Ccr5 But Not Ccr1 Deficiency Reduces Development of Diet-Induced Atherosclerosis in Mice


Objective—Chemokines and their receptors are crucially involved in the development of atherosclerotic lesions by directing monocyte and T cell recruitment. The CC-chemokine receptors 1 (CCR1) and 5 (CCR5) expressed on these cells bind chemokines implicated in atherosclerosis, namely CCL5/RANTES. Although general blockade of CCL5 receptors reduces atherosclerosis, specific roles of CCR1 and CCR5 have not been unequivocally determined.

Methods and Results—We provide two independent lines of investigation to dissect the effects of Ccr1 and Ccr5 deletion in apolipoprotein E–deficient (ApoE<sup>−/−</sup>) mice in a collaboration between Aachen/Germany and Geneva/Switzerland. Different strains of ApoE<sup>−/−</sup>Ccr5<sup>−/−</sup> mice, ApoE<sup>−/−</sup>Ccr1<sup>−/−</sup> mice or respective littermates, were fed a high-fat diet for 10 to 12 weeks. Plaque areas were quantified in the aortic roots and thoracoabdominal aortas. Concordantly, both laboratories found that lesion formation was reduced in ApoE<sup>−/−</sup>Ccr5<sup>−/−</sup> mice. Plaque quality and immune cells were assessed by immunohistochemistry or mRNA analysis. Whereas lesional macrophage content, aortic CD4, and Th1-related Tim3 expression were reduced, smooth muscle cell (SMC) content and expression of interleukin-10 in plaques, lesional SMCs, and splenocytes were elevated. Protection against lesion formation by Ccr5 deficiency was sustained over 22 weeks of high-fat diet or over 26 weeks of chow diet. Conversely, plaque area, T cell, and interferon-γ content were increased in ApoE<sup>−/−</sup>Ccr1<sup>−/−</sup> mice.

Conclusion—Genetic deletion of Ccr5 but not Ccr1 in ApoE<sup>−/−</sup> mice protects from diet-induced atherosclerosis, associated with a more stable plaque phenotype, reduced mononuclear cell infiltration, Th1-type immune responses, and increased interleukin-10 expression. This corroborates CCR5 as a promising therapeutic target. (Arterioscler Thromb Vasc Biol. 2007;27:373-379.)

Key Words: atherosclerosis ■ chemokine receptors ■ inflammation ■ macrophage ■ T lymphocyte

Atherosclerosis is a chronic immunoinflammatory disease characterized by the formation of arterial lesions composed of lipids, fibrous elements, and immune cell infiltrates. Endothelial dysfunction attributable to different risk factors, such as diabetes, hypercholesterolemia, smoking, or hypertension, triggers the recruitment of lymphocytes and monocytes to the intima. Damaged endothelial cells (ECs) express and secrete proinflammatory molecules that can activate and attract leukocytes from the circulation to the nascent lesion. By secreting cytokines and chemokines, emigrated macrophages and lymphocytes induce smooth muscle cell (SMC) immigration to the intima and perpetuate the chronic inflammatory process through extravasation and activation of additional leukocytes. This continued influx of inflammatory cells from the circulation into the arterial intima is a critical determinant throughout different stages of atherogenesis and thus presents an attractive therapeutic target.6,7

Chemokines are small chemoattractant cytokines, bind to G protein–coupled receptors with 7 transmembrane domains, and attract leukocytes to sites of inflammation by triggering firm adhesion and directing transendothelial migration.6,7 In particular, this is instrumental in mediating distinct steps of leukocyte recruitment to atherosclerotic lesions.6,8 The chemokine (C-C motif) receptors Ccr1 and Ccr5 bind to chemokines present in arterial plaques, eg, CCL5/regulated on activation normal T cell expressed and secreted (RANTES), CCL3/macrophage inflammatory protein (MIP)-1α, or CCL4/MIP-1β.6,7 CCR1 and CCR5 are expressed on various cell types implicated in atherosclerosis, eg, monocytes/macrophages, T lymphocytes, or Th1-type cells, and are special-
ized in mediating RANTES-triggered arrest and transendothelial diapedesis of these cells. Further, to ligand binding and leukocyte attraction, these receptors can induce activation and detrimental effects of emigrated cells at the lesion sites. RANTES is detectable in atherosclerotic plaques and deposited on early atherosclerotic endothelium by activated platelets, where it triggers arrest of circulating monocytes. Blocking Ccr1 and Ccr5 by administration of Met-RANTES, a peptidic RANTES receptor antagonist, prevents RANTES-triggered monocyte arrest and modulates the inflammatory process during atherogenesis. Indeed, mice treated with Met-RANTES showed a reduction in atherosclerotic lesion formation and a more stable plaque phenotype. Although Ccr5 has been implicated in immunoinflammatory processes, favoring a Th1-type immune response, it has been shown that Ccr5 deletion in ApoE−/− mice fed a normal diet did not reduce spontaneous formation of early-stage atherosclerotic lesions. In contrast, recent data revealed that reconstitution of Ldrl−/− mice with Ccr5−/− bone marrow improved atherosclerotic plaque quality with little effects on lesion size. In the same model, deficiency in bone marrow Ccr1 did not protect but enhanced atherosclerosis progression in Ldrl−/− mice. Similarly, deletion of Ccr5 but not Ccr1 led to a reduction in neointima formation after arterial wire-injury in ApoE−/− mice. However, the specific role of Ccr5 and Ccr1 in diet-induced atherosclerosis remains to be determined. Hence, we decided to engage in two independently initiated lines of investigation to test the impact of Ccr5 deletion on the diet-induced development of more advanced lesions in ApoE−/− mice, leading to a collaborative effort of two groups in Geneva (Switzerland) and Aachen (Germany). In Geneva, ApoE−/− Ccr5−/− mice were generated using Ccr5−/− mice reported by Luckow et al (which are protected against transplant arteriopathy) and fed a high-cholesterol diet for 10 weeks. In Aachen, ApoE−/− Ccr5−/− mice were independently generated using Ccr5−/− mice reported by Kuziel et al and fed a high-fat diet for 12 to 22 weeks or chow diet for 26 weeks, including ApoE−/− Ccr1−/− mice in a comparative analysis. To strengthen the validity of our results, we performed a mutually unbiased data analysis and consented to share and combine our data into the present report. Our results clearly illustrate that deficiency in Ccr5 but not Ccr1 protects against atherosclerotic lesion formation and cell accumulation.

**Materials and Methods**

**Animals**
Ccr5−/− and Ccr1−/− mice were kindly provided by Drs W.A. Kuziel and P.M. Murphy, respectively, and crossed with ApoE−/− mice in Aachen. ApoE−/− Ccr1−/− and ApoE−/− Ccr5−/− mice and respective ApoE−/− littermates were in a C57BL/6J background. In Geneva, ApoE−/− Ccr5−/− mice were generated by crossing C57−/− mice, backcrossed for 10 generations to C57BL/6NCrI (Charles River) with ApoE−/− mice in a C57BL/6J background. Genotyping for Ccr5 and ApoE was performed by polymerase chain reaction analysis of DNA extracted from tail biopsies. One forward and 2 reverse primers were used for Ccr5 genotyping. Ccr5 WT/KO for: 5′-GATGTTCTTCAAGGTTCAAG-3′, Ccr5 WT rev 5′-GTCACAGTCCAGTTCACAAG-3′ (1076 bp); Ccr5 KO rev: 5′-CCCCTCATCCTGTTCACTCC-3′ (745 bp).

As a model of atherosclerosis, 9- to 12-week-old female littermate mice were fed a high-cholesterol diet (20.1% fat, 1.25% cholesterol; No. D12108, Research Diets) for 10 weeks (Geneva), a high-fat diet (21% fat; 0.15% cholesterol; Altromin, also used in ApoE−/− Ccr5−/−) for 12 or 22 weeks or a Chow diet for 26 weeks (Aachen). Mice were euthanized for histological analysis, proliferation, cytokine assays (n=8 per group for 12 weeks in Aachen; n=6 per group for 22 to 26 weeks in Aachen; n=6 for ApoE−/−, n=9 for ApoE−/− Ccr5−/− in Geneva) or mRNA analysis. All mice (ApoE−/−, ApoE−/− Ccr1−/−, ApoE−/− Ccr5−/−) were healthy without signs of disease during the study. At the end, weight was determined and blood was collected. Leukocyte counts and lipid levels were determined by routine methods. All animal studies were approved by local authorities.

**Atherosclerotic Lesion Quantification**

The extent of atherosclerosis was assessed in aortic roots and thoracoabdominal aortas by staining for lipid deposition with oil-red-O, as previously described and quantified by computerized image analysis (MetaMorph v6.0, Universal Imaging Corporation or Diskus software). Briefly, atherosclerotic lesion areas were measured in 5-μm transversal sections through the heart and aortic roots. For each aortic root, the average of oil-red-O-stained areas from 6 sections separated by 50 μm from each other was calculated. The thoracoabdominal aorta was opened longitudinally along the ventral midline, and lesion areas in each preparation were stained with oil-red-O. The percentage of lipid deposition was calculated by dividing the stained area by the total thoracoabdominal aortic surface.

**Immunhistochemical Analysis**

Aortic root sections were reacted with mAb to MOMA-2 (MCA519G; Serotec), smoothelin (N-15), CD3-e (48 to 2B), IL-10 (M-18; all Santa Cruz) for quantification of cell contents; and with mAb to CCR5 (MAB1800), IFN-γ (MAB4851), R&D, or α-smooth muscle actin/SMA (1A4; Dako) for immunostaining. Binding of primary antibodies was detected with fluorescein isothiocyanate- or PE-conjugated Abs (Sigma) or alkaline phosphatase substrate (Vector Labs). After subtracting isotype controls, specific immunostaining was expressed as percentage of disease during the study. At the end, weight was determined and blood was collected.

**Messenger RNA Expression Analysis**

Total murine RNA was extracted from the aorta (from the beginning of the aortic arch to the iliac bifurcation) using TRI Reagent (MRC Inc) according to the manufacturer’s instructions. Reverse transcription was performed using Omniscript Kit (Qiagen) to obtain cDNA. Real-time PCR (ABI Prism 7000 Sequence Detection System, Applied Biosystems) was used to determine mRNA levels for Tim3 (T lymphocyte immunoglobulin domain, mucin domain) to detect proinflammatory Th1 cells, for Gata3, a Th2 lymphocyte cell-specific transcription factor, for collagenase/elastase digestion, cultured in SMC growth.
medium 2 (PromoCell), and used between passage 2 to 9. Proliferation was assessed using a CyQUANT NF cell proliferation assay kit (Molecular Probes). Total RNA was isolated with RNase-Free Micro isolation kit (Ambion) and reverse-transcribed into cDNA by Mo-MLV RT (Invitrogen). Real-time PCR (MJ Research Opticon) was performed using the DNA-binding dye SYBR Green I for detecting products and primers for IL-10 (rev 5′-TGAGGAATGAGTGCAGTATTG-3′, for 5′-TGACTACCAAGGCAAGATTGTCAGC-3′), for 5′-CACTCAAGATGGTCAGC-3′). Levels of IL-10 mRNA relative to GAPDH were calculated by comparative Ct methods.

Statistical Analysis
Data are mean±SEM. Analysis by $t$ test or nonparametric Mann–Whitney test was performed with GraphPad Prism4 or SigmaStat v3.1. Differences with $P<0.05$ were regarded significant.

Results
Effect of Ccr5 Deficiency on Atherosclerotic Plaque Development
The role of Ccr5 in atherosclerosis was independently investigated in two arms of the study using different strains of ApoE$^{-/-}$ mice in Aachen and Geneva. After 12 weeks of high-fat diet (Aachen) or 10 weeks of high-cholesterol diet (Geneva), female ApoE$^{-/-}$/Ccr5$^{-/-}$ and ApoE$^{-/-}$ mice did not significantly differ in weight or lipid levels (supplemental Table I, available online at http://atvb.ahajournals.org). Atherosclerotic lesions were stained for lipid deposition by oil-red-O and quantified in the aortic roots and the thoracoabdominal aortas. Results from Aachen revealed that plaque areas were substantially reduced (by $>50\%$) in the aortic root and in the thoracoabdominal aorta of ApoE$^{-/-}$/Ccr5$^{-/-}$ mice as compared with ApoE$^{-/-}$ littermates (Figure 1A). In striking accordance, the data independently obtained in Geneva demonstrated identical effects. Female ApoE$^{-/-}$/Ccr5$^{-/-}$ mice revealed a marked reduction of atherosclerotic lesion area for both aortic roots and thoracoabdominal aortas, corresponding to a $>50\%$ reduction (Figure 1B). Figure 1C depicts representative images of thoracoabdominal aortas obtained in Aachen or Geneva. Strikingly, the reduction of lesion areas in ApoE$^{-/-}$/Ccr5$^{-/-}$ mice was sustained at later time points (22 weeks) and was also observed when feeding a chow diet (for 26 weeks) in both the aortic root and the thoracoabdominal aorta (Figure 1D).

Cellular Composition of Atherosclerotic Lesions
The cellular composition of atherosclerotic lesions rather than plaque size appears crucial in both atherogenesis and plaque rupture. To investigate the influence of Ccr5 deletion on the atherosclerotic lesion quality, quantitative immunohistochemistry analysis on aortic roots was performed. Notably, the content of monocytes/macrophages (Figure 2A) and T lymphocytes (Figure 2B) was reduced by 77% and 24%, respectively, in ApoE$^{-/-}$/Ccr5$^{-/-}$ mice compared with ApoE$^{-/-}$ mice. Similar results were obtained with CD4 (data not shown). Conversely, the content of SMCs, which reflects plaque stability, was significantly increased in plaques of ApoE$^{-/-}$/Ccr5$^{-/-}$ mice compared with ApoE$^{-/-}$ mice (Figure 2C). In parallel, the expression of IL-10, known as an antiinflammatory cytokine, was increased in ApoE$^{-/-}$/Ccr5$^{-/-}$ compared with ApoE$^{-/-}$ littermates (Figure 2D) and was detectable in colocalization with SMCs (Figure 2E). CCR5 was also present in some plaque SMCs of ApoE$^{-/-}$ littermates (Figure 2F). Whereas apoptosis assessed by TUNEL staining was unaltered in plaques of ApoE$^{-/-}$/Ccr5$^{-/-}$ mice (Figure 2G) and proliferation of aortic SMCs isolated from ApoE$^{-/-}$/Ccr5$^{-/-}$ mice was not increased (102.1±8.8% of controls; n=5), their expression of IL-10 transcripts was upregulated (251.9±2.6% of controls; n=3; $P<0.01$). This points to the presence of SMCs with an antiinflammatory phenotype expressing IL-10 as an underlying mechanism.

Messenger RNA Analysis of T Cell Markers and Proinflammatory Mediators
To confirm the findings obtained by immunohistochemical analysis of aortic roots, we next studied cell type–specific transcript expression in the whole thoracoabdominal aorta using quantitative real-time PCR. In line with a reduced CD3$^+$ T cell content, ApoE$^{-/-}$/Ccr5$^{-/-}$ mice showed significantly lower aortic mRNA levels for CD4 than littermate controls (Figure 3A). To determine the different T cell subtypes in more detail, we analyzed subset-specific mRNA expression. These experiments revealed significantly lower mRNA levels for Tim-3, known as a marker of Th1 lymphocyte (Figure 3B), little change in Gata3 (Th2 cell-marker), and lower Foxp3 (regulatory T cell marker) transcript expression in aortic tissue of ApoE$^{-/-}$/Ccr5$^{-/-}$ mice (Figure 3C and 3D). Transcripts for IL-4 were below the detection limit (data not shown).
Proliferation and Cytokine Secretion From Splenocytes and Lymph Node Cells

To test whether atheroprotective effects of Ccr5 deletion were related to an attenuation of the chronic inflammatory reaction to high-fat diet, we performed proliferation assays. Compared with cells from ApoE−/−/Ccr5−/− mice, stimulated splenocytes and lymph node cells from ApoE−/−/Ccr5−/− mice exhibited a significant reduction (by 25%) of their proliferative responses (data not shown). Supernatants were examined for proinflammatory cytokines (IFN-γ, TNF-α) and antiinflammatory cytokines (IL-10, TGF-β) by ELISA. As for the proliferative response, splenocytes from ApoE−/−/Ccr5−/− mice and controls were observed for the secretion of IFN-γ by lymph node cells (Figure 4B) or for TNF-α secretion by either cell type (data not shown). Conversely, deletion of Ccr5 significantly increased the secretion of the antiinflammatory cytokine IL-10 in splenocytes and lymphoid cells (Figure 4C and 4D) but did not alter TGF-β secretion, whereas IL-4 levels were below the detection limit (data not shown). Thus, ApoE−/−/Ccr5−/− mice display attenuated systemic Th1-type immune responses and a shift to a more antiinflammatory cytokine profile, possibly explaining reduced lesion formation.

Comparison With Ccr1-Deficient Mice

To dissect whether the proatherogenic role of RANTES is specifically mediated via Ccr5, as indicated by our data, we comparatively analyzed the involvement of Ccr1 as the other major RANTES receptor. ApoE−/−/Ccr1−/− mice and littermate controls were fed a high-fat diet for 12 weeks. Weight and lipid levels did not differ between ApoE−/−/Ccr1−/− and ApoE−/−/Ccr5−/− mice (supplemental Table I). Notably, quantification of lipid-stained atherosclerotic lesion areas revealed a marked increase in plaque size in the aortic root and thoracoabdominal aorta of ApoE−/−/Ccr1−/− versus control mice (Figure 5A and 5B). Moreover, quantitative immunohistochemistry demonstrated a significant increase in CD3+ lymphocytes and in IFN-γ-expressing cells (Figure 5C and 5D), suggestive of a predominant Th1-type infiltrate, and little change in SMC content, whereas macrophage content and IL-10 expression were not significantly affected (Figure 5E through 5G). Hence, deletion of Ccr1 results in a remarkably distinct and rather contrary phenotype with increased atherosclerosis and T cell infiltration.

Discussion

Given the emerging role of chemokines and their receptors in immunoinflammatory diseases, particularly in the pathogenesis of atherosclerosis, it is expected that the role of Ccr5 in atherogenesis would be further elucidated by additional studies. The results obtained in this study provide a framework for understanding the role of Ccr5 in the development and progression of atherosclerosis. The findings also suggest that targeted therapies aimed at modulating the Ccr5 axis may represent a potential strategy for the prevention and treatment of atherosclerosis. Further investigations are needed to confirm these observations and to explore the clinical relevance of these findings.
of atherosclerosis,2 and the importance of RANTES in lesion development,12,13 we were prompted to clarify the involvement of the RANTES receptors Ccr5 or Ccr1 by genetic deletion in atherosclerosis-prone mouse models. Although deficiency in Ccr5 has been described to leave early stages of spontaneous atherogenesis in ApoE−/−/− mice unaffected17 and the absence of bone marrow Ccr5 reduced macrophage infiltration with only minor effects on lesion area in Ldlr−/−/− mice,19 this issue has not been conclusively addressed. Herein, ApoE−/−/− mice, ApoE−/−/−Ccr5−/−/−, or ApoE−/−/−Ccr1−/−/− mice were fed a high-cholesterol or high-fat diet for 10 to 12 weeks. This treatment is known to induce atherosclerotic plaques along the thoracoabdominal aorta and more advanced lesions in the aortic root,26 and results in a more severe phenotype of atherosclerosis than in previously used models. In case of Ccr5, we have also taken advantage of a unique set-up, which allowed us to combine independently obtained data on different ApoE−/−/− mice into the present report, and to evaluate lesion formation at later time points (22 weeks with high-fat diet or 26 weeks with chow-diet).

Our results unequivocally demonstrate that the genetic deletion of Ccr5 in ApoE−/−/− mice substantially reduced atherosclerotic plaque size in the thoracoabdominal aorta and in the aortic root in both arms (Aachen and Geneva) of the study. Notably, the protective effect of CCR5 deficiency was also sustained at later time points and was observed with a high-fat diet and with a normal chow-diet. In addition, we found a significant improvement of plaque quality toward a more stable phenotype in the aortic root of ApoE−/−/−Ccr5−/−/− mice, as evident by reduced T cell/monocyte infiltration and higher SMC and IL-10 content. This may lead to a less inflammatory status and lower susceptibility to rupture and is in accordance with reduced matrix protease expression, increased collagen and IL-10 content in Ccr5−/−/−Ldlr−/−/− chimeras, in arterial grafts and in neointimal lesions of Ccr5−/−/− mice.18,20,22

Considering that most T helper lymphocytes present within atherosclerotic lesions are of the Th1 subpopulation and can secrete proinflammatory cytokines,2 we hypothesized that Ccr5 deletion would reverse the proinflammatory dysbalance by shifting the content of immune and particular T cell subtypes within atherosclerotic plaques. Indeed, mice bearing the deficiency in Ccr5 displayed reduced aortic content of total T helper lymphocytes, as shown by expression of respective markers. Further subanalysis revealed that this was mainly related to a decrease in proinflammatory Th1-type lymphocytes, whereas Th2-type lymphocytes and regulatory T cells with antiinflammatory properties were not affected.

When assessing the systemic inflammatory response by cell proliferation and cytokine analysis, cells isolated from

Figure 4. Secretion of index cytokines by splenocytes and lymph node cells. The secretion of proinflammatory IFN-γ (A and B) and antiinflammatory IL-10 (C and D) was measured by ELISA in splenocytes (A and C) and lymph node cells (B and D) from ApoE−/− Ccr5−/− and ApoE−/− mice. *P<0.05.

Figure 5. Effect of Ccr1 deficiency on atherosclerotic plaque development. Female ApoE−/−Ccr1−/− and ApoE−/− mice were fed a high-fat-diet for 12 weeks, aortic roots (A) and thoracoabdominal aortas (B) were stained for lipids, and lesion areas were quantified by planimetry. The composition of plaques was analyzed by immunohistochemistry for the content of T cells (CD3, C), IFN-γ (D, counter-staining of nuclei with DAPI), SMCs (smoothelin, E), macrophages (MOMA-2, F), and IL-10 (G). *P<0.05.
the spleen or lymph nodes of ApoE−/−Ccr5−/− mice displayed less proliferation and secretion of the proinflammatory cytokine IFN-γ compared with controls. This was accompanied by increased expression of antiinflammatory IL-10 in SMCs, confirming a shift of the immune balance toward a more atheroprotective state in ApoE−/−Ccr5−/− mice.20

The role of RANTES in atherogenic recruitment after deposition by activated platelets or microparticles has been well documented.12,28 Notably, blocking Ccl5/RANTES receptors, which reduced atherosclerotic lesions and stabilized plaque composition, had implicated this chemokine in the development and progression of atherosclerotic lesions.13 Because it remained unclear which receptor is most important in mediating the effects of RANTES in atherosclerosis, we investigated the role of another RANTES receptor present on the surface of leukocytes, which shares several ligands with Ccr5. Surprisingly, rather than reducing atherogenesis, deletion of Ccr1 increased atherosclerotic lesion development and T cell infiltration. Although specialized functions of Ccr1 and Ccr5 in mediating distinct steps of RANTES-triggered recruitment of monocytes and activated T cells have been shown, the differential involvement in atherosclerosis is more likely attributable to contrary effects on the Th1/Th2 balance, with Ccr1 deletion favoring a proatherogenic Th1-type response.20,21

In line with our data, clinical reports have shown that patients with a CCR5 deletion allele are protected against advanced coronary artery disease or myocardial infarction.29,30 Likewise, Ccr5 deficiency attenuated wire-induced neointima formation, macrophage and T cell infiltration attributable to an upregulation of antiinflammatory IL-10.17 Here, we demonstrated the effect of Ccr5 deletion on the recruitment of different lymphocyte subsets. Infiltrating T cells of a CD4+ subtype are present throughout all stages of atherosclerosis and specialized to recognize antigens in association with MHC class II proteins. Within lesions, disease-related antigens such as oxidized LDL or heat-shock protein 60 may enhance immune responses by CD4+ T cells.31,32 The inflammatory context regulated by antigen and cytokine concentrations determines the fate of developing Th cells toward the Th1 or Th2 lineage33 with principal inducers being IL-12 for Th1 cells and IL-10 for Th2 cells.34 Th1 cells responsible of cell-mediated immunity are the most prevalent subtype of CD4+ cells in atherosclerotic lesions, activate macrophages, and initiate inflammatory responses by secreting IFN-γ,35 whereas Th2 cells can provide help for Ab production by B cells and balance Th1 responses by secreting IL-4 or IL-10.36 Deletion of Ccr5 reduced the secretion of proinflammatory IFN-γ by immune cells and enhanced expression of antiinflammatory IL-10, thus counteracting the Th1/Th2 disequilibrium of atherosclerotic inflammation. Given that SMCs constitute a major source for IL-10 in the lesions, and that Ccr5 deletion in bone marrow cells had little effect on plaque area,18 it is conceivable that Ccr5 deficiency in nonhematopoietic cells, namely in Ccr5+ SMCs,37 recruited to the lesions accounts for a main part of the antiinflammatory protection.

In contrast, Ccr1 appeared to play a protective role in atherosclerosis, thus confirming findings by Potteaux et al that atherosclerosis development is enhanced in mice with Ccr1−/− bone marrow cells.19 Together, this implies that the Ccr1-mediated protection is attributable to its expression in hematopoietic cells, possibly regulating the trafficking of macrophage and dendritic cell subsets, rather than in local plaque cells. The protective role of Ccr1 has also been shown in a model of arterial injury, where Ccr1 deletion induced a shift toward a Th1 response with an increase in lesional IFN-γ and a decrease of CD4/IL-10-positive cells.20

The antipodal effects of Ccr1 and Ccr5 deletion are quite surprising. Indeed, both are expressed on lymphocytes and macrophages and share several ligands. The function of chemokines as agonist for one receptor and antagonist for another may potentially explain the contrary influence of Ccr1 or Ccr5 deletion on the development of atherosclerosis. Such mechanisms could affect the Th1/Th2 balance as seen for CCL26/eotaxin-3, a CC chemokine that binds CCR3 and CCR2. Through interaction with CCR3, eotaxin-3 attracts eosinophils, basophils, and Th2 cells, but acts as an antagonist for monocyte recruitment through CCR2.38 It is conceivable that shared ligands may exert differential responses via CCR1 or CCR5.

In conclusion, we independently show in two parallel studies (in Aachen and Geneva) that deletion of Ccr5 reduced atherosclerotic lesion extent accompanied by a more stable plaque quality. These benefits were attributable to an attenuation of the proinflammatory milieu in the lesions and to a shift of the T helper cell subsets away from a Th1-type response, possibly mediated by increased secretion of IL-10. In contrast, lack of Ccr1 enhanced atherosclerotic plaque development and exacerbated T lymphocyte recruitment. Therefore, therapies aiming at a selective blockade of Ccr5 but not Ccr1 may be a promising approach to prevent ongoing inflammatory processes sustaining or aggravating atherosclerosis. Indeed, the HIV entry inhibitor and Ccr5 antagonist TAK-779 has recently been found to attenuate atherosclerosis in Ldlr−/− mice,39 confirming CCR5 as a conjunct target for HIV therapy and atherosclerosis.40

Acknowledgments
The technical assistance of M. Roller, M. Garbe, S. Wilbertz, and S. Chilla is highly appreciated.

Sources of Funding
This work was supported by Deutsche Forschungsgemeinschaft (WE1913/5-2, WE1913/7-1 to C.W., LU612/4-1, GRK438 to B.L.), by the Interdisciplinary Center For Clinical Research Biomat (A.Z., C.W.), and by the Swiss National Science Foundation (F.M.). The authors (V.B., S.S., F.B., G.P., F.M.) belong to the European Vascular Genomics Network (http://www.evgn.org) a Network of Excellence supported by the European Community’s sixth Framework Program for Research Priority 1 “Life sciences, genomics and biotechnology for health”.

Disclosures
None.

References


Ccr5 But Not Ccr1 Deficiency Reduces Development of Diet-Induced Atherosclerosis in Mice


Arterioscler Thromb Vasc Biol. 2007;27:373-379; originally published online November 30, 2006;
doi: 10.1161/01.ATV.0000253886.44609.ae

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/27/2/373

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2006/11/30/01.ATV.0000253886.44609.ae.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
# Online Data Supplement

## Table 1
Weights and lipid levels after 12 weeks of high fat diet

<table>
<thead>
<tr>
<th></th>
<th>weight (g)</th>
<th>total cholesterol (mg/dL)</th>
<th>triglycerides (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apoe&lt;sup&gt;-/-&lt;/sup&gt;Ccr5&lt;sup&gt;+/+&lt;/sup&gt; (Geneva)</strong></td>
<td>22.3±0.4</td>
<td>700.3±52.6</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Apoe&lt;sup&gt;-/-&lt;/sup&gt;Ccr5&lt;sup&gt;-/-&lt;/sup&gt; (Geneva)</strong></td>
<td>22.9±2.0</td>
<td>678.9±25.6</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Apoe&lt;sup&gt;-/-&lt;/sup&gt;Ccr5&lt;sup&gt;+/+&lt;/sup&gt; (Aachen)</strong></td>
<td>23.3±0.4</td>
<td>934.8±32.4</td>
<td>56.3±12.8</td>
</tr>
<tr>
<td><strong>Apoe&lt;sup&gt;-/-&lt;/sup&gt;Ccr5&lt;sup&gt;-/-&lt;/sup&gt; (Aachen)</strong></td>
<td>26.5±1.2</td>
<td>800.7±110.7</td>
<td>61.6±7.0</td>
</tr>
<tr>
<td><strong>Apoe&lt;sup&gt;-/-&lt;/sup&gt;Ccr1&lt;sup&gt;+/+&lt;/sup&gt; (Aachen)</strong></td>
<td>27.4±1.9</td>
<td>667.0±38.0</td>
<td>54.3±5.2</td>
</tr>
<tr>
<td><strong>Apoe&lt;sup&gt;-/-&lt;/sup&gt;Ccr1&lt;sup&gt;-/-&lt;/sup&gt; (Aachen)</strong></td>
<td>24.3±0.9</td>
<td>708.3±47.4</td>
<td>75.3±6.7</td>
</tr>
</tbody>
</table>

n.d. not determined