evans blue-TTC double staining methods as previously described [20]. Briefly, Evan’s blue dye (2%; Sigma) was intravenously injected to delineate the in vivo AAR, and the heart was rapidly excised and rinsed in 0.9% NaCl. Hearts from both assays were frozen and sliced into 2-mm transverse sections from apex to base (5 slices/heart). The sections were incubated at 37°C with 1% triphenyltetrazolium chloride (TTC) in phosphate buffer (pH 7.4) for 15 min; fixed in 10% formaldehyde solution; and photographed with a digital camera (Nikon Coolpix) to distinguish continuously perfused tissue (blue), stained ischemic viable tissue (red) and unstained necrotic tissue (white). After their determination, AAR and left ventricular infarct size (IS) were respectively expressed as percentage of ventricle surface (AAR/V) and AAR (I/AAR) using Meta Morph software (RPI, Co., Inc., Natick, MA, USA).

Determination of serum levels of cardiac troponin I (cTnI), lactic hydrogenase (LDH) and chemokine attractants

Circulating cTnI levels at 1 day post AMI were measured using a high sensitivity ELISA kit (Life Diagnostics Inc.). To determine the extent of myocardial injury, the release of LDH in serum of mice at 1 day after AMI was measured using commercially available kits and spectrophotometer; values were expressed in international units (IU) per liter. Serum levels of CXCL1, CXCL2, macrophage inflammatory protein-1 alpha (MIP)-1α, stromal-derived factor (SDF)-1, monocyte chemotactic protein (MCP)-1 and CCL5 at 1, 3, 7 days after AMI were measured by colorimetric enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota, USA).

Western blot analysis

Homogenized mice myocardium was lysed in 200 μL RIPA lysis buffer (Beyotime, P0013B) with 1% phenylmethyl sulfonylfluoride and 4% complete protease inhibitor cocktail mix (Roche, Mannheim, Germany). Extracts were centrifuged at 14,000 g for 15 min at 4°C. Eighty micrograms of total protein were used for sodium dodecyl sulphate-polyacrylamide gel electrophoresis, followed by transfer to nitrocellulose membrane (Millipore Corp., Billerica, MA, USA). Membranes were then blocked with 5% non-fat dried milk in PBS for 1 h with gentle shaking. Membranes were incubated first with CCR7, CCL21, MMP-2 and MMP-9 (Santa Cruz Biotechnology) overnight at 4°C, then placed in 1% BSA in PBS overnight at 4°C with shaking, and washed and incubated with secondary antibodies for 2 h at room temperature. Finally, the samples were visualized by enhanced chemiluminescence [22]. After scanning, band density was analyzed using Image J 1.33 software (National Institutes of Health, Bethesda, MD, USA).

Sirius Red staining for collagen content

Mouse heart sections were rinsed with water and incubated with 0.1% Sirius red (Sigma) in saturated picric acid for 90 min, rinsed twice with 0.01N HCl for 1 min and then immersed in water. After dehydroxylation with ethanol for 30 seconds and cover-slipping, the sections were photographed with identical exposure settings under ordinary polychromatic or polarized light microscopy. Total collagen content was evaluated under polychromatic light (Sirius red). Quantifications were performed using the MetaMorph software, and expressed as percentage of stained area per total lesion area.

Echocardiography

All mice underwent transthoracic echocardiography at 7 and 21 days after surgery (prior to killing) using an echocardiography system with a Sono 4500 and a 15-16 MHz transducer (PHILIPS Corporation). Left ventricular dimensions (end-diastolic diameter, LVEDD; and end-systolic diameter, LVEDD) were measured perpendicular to the long axis of the ventricle at the mid-chest level. Left ventricular fractional shortening (LVFS) (%) and left ventricular ejection fraction (LVEF) (%) were calculated automatically by the echocardiography equipment.

Statistical analysis

Data are presented as mean ± standard error and were compared using the Student’s t test (SPSS 16.0 software). A P-value <0.05 was considered statistically significant.
Results

Increased circulating levels of CCL21 and cardiac levels of CCR7 after AMI

As compared to those in sham-operated mice, serum levels of CCL21 and cardiac levels of CCL21 and CCR7 were increased 1-7 days after AMI induction in antibody untreated mice (Fig. 1B and 1C-E, respectively), suggesting that AMI might be associated with activated CCL21/CCR7.

Blocking CCL21 reduced infarct size at 1 and 7 days after AMI

As shown in Fig. 2, area at risk (AAR) relative to ventricle area (V) were similar among different groups, while blocking CCL21 induced significant reduction in infarct size (I/AAR) at 1 and 7 days post AMI. 1 day post AMI, mice treated with anti-CCL21 mAb exhibited reduced serum levels of cTnI and LDH relative to mice treated with isotype control (Fig. 2, panels A-B, C-D, and E-F, respectively).

Blocking CCL21 reduced neutrophil and macrophage recruitment in infarcted hearts after AMI

Myocardial infarction is characterized by infiltration of inflammatory cells into injured myocardium. Neutrophil and macrophage infiltration therefore was analyzed at different

Fig. 1. AMI significantly increased circulating levels of CCL21 and cardiac levels of CCR7. Left anterior coronary artery permanent ligation was performed in male C57BL/6 mice. Data are expressed as mean ± SEM. (A) Timeline of study protocol. (B) Serum CCL21 levels of sham-operated and coronary artery ligation treated mice at different time points post myocardial infarction. (C) Protein levels of CCR7 and CCL21 within the infarcted myocardium. (D) Quantification of CCL21 within the infarcted myocardium. (E) Quantification of CCR7 within the infarcted myocardium.
time points (1, 3 and 7 days) to assess whether neutralization of CCL21 modified leucocyte recruitment post AMI. Neutrophil infiltration was significantly increased in isotype-treated mice at 1-7 days after AMI as compared with those in sham-operated mice (Fig. 3A). Compared to isotype controls, anti-CCL21 mAb treatment significantly reduced neutrophil infiltration at 1, 3 and 7 days post AMI and macrophage (CD68+ cell) recruitment at 3 days post AMI (Fig. 3A and 3B, respectively).

Blocking CCL21 reduced serum levels of neutrophil and monocyte chemoattractants after AMI

CXCR chemokines play an important role in regulating neutrophil chemotaxis and activation in ischemic tissues [23]. Previous studies suggested that CCL21 could induce neutrophil and monocyte chemotaxis [24, 25]. To investigate whether CCL21 neutralization might influence neutrophil and macrophage recruitment into infarcted hearts, we analyzed the possible reduction of serum levels of other chemo attractants (i.e. CXCL1, CXCL2, SDF-1, MIP-1α, MCP-1 and CCL5) [26, 27]. Serum levels of CXCL1, CXCL2, MCP-1 and CCL5 were significantly increased in isotype-treated mice at 1 and 3 days after AMI as compared to those in sham-operated mice (Fig. 4A, B, E, F). As compared to isotype-treated mice, anti-
CCL21 mAb treatment attenuated the post AMI increase in serum levels of: 1. CXCL1, CXCL2, MIP-1α and MCP-1 at 1 day (Fig. 4A, B, D, E); 2. CCL5 at 1 and 3 days (Fig. 4F).

Blocking CCL21 transiently reduced MMP-9 and collagen amounts, but not MMP-2 in infarcted hearts after AMI.

Cardiac repair following AMI is closely associated with the inflammatory response. The recruited inflammatory cells clean the wound of tissue debris, whereas fibroblasts...
and endothelial cells infiltrate, proliferate, remodel the extracellular matrix, and determine scar formation. To explore whether the anti-CCL21 mAb treatment modulates molecular mediators of post-infarction cardiac remodeling, we assessed the cardiac content of matrix metalloproteinase (MMP)-2, MMP-9, and total collagen content. Treatment with the anti-CCL21 mAb did not affect MMP-2 amounts but significantly reduced MMP-9 content at 7 days after AMI as compared to isotype-treated mice (Fig. 5A and B, and 5A and C, respectively) and remained low. This is clinically relevant because during the proliferative phase of healing, activated myofibroblasts produce extracellular matrix proteins and the molecular and cellular changes associated with ventricular remodeling, which is closely associated with the progression of heart failure, increased incidence of arrhythmias, and poor prognosis in patients surviving AMI. In addition, treatment with the anti-CCL21 mAb reduced amounts of total collagen at 7 and 21 days after AMI as compared to isotype-treated mice (Fig. 5D).

![Fig. 5](image)

**Fig. 5.** Treatment with anti-CCL21 mAb reduced MMP-9, but not MMP-2 release in infarcted hearts. (A) Cardiac expression levels of MMP-2 of sham-operated (Sham, sacrificed at 7 days) and infarcted hearts at 7 days after AMI. Data are expressed as mean ± SEM. Quantification of cardiac MMP-2 (B) and MMP-9 (C) in sham-operated (Sham, sacrificed at 7 days) and infarcted hearts at 7 days after AMI. (D) Quantification of total collagen in frozen sections of sham-operated (Sham, sacrificed at 7 days) and infarcted hearts at different time points post AMI (at 7 and 21 days). Data are expressed as mean ± SEM (n=6).

![Fig. 6](image)

**Fig. 6.** (A) LVEED, (B) LVESD, and (C) LVEF of isotype IgG- (1 mg/mouse) or anti-CCL21 mAb-treated mice (1 mg/mouse) at 7 and 21 days after AMI. Data are expressed as mean ± SEM.
Blocking CCL21 limited the cardiac enlargement and dysfunction after AMI

Overactive matrix-degrading processes induced by local activation of MMPs by proinflammatory mediators, such as MCP-1, are generally associated with decreased tensile strength, leading to left ventricular dilatation and systolic dysfunction. Herein, cardiac remodeling was measured non-invasively using echocardiography. As shown in Fig. 6, administration of the antibody directed against CCL21 after AMI significantly limited cardiac enlargement and dysfunction at 7 and 21 days. At 21 days, the anti-CCL21 treatment effectively prevented LV enlargement in comparison to isotype IgG (Fig. 6A and B). The LVEF and LVFS were also significantly greater in the anti-CCL21 treated animals as compared to control group (Fig. 6C and D).

Discussion

The inflammatory reaction plays an important role in the pathogenesis, progression and prognosis of cardiovascular diseases [28]. Recruitment of inflammatory cells in the ischemic myocardium is mediated by the family of CC-chemokines and their receptors. To this end, up-regulation of circulatory and cardiac chemokines has been considered as a vital mechanism in leukocyte recruitment, early vascular inflammation, and atherogenesis. For instance, selective inhibition of CC chemokines CCL5 with Evasin-4 effectively reduced cardiac injury/inflammation and improved survival [29]. In the present study, we observed significant increase of both CCL21 and CCR7 after AMI as compared with sham-operated mice. In particular, systemic levels of CCL21 peaked in the early period of AMI (1 day). This is in agreement with a previous study showing increased expression of the CCR7 ligand CCL21 both systemically and within the failing myocardium in human heart failure [17]. Damas et al. also found increased levels of CCL19 and CCL21 in coronary artery disease, providing further evidence for the role of CCL21 in inflammation and immune cell recruitment in cardiovascular diseases [10].

A growing body of evidence suggests that prolongation or expansion of the post-infarction inflammatory response results in worse remodeling and dysfunction following AMI [30-32]. Therefore, timely termination of chemokine signals might be mediated through the concerted action of multiple suppressive pathways that prevent extension of injury and protect from adverse remodeling [28, 33]. In early phase post AMI, neutrophils were considered as first cells recruited to the infarction area within hours, which release proteolytic enzymes and reactive oxygen species, and directly injure surrounding cells [19]. Accumulated evidence suggested that circulating neutrophil number was a prognostic factor for future cardiovascular events [34, 35]. In turn, neutrophils depletion in animals undergoing MI effectively decreased infarct size and prevented ventricular remodeling. A study using gene-deficient mice revealed that CCR1, a receptor for a receptor for CCL3/MIP-1 and CCL5, exert positive role in improving cardiac function and preserve cardiac remodeling after MI by attenuating the neutrophil-induced myocardial injury and promote tissue healing [36].

Chemotaxis and activation of neutrophils in infarction tissues were regulated by a series of chemokines [37]. In this study, we found that serum levels of neutrophil chemo attractants (MIP-1α, SDF-1, CXCL1 and CXCL2) significantly increased at 1 day post AMI accompanied with increased neutrophil numbers in myocardium. Those neutrophil chemo attractants are reported to have important roles in AMI. CCL3/MIP-1α level was reported to be elevated during acute coronary syndromes and show strong prognostic power for future ischemic events and served as a mediator in the ischemic process itself [38]. SDF-1 expression was also reported to be elevated within 24 hours after AMI and served as key factors in mobilization of endogenous bone marrow cells towards infarcted myocardium [39]. We also found that blocking CCL21 effectively attenuated increased serum levels of neutrophil chemo attractants (CXCL1, CXCL2 and MIP-1α) and neutrophil infiltration in myocardium at 1 day post AMI. In addition, the anti-CCL21 mAb treatment effectively reduced infarction size and attenuated AMI induced increase in serum levels of cTnI and LDH. On the
basis of these observations, CCL21 might play a vital role in mediating the recruitment of neutrophils to the infarcted myocardium in the early phase post-AMI by coordinating with other classical chemo attractants. In line with our study, Montecucco et al. [40] found that blocking CCL5 reduced infarct size in chronic ischemia, and that the cardio protective effects of CCL5 neutralization during early phases of chronic ischemia were associated with the disruption of the CCL2/CCR2 axis, or with CCR1 deficiency. In addition, a recent study found that CCL19, CCL21 and CCR7 were involved in promoting monocyte adhesion and migration [41]. Further studies are needed to examine whether similar mechanisms could be seen in the post-AMI inflammatory response.

Despite reperfusion strategies, patients with large AMI who survive the initial ischemic event are at higher risk of ventricular remodeling [42]. The healing process in post-AMI ventricular remodeling can be divided into 3 partially overlapping phases, namely inflammatory, proliferative, and maturation [15]. Healing of myocardial infarction is dependent on a chemokine-driven inflammatory response that ultimately results in replacement of necrotic cardiomyocytes with a collagen-based scar. The transition from the inflammatory to reparative phase is associated with the activation of pathways that turns off inflammation and promotes extracellular matrix (ECM) scar formation [43]. MMPs are important proteolytic enzymes that lead to degradation of the extracellular matrix and to changes in cardiomyocytes in both infarcted and non-infarcted myocardium [44]. Moreover, Matrix metalloproteinases (MMPs) have recently emerged as modulators of cardiovascular inflammation [45, 46]. In addition to ECM, MMP substrates also include a multitude of cytokines, chemokines, growth factors, and adhesion molecules [47]. For instance, MMP-9 could activate a series of ELR-positive CXC chemokines, including CXCL5, CXCL6, and CXCL8 [48].

A magnitude of evidence from clinical studies strongly suggests that increased MMPs correlate with adverse pathophysiology and clinical outcomes post AMI [49, 50]. Targeted MMP-9 deletion in mice was proved to improve left ventricular function by stimulating neovascularization in remodeling myocardium post-MI [51] and attenuate left ventricular remodeling and myocardial contractile dysfunction in heart failure [52]. Li et al. found that NOD2 deficiency protects against cardiac dysfunction and remodeling after MI by reducing the levels of cytokines, macrophage infiltration and matrix MMP-9 activity [53]. In our study, anti-CCL21 mAb treatment also attenuated AMI induced increase in MMP-9 and collagen deposition 7 days post AMI. MMP-9 is secreted by neutrophils early post-MI (1-2 day), and by macrophages, lymphocytes, and fibroblasts at later phases post-MI [54]. In line with this, blockade of CCL21 also significantly attenuated increased levels of macrophage (CD68+ cell) at 3 days post AMI. Based on these data, we speculate that the greater preservation in left ventricular dimensions and improved function post-AMI might benefit from the inhibited inflammation response mediated by MMP-9 in later period post-AMI. In addition, CCL21 has been implicated in beneficial remodeling processes within the kidney by enhancing the degree and firmness of cell adhesion and increases in cell spreading and the formation of cell-cell contacts [55]. These effects might actively contribute to the anti-CCL21 mAb-mediated improvement on cardiac function in the present study.

The present study has some limitations such as the lack of confirmation of findings in patients. Nonetheless, our results suggest that anti-CCL21 mAb treatment reduces infarct size, leukocyte infiltration and collagen deposition post myocardial infarction, which limits cardiac enlargement and dysfunction. Anti-inflammatory/anti-CCL21 strategies targeting ventricular remodeling following AMI therefore appear clinically relevant.

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Disclosure Statement

The authors declare no conflict of interest.

References

1. Heusch G, Libby P, Gersh B, Yellon D, Bohm M, Lopaschuk G, Opie L: Cardiovascular remodelling in coronary artery disease and heart failure. Lancet 2014;383:1933-1943.
2. Pu J, Mintz GS, Brilakis ES, Barnerjee S, Abdel-Karim AR, Maini B, Biro S, Lee JB, Stone GW, Weisz G, Maehara A: In vivo characterization of coronary plaques: novel findings from comparing greyscale and virtual histology intravascular ultrasound and near-infrared spectroscopy. Eur Heart J 2012;33:372-383.
3. Ueland T, Gullestad L, Nycomed A, Yndestad A, Aukrust P, Askved et al.: Inflammatory cytokines as biomarkers in heart failure. Clin Chim Acta DOI:10.1016/j.cca.2014.09.001.
4. Toldo S, Mezzaroma E, Bressi E, Marchetti C, Carbone S, Sonnino C, Van Tassell BW, Abbate A: Interleukin-1beta blockade improves left ventricular systolic/diastolic function and restores contractility reserve in severe ischemic cardiomyopathy in the mouse. J Cardiovas Pharmacol 2014;64:1-6.
5. Liehn EA, Kanzler I, Korschall S, Kroh A, Simsek Yilmaz S, Sonmez TT, Bucala R, Bernhagen J, Weber C: Compartmentalized protective and detrimental effects of endogenous macrophage migration-inhibitory factor mediated by CXCR2 in a mouse model of myocardial ischemia/reperfusion. Arterioscler Thromb Vasc Biol 2013;33:2180-2186.
6. Cochain C, Auvynet C, Poupel L, Vilar J, Dupeaux E, Recalde A, Zouggari Y, Yin KY, Bruneval P, Renault G, Marchiol C, Bonnin P, Levy B, Bonecchi R, Locati M, Combadiere C, Silvestre JS: The chemokine decoy receptor D6 prevents excessive inflammation and adverse ventricular remodeling after myocardial infarction. Arterioscler Thromb Vasc Biol 2012;32:2206-2213.
7. Forster R, Davalos-Misslitz AC, Rot A: CCR7 and its ligands: balancing immunity and tolerance. Nat Rev Immunol 2008;8:362-371.
8. Menning A, Hopken UE, Siegmund K, Lipp M, Hamann A, Huehn J: Distinctive role of CCR7 in migration and functional activity of naive- and effector/memory-like Treg subsets. Eur J Immunol 2007;37:1575-1583.
9. Pierce EM, Carpenter K, Jakubzick C, Morelli L, Enderle A, Recalde A, Zouggari Y, Yin KY, Bruneval P, Renault G, Marchiol C, Bucala R, Bernhagen J, Weber C: Compartmentalized protective and detrimental effects of endogenous macrophage migration-inhibitory factor mediated by CXCR2 in a mouse model of myocardial ischemia/reperfusion. Arterioscler Thromb Vasc Biol 2013;33:2180-2186.
10. Sanchez-Sanchez N, Riol-Blanco L, Rodriguez-Fernandez JL: The multiple personalities of the chemokine receptor CCR7 in dendritic cells. J Immunol 2006;176:5153-5159.
11. Yang W, Nurbaeva MK, Schmehid E, Russo A, Almilaji A, Szteyn K, Yan J, Faggio C, Shumilina E, Lang F: Akt2- and ETS1-dependent IP3 receptor 2 expression in dendritic cell migration. Cell Physiol Biochem 2014;33:222-236.
12. Yang Y, Cui J, Gao F, Li B, Liu C, Zhang P, Huang Y, Liu W, Liu H, Cai J: Whole body irradiation induces cutaneous dendritic cells depletion via NF-kappaB activation. Cell Physiol Biochem 2013;32:200-209.
13. Aastrup E, Ranheim T, Damas J, Davi G, Santilli F, Jensenius M, Vitale G, Aukrust P, Olano JP, Otterdal K: Increased expression of the homeostatic chemokines CCL19 and CCL21 in clinical and experimental atherosclerosis: possible pathogenic role in plaque destabilization. Arterioscler Thromb Vasc Biol 2007;27:614-620.
Jiang et al.: CCR21 Blockade Attenuates Post-MI Injury

17 Yndestad A, Finsen AV, Ueland T, Husberg C, Dahl CP, Oie E, Vinge LE, Sjaastad I, Sandanger O, Ranheim T, Dickstein K, Kjekshus J, Damas JK, Fiane AE, Hilfliger-Kleiner D, Lipp M, Gullestad L, Christensen G, Aukrust P: The homeostatic chemokine CCL21 predicts mortality and may play a pathogenic role in heart failure. PLoS One 2012;7:e33038.

18 Finsen AV, Ueland T, Sjaastad I, Ranheim T, Ahmed MS, Dahl CP, Askevold ET, Aakhus S, Husberg C, Fiane AE, Lipp M, Gullestad L, Christensen G, Aukrust P, Yndestad A: The Homeostatic Chemokine CCL21 Predicts Mortality in Aortic Stenosis Patients and Modulates Left Ventricular Remodeling. PLoS One 2014;9:e112172.

19 Liehn EA, Postea O, Curaj A, Marx N: Repair after myocardial infarction, between fantasy and reality: the role of chemokines. J Am Coll Cardiol 2011;58:2357-2362.

20 Yu LH, Kim MH, Park TH, Cha KS, Kim YD, Quan ML, Rho MS, Seo SY, Jung JS: Improvement of cardiac function and remodeling by transplanting adipose tissue-derived stromal cells into a mouse model of acute myocardial infarction. Int J Cardiol 2010;139:166-172.

21 Wang WE, Yang D, Li L, Wang W, Peng Y, Chen C, Chen P, Xia X, Wang H, Jiang J, Liao Q, Li Y, Xie G, Huang H, Guo Y, Ye L, Duan DD, Chen X, Houer SR, Zeng C: Prolyl hydroxylase domain protein 2 silencing enhances the survival and paracrine function of transplanted adipose-derived stem cells in infarcted myocardium. Circ Res 2013;113:288-300.

22 Cao CM, Zhang Y, Weislede N, Ferrante C, Wang XH, Lv F, Zhang Y, Song RS, Hwang M, Jin L, Guo J, Peng W, Li G, Nishi M, Takeshima H, Ma J, Xiao RP: MG53 constitutes a primary determinant of cardiac ischemic preconditioning. Circulation 2010; 121:2565-2574.

23 Bizzarri C, Beccari AR, Bertini R, Cavicchia MR, Giorgini S, Allegretti M: ELR+ CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets. Pharmacol Ther 2006;112:139-149.

24 Ley K: Arrest chemokines. Microcirculation 2003;10:289-295.

25 Beauvillain C, Cunin P, Doni A, Scotet M, Jaillon S, Loiry ML, Magistrelli G, Masternak K, Chevailler A, Delneste Y, Jeannin P: CCR7 is involved in the migration of neutrophils to lymph nodes. Blood 2011;117:1196-1204.

26 McColl SR, Clark-Lewis I: Inhibition of murine neutrophil recruitment in vivo by CXC chemokine receptor antagonists. J Immunol 1999;163:2829-2835.

27 Rollins BJ: Chemokines. Blood 1997;90:909-928.

28 Aukrust P, Halvorsen B, Yndestad A, Ueland T, Oie E, Otterdal K, Gullestad L, Damas JK: Chemokines and cardiovascular risk. Arterioscler Thromb Vasc Biol 2008;28:1909-1919.

29 Braunersreuther V, Montecucco F, Pelli G, Galan K, Proudfoot AE, Belin A, Vuilleumier N, Burger F, Lenglet S, Caffa I, Soncini D, Nencioni A, Vallee JP, Mach F: Treatment with the CC chemokine-binding protein Evasin-4 improves post-infarction myocardial injury and survival in mice. Thromb Haemost 2013;110:807-825.

30 Bujak M, Frangogiannis NG: The role of TGF-beta signaling in myocardial infarction and cardiac remodeling. Cardiovasc Res 2007;74:184-195.

31 Saxena A, Bujak M, Frunza O, Dobaczewski M, Gonzalez-Quesada C, Lu B, Gerard C, Frangogiannis NG: CXCR3-independent actions of the CXC chemokine CXCL10 in the infarcted myocardium and in isolated cardiac fibroblasts are mediated through proteoglycans. Cardiovasc Res DOI:10.1093/cvr/cvu138.

32 Bujak M, Dobaczewski M, Gonzalez-Quesada C, Xia Y, Leucker T, Zymek P, Veeranna V, Tager AM, Luster AD, Frangogiannis NG: Induction of the CXC chemokine interferon-gamma-inducible protein 10 regulates the reparative response following myocardial infarction. Circ Res 2009;105:973-983.

33 Hayashidani S, Tsutsui H, Shiomi T, Ikeuchi M, Matsusaka H, Suematsu N, Wen J, Egashira K, Takeshita A: Anti-monocyte chemoattractant protein-1 gene therapy attenuates left ventricular remodeling and failure after experimental myocardial infarction. Circulation 2003;108:2134-2140.

34 Toor IS, Jaumdally RJ, Moss MS, Babu SB: Preprocedural neutrophil count predicts outcome in patients with advanced peripheral vascular disease undergoing percutaneous transluminal angioplasty. J Vasc Surg 2008;48:1504-1508.

35 Haumer M, Amighi J, Exner M, Miekusch W, Sabeti S, Schlager O, Schwarzinger I, Wagner O, Minar E, Schillinger M: Association of neutrophils and future cardiovascular events in patients with peripheral artery disease. J Vasc Surg 2005;41:610-617.
36 Liehn EA, Merx MW, Postea O, Becher S, Djalali-Talab Y, Shagdarsuren E, Kelm M, Zernecke A, Weber C: Ccr1 deficiency reduces inflammatory remodelling and preserves left ventricular function after myocardial infarction. J Cell Mol Med 2008;12:496-506.

37 Frangogiannis NG: The inflammatory response in myocardial injury, repair; and remodelling. Nat Rev Cardiol 2014;11:255-265.

38 de Jager SC, Kraaijveld AO, Grauss RW, de Jager W, Liem SS, van der Hoeven BI, Prakken BJ, Putter H, van Berkel TJ, Atsma DE, Schalij MJ, Jukema JW, Biessen EA: CCL3 (MIP-1 alpha) levels are elevated during acute coronary syndromes and show strong prognostic power for future ischemic events. J Mol Cell Cardiol 2008;45:446-452.

39 Schuh A, Konschalla S, Kroh A, Schober A, Marx N, Sonmez TT, Zenke M, Sasse A, Liehn EA: Effect of SDF-1 alpha on endogenous mobilized and transplanted stem cells in regeneration after myocardial infarction. Curr Pharm Des 2014;20:1964-1970.

40 Montecucco F, Braunersreuther V, Lenglet S, Delattre BM, Pelli G, Buatois V, Guilhot F, Galan K, Vuilleumier N, Ferlin W, Fischer N, Vallejo JP, Kosco-Vilbois M, Mach F: CC chemokine CCL5 plays a central role impacting infarct size and post-infarction heart failure in mice. Eur Heart J 2012;33:1964-1974.

41 Cai W, Tao J, Zhang X, Tian X, Liu T, Feng X, Bai J, Han Y: Contribution of Homeostatic Chemokines CCL19 and CCL21 and Their Receptor CCR7 to Coronary Artery Disease. Arterioscler Thromb Vasc Biol DOI:10.1161/ATVBAHA.113.303081.

42 Dobaczewski M, Xia Y, Bujak M, Gonzalez-Quesada C, Frangogiannis NG: CCR5 signaling suppresses inflammation and reduces adverse remodeling of the infarcted heart, mediating recruitment of regulatory T cells. Am J Pathol 2010;176:2177-2187.

43 Dobaczewski M, Gonzalez-Quesada C, Frangogiannis NG: The extracellular matrix as a modulator of the inflammatory and reparative response following myocardial infarction. J Mol Cell Cardiol 2010;48:1904-511.

44 Lindsey ML: MMP induction and inhibition in myocardial infarction. Heart Fail Rev 2004;9:7-19.

45 Berry E, Hernandez-Anzaldo S, Gobbi F, Lehner R, Murakami M, Gelb MH, Sassi A, Wang X, Fernandez-Patron C: Matrix metalloproteinase-2 negatively regulates cardiac secreted phospholipase A2 to modulate inflammation and fever. J Am Heart Assoc 2015;4:100186.

46 Iyer RP, Patterson NL, Zouein FA, Ma Y, Dilve V, de Castro Bras LE, Lindsey ML: Early matrix metalloproteinase-12 inhibition worsens post-myocardial infarction cardiac dysfunction by delaying inflammation resolution. Int J Cardiol 2015;185:198-208.

47 Lindsey ML, Zamilpa R: Temporal and spatial expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases following myocardial infarction. Cardiovasc Ther 2012;30:31-41.

48 Van Den Steen PE, Wuys A, Husson SJ, Proost P, Van Damme J, Van Den Steen PE, Wuys A, Husson SJ, Proost P, Van Damme J, Odenakker G: Gelatinase B/MMP-9 and neutrophil collagenase/MMP-8 process the chemokines human GCP-2/CXCL6, ENA-78/CXCL5 and mouse GCP-2/LIX and modulate their physiological activities. Eur J Biochem 2003;270:3739-3749.

49 Kelly D, Cockerill G, Ng LL, Thompson M, Khan S, Samani NJ, Squire IB: Plasma matrix metalloproteinase-9 and left ventricular remodelling after acute myocardial infarction in man: a prospective cohort study. Eur Heart J 2007;28:711-718.

50 van den Borne SW, Cleutjens JP, Hanemaaijer R, Cremers EE, Smits JE, Daemen MJ, Blankesteijn WM: Increased matrix metalloproteinase-8 and -9 activity in patients with infarct rupture after myocardial infarction. Cardiovasc Pathol 2009;18:37-43.

51 Lindsey ML, Escobar GP, Dobrucki LW, Gosnold DW, Bouges S, Mingoa JT, McClister DM, Su H, Gannon J, MacGillivray C, Lee RT, Sinusas AJ, Spinale FG: Matrix metalloproteinase-9 gene deletion facilitates angiogenesis after myocardial infarction. Am J Physiol Heart Circ Physiol 2006;290:H232-239.

52 Moshal KS, Rodriguez WE, Sen U, Tyagi SC: Targeted deletion of MMP-9 attenuates myocardial contractile dysfunction in heart failure. Physiol Res 2008;57:379-384.

53 Li X, Li F, Chu Y, Wang Z, Zhang H, Hu Y, Zhang Y, Wang Z, Wei X, Jian W, Zhang X, Yi F: NOD2 deficiency protects against cardiac remodeling after myocardial infarction in mice. Cell Physiol Biochem 2013;32:1857-1866.

54 Halade GV, Jin YF, Lindsey ML: Matrix metalloproteinase (MMP)-9: a proximal biomarker for cardiac remodeling and a distal biomarker for inflammation. Pharmacol Ther 2013;139:32-40.

55 Banas B, Wornle M, Merkle M, Gonzalez-Rubio M, Schmid H, Kretzler M, Pfeifer M, Perez de Lema G,) Schondorff D: Binding of the chemokine SLC/CCL21 to its receptor CCR7 increases adhesion properties of human mesangial cells. Kidney Int 2004;66:2256-2263.