Chemokines
Key Regulators of Mononuclear Cell Recruitment in Atherosclerotic Vascular Disease

Christian Weber, Andreas Schober, Alma Zernecke

Abstract—Understanding the increasingly complex role of chemokines in various manifestations of atherosclerotic vascular disease and the apparent redundancy in their expression requires improved concepts defining the specialization and cooperation of chemokines in regulating the recruitment of mononuclear cells to vascular lesions. In an attempt to elaborate such models, this review highlights recent insights into the functional role of chemokines in mediating distinct steps during the atherogenic recruitment of monocytes and T cells obtained in genetically deficient mice and in suitable models. A particular focus is placed on the contribution of platelet chemokines deposited on endothelium for monocyte arrest, on differences in the involvement of chemokines between recruitment to native lesions and to neointimal lesions after arterial injury, and on closely related functions of macrophage migration inhibitory factor, a cytokine with considerable structural homology to chemokines. As an evolving aspect of atherosclerotic vascular disease, a role of chemokines, foremost stromal cell-derived factor-1, in the recruitment of mononuclear progenitors of vascular cells during neointimal hyperplasia, endothelial recovery, and angiogenesis is discussed. The functional diversity and pleiotropy of chemokines in and beyond mononuclear cell recruitment awaits further elucidation to enable therapeutic targeting of atherogenesis by context-specific blockade of nonoverlapping chemokine receptor pairs. (Arterioscler Thromb Vasc Biol. 2004;24:1997-2008.)

Key Words: atherosclerosis ■ chemokines ■ leukocytes ■ receptors

Inflammation has emerged as a crucial force driving the initiation and progression of atherosclerotic lesion formation. Hypercholesterolemia as the best-documented risk factor contributing to atherogenesis instigates early endothelial activation or dysfunction accompanied by the expression of adhesion molecules and chemokines, and thereby leads to early subintimal infiltration with mononuclear cells, the first morphological sign of inflammation in the arteries. The mononuclear cells found in the lesions comprise ≈80% monocyte-derived macrophages, which transform into foam cells characteristic for fatty-streak lesions, and ≈10% to 20% lymphocytes predominantly of the Th1 helper subtype of T memory cells. In addition, the presence of mast cells and of dendritic cells interacting with T cells has been convincingly revealed in atherosclerotic lesions. Recently, the recruitment of a blood-borne progenitor cell subpopulation eventually giving rise to neointimal smooth muscle cells (SMC) and endothelial cells (EC) has been directly demonstrated in various models of atherosclerosis.

The nonrandom attraction of mononuclear cells to specific tissue targets is governed by sequential steps in the interaction with the vessel wall, namely rolling mediated by selectin-carbohydrate interactions, integrin-dependent arrest, and transendothelial diapedesis triggered by chemokines. The refinement of this multistep model has revealed that these functions are to some degree mutually overlapping. For instance, integrins can mediate rolling interactions, whereas selectin interactions can serve as a prerequisite for transmigration, and some chemokines, such as fractalkine, can mediate arrest independent of and possibly before integrin interactions. The immobilization of chemokines to endothelial surface proteoglycans appears crucial for the efficacy of their arrest function and has also been implicated in the migratory response under shear flow (chemorheotaxis). This review discusses the role of chemokines in mononuclear cell recruitment to native atherosclerotic lesions and distinctive differences in their contribution to neointimal recruitment after vascular injury.

Chemokine Expression in Atherosclerotic Lesions
Chemokines constitute a family of structurally related and secretable basic chemotactic cytokines, which are classified in subgroups (CC, CXC, C, CXXXC) according to the position of the N-terminal cysteines. As the first and prototypic CC chemokine, MCP-1 (CCL2) has been detected in...
human atherosclerotic lesions and is induced primarily in mediol and neointimal SMC as well as in monocytes/macrophages in animal models of atherosclerosis with dietary-induced hypercholesterolemia and after acute vascular injury.10–13 The CC chemokines TARC (CCL17), PARC (CCL18), and MDC (CCL22) have been identified in macrophage-rich areas of atherosclerotic lesions,14–16 whereas ELC (CCL19) expression has been detected in both SMC and monocyte-derived macrophages of human plaques.16 Also, the presence of the typically T cell–derived CC chemokines MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5), and I-309 (CCL1) could be demonstrated in atherosclerotic plaques.17–19 A novel pathway of vascular inflammation has also been suggested by identifying the overexpression of eotaxin and its receptor CCR3 in atherosclerotic lesions.20 However, CXC chemokines with (IL-8/CXCL8, GRO-/H9251α/CXCL1) or without ELR motif (Mig/CXCL9, IP10/CXCL10, I-TAC/CXCL11, SDF-/H9251α/CXCL12) and the transmembrane chemokines CXCL16 and fractalkine (CX3CL1) are detectable in atherosclerotic lesions.16,21–28 The expression, cellular localization, and target cells of chemokines in atherosclerotic plaques, their abbreviations, and functional involvement in mouse models of atherosclerosis or arterial injury are summarized in Tables 1 and 2. These data emphasize the variety and abundance of chemokines expressed in atherosclerotic lesions.

### Genetic Deletion of Chemokines and Their Receptors in Murine Atherosclerosis Models

Given the pivotal role of monocytes in the process of lesion formation, it was not surprising that the direct evidence for the critical function of chemokines in atherogenesis came from the genetic deletion of the CC chemokine MCP-1 or its receptor CCR2, which mediate the attraction of monocytes but not neutrophils. The absence of MCP-1 or CCR2 in an atherogenic, ie, either low-density lipoprotein receptor-deficient (LDLR-/H9251−) or apolipoprotein E-deficient (apoE−/−) background protects mice from the development of atherosclerotic lesions.29–33 The fact that MCP-1 was the first chemokine shown to play a pivotal role in atherogenesis likely reflects the fact that MCP-1−/− mice are viable and that effects of MCP-1 are mediated through a single receptor, CCR2. Transplantation of apoE-Leiden mice with bone marrow deficient in CCR2 confirmed the role of CCR2 expressed on monocytes.32 Conversely, mice deficient in KC and CXCR2 are not viable or extremely susceptible to infection, respectively. As an alternative to genetic recombination, the repopulation of atherosclerosis-prone LDLR-/− mice with bone marrow deficiency in CXCR2, the receptor for the neutrophil chemokines IL-8 and GRO-α, resulted in a substantial reduction of atherosclerosis.33 Because neutrophils are not present in atherosclerotic lesions, this study implied an involvement of CXCR2 in the atherogenic recruitment of monocytes or other bone marrow–derived cells. CXCR2 is expressed on monocytes,34,35 and the CXCR2 ligands IL-8 and GRO-α have been shown to enhance adhesion of monocytes to matrix proteins or EC activated with modified lipoproteins, respectively.36,37

Moreover, these ELR CXC chemokines have been implicated in angiogenesis,38,39 and by promoting plaque neo-vascularization, this may provide an alternative explanation for the contribution of CXCR2 to atherosclerosis. More recently, the genetic deletion of CCR2 and/or CXCR3 has suggested nonredundant roles of these receptors in the formation of atherosclerotic lesions.40 Moreover, fractalkine is expressed in lesional EC and most robustly in SMC located directly beneath lesional macrophages, but not in macrophages themselves.25,26 and genetic deletion of its receptor CXCR1 reduced macrophage infiltration and retarded lesion development in the aorta to convincingly implicate CXCR1 in atherogenesis.41 Although the targeted disruption of the RANTES receptor CCR5 failed to protect from atherosclerotic lesion formation,42 data on a deletion of the other RANTES receptors CCR1 and CCR3 are not yet available.

### Table 1. Abbreviations Used

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADAM</td>
<td>Disintegrin and metalloproteinase domain</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>apoE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>CCL</td>
<td>CC chemokine ligand</td>
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<tr>
<td>CCR</td>
<td>CC chemokine receptor</td>
</tr>
<tr>
<td>CLF</td>
<td>Chemokine-like function</td>
</tr>
<tr>
<td>CTAP-III</td>
<td>Connective tissue-activating peptide III</td>
</tr>
<tr>
<td>CXCL</td>
<td>CC chemokine ligand</td>
</tr>
<tr>
<td>CXCR</td>
<td>CXC chemokine receptor</td>
</tr>
<tr>
<td>EC</td>
<td>Endothelial cell</td>
</tr>
<tr>
<td>ELC</td>
<td>Epstein–Barr virus-induced molecule 1 ligand</td>
</tr>
<tr>
<td>EPC</td>
<td>Endothelial progenitor cell</td>
</tr>
<tr>
<td>GRO</td>
<td>Growth-related oncogene</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon-γ</td>
</tr>
<tr>
<td>IL-8</td>
<td>IL-8</td>
</tr>
<tr>
<td>IP10</td>
<td>IFN-γ-inducible protein 10 kDa</td>
</tr>
<tr>
<td>I-TAC</td>
<td>IFN-inducible T cell alpha chemotactant</td>
</tr>
<tr>
<td>KC</td>
<td>Keratinocyte chemokine</td>
</tr>
<tr>
<td>LDL-R</td>
<td>Low-density lipoprotein receptor</td>
</tr>
<tr>
<td>MCP</td>
<td>Monocyte chemotactic protein</td>
</tr>
<tr>
<td>MDC</td>
<td>Macrophage-derived chemokine</td>
</tr>
<tr>
<td>MIF</td>
<td>Macrophage migration inhibitory factor</td>
</tr>
<tr>
<td>Mig</td>
<td>Monokine induced by IFN-γ</td>
</tr>
<tr>
<td>MIP</td>
<td>Macrophage inflammatory protein</td>
</tr>
<tr>
<td>NAP-2</td>
<td>Neutrophil-activating peptide-2</td>
</tr>
<tr>
<td>PARC</td>
<td>Pulmonary and activation-regulated chemokine</td>
</tr>
<tr>
<td>PF4</td>
<td>Platelet factor 4</td>
</tr>
<tr>
<td>RANTES</td>
<td>Regulated on activation normal T cell expressed and secreted</td>
</tr>
<tr>
<td>SDF-1</td>
<td>Stromal cell-derived factor-1</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
</tr>
<tr>
<td>SR-PSOX</td>
<td>Scavenger receptor for phosphatidyl serin and oxidized lipoprotein</td>
</tr>
<tr>
<td>TARC</td>
<td>Thymus-activation-regulated chemokine</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
</tr>
</tbody>
</table>

Moreover, these ELR CXC chemokines have been implicated in angiogenesis,38,39 and by promoting plaque neo-vascularization, this may provide an alternative explanation for the contribution of CXCR2 to atherosclerosis. More recently, the genetic deletion of CCR2 and/or CXCR3 has suggested nonredundant roles of these receptors in the formation of atherosclerotic lesions.40 Moreover, fractalkine is expressed in lesional EC and most robustly in SMC located directly beneath lesional macrophages, but not in macrophages themselves.25,26 and genetic deletion of its receptor CXCR1 reduced macrophage infiltration and retarded lesion development in the aorta to convincingly implicate CXCR1 in atherogenesis.41 Although the targeted disruption of the RANTES receptor CCR5 failed to protect from atherosclerotic lesion formation,42 data on a deletion of the other RANTES receptors CCR1 and CCR3 are not yet available.
With the apparent redundancy of chemokine expression and function in atherosclerotic lesions in mind, it is not easily conceivable why a deletion of individual chemokines or their receptors would each lead to a marked reduction in lesion formation and is not compensated by other chemokine receptor pairs. This would indicate that MCP-1, IL-8, and fractalkine do not act independently, but rather in concert to efficiently recruit circulating monocytes into lesions, and further insinuates that a functional specialization of chemokines and their receptors at distinct steps of the atherogenic recruitment process exists to allow for nonredundant roles.

Previous evidence suggested that a hierarchical involvement of GRO-α and MCP-1 orchestrates monocyte arrest versus transmigration on activated EC, respectively. Although a preferential immobilization of endogenous GRO-α via endothelial heparan sulfate proteoglycans stipulated its functional specialization in arrest on activated endothelium, the MCP-1/CCR2 axis did not appear to be involved in triggering arrest but was well-suited to mediate subsequent transendothelial migration under flow conditions (Figure 1).

This may reflect different patterns in the presentation or directional secretion of MCP-1. The concept that immobilization to specific proteoglycans may lead to a local enrichment of chemokines to achieve concentrations sufficient to trigger arrest is supported by findings that exogenous IL-8 and MCP-1 at high concentrations can induce firm arrest of monocytes on EC under physiological flow. Moreover, a simultaneous requirement of apically presented chemokines and shear flow for transendothelial migration has been demonstrated for certain chemokines and termed chemorheotaxis. Hence, the functional specialization of GRO and MCP-1 may reflect a specific coupling to distinct signaling pathways instrumental in mediating mechanisms either permissive to triggering arrest (eg, integrin clustering) or transmigration/chemorheotaxis. Importantly, this model establishing a division of labor for chemokines could be confirmed in a study on monocyte accumulation on early atherosclerotic endothelium in carotid arteries of apoE−/− mice. Here, KC and CXCR2 were able to capture monocytes in flow, whereas no evidence for an involvement of MCP-1 and CCR2 could be obtained. However, genetic deletion of CXCR2, MCP-1, or CCR2 could provide its inhibition by overexpression of a truncated MCP-1 peptide antagonist protected against atherosclerotic cell recruitment.

### TABLE 2. Chemokines Expressed in Atherosclerosis

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Localization</th>
<th>Target</th>
<th>Receptor</th>
<th>Mouse Model</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>CC Chemokine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-309/CCL1</td>
<td>EC</td>
<td>MM</td>
<td>CCR8</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>MIP-1α/CCL3</td>
<td>TC</td>
<td>MM, TC</td>
<td>CCR1/5</td>
<td>met-RANTES</td>
<td>17</td>
</tr>
<tr>
<td>MIP-1β/CCL4</td>
<td>TC</td>
<td>MM, aTC, SMC</td>
<td>CCR5</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>RANTES/CCL5</td>
<td>TC</td>
<td>MM, aTC, DC</td>
<td>CCR1/3/5</td>
<td>met-RANTES</td>
<td>18, 63, 64, 67</td>
</tr>
<tr>
<td>Eotaxin/CCL11</td>
<td>SMC</td>
<td>B, DC</td>
<td>CCR3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>MCP-4/CCL13</td>
<td>MM, EC</td>
<td>MM, aTC</td>
<td>CCR2/3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>TARC/CCL17</td>
<td>MM, DC</td>
<td>Th2, DC</td>
<td>CCR4</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>PARC/CCL18</td>
<td>MM, DC</td>
<td>aTC</td>
<td>CCR7</td>
<td>14</td>
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</tr>
<tr>
<td>ELC/CCL19</td>
<td>MM, DC</td>
<td>aTC</td>
<td>CCR7</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>MDC/CCL22</td>
<td>MM, DC</td>
<td>aTC</td>
<td>CCR7</td>
<td>16</td>
<td></td>
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<tr>
<td>CXC Chemokine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GRO/KC/CXCL1</td>
<td>MM</td>
<td>MM, EC, SMC</td>
<td>CXCR1/2</td>
<td>KC mAb, CCR2−/−</td>
<td>21, 22, 33, 122</td>
</tr>
<tr>
<td>IL-8/CXCL8</td>
<td>MM</td>
<td>MM, EC, SMC</td>
<td>CXCR1/2</td>
<td>BM-Transplantation</td>
<td></td>
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<tr>
<td>Mig/CXCL9</td>
<td>MM, EC, SMC</td>
<td>aTC</td>
<td>CXCR3</td>
<td>CXCR3−/−</td>
<td>24, 49</td>
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<tr>
<td>IP10/CXCL10</td>
<td></td>
<td>MM</td>
<td>CXCR3</td>
<td>CXCR3−/−</td>
<td>24, 49</td>
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<tr>
<td>I-TAC/CXCL11</td>
<td>MM, EC</td>
<td>MM, aTC, SMC</td>
<td>CXCR3</td>
<td>CXCR3−/−</td>
<td>24, 49</td>
</tr>
<tr>
<td>SDF-1α/CXCL12</td>
<td>MM, SMC</td>
<td>TC, vascular progenitors</td>
<td>CXCR4</td>
<td>SDF-1α mAb</td>
<td>8, 24, 104</td>
</tr>
<tr>
<td>SR-PSOX/CXCL16</td>
<td>MM, DC, SMC</td>
<td></td>
<td>CXCR6</td>
<td></td>
<td>27, 28</td>
</tr>
<tr>
<td>CX3C Chemokine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractalkine/CX3CL1</td>
<td>SMC</td>
<td>MM, aTC</td>
<td>CX6CR1</td>
<td>CX6CR1−/−</td>
<td>16, 25, 26, 40</td>
</tr>
<tr>
<td>CLF</td>
<td>MM, EC, SMC</td>
<td>MM, TC</td>
<td>Unknown</td>
<td>MIF mAb</td>
<td>85, 94–97</td>
</tr>
</tbody>
</table>

EC indicates endothelial cell; MM, monocyte/macrophage; SMC, smooth muscle cell; TC, T cell; NK, natural killer cell; mAb, monoclonal antibody; a, activated; DC, dendritic cell; B, B cell; Th2, T-helper cell; r, resting.

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**Functional Specialization of Chemokines in Atherogenic Monocyte and T Cell Recruitment**

With the apparent redundancy of chemokine expression and function in atherosclerotic lesions in mind, it is not easily conceivable why a deletion of individual chemokines or their receptors would each lead to a marked reduction in lesion formation and is not compensated by other chemokine receptor pairs. This would indicate that MCP-1, IL-8, and fractalkine do not act independently, but rather in concert to efficiently recruit circulating monocytes into lesions, and further insinuates that a functional specialization of chemokines and their receptors at distinct steps of the atherogenic recruitment process exists to allow for nonredundant roles.
Figure 1. Chemokines and receptors involved in mononuclear cell recruitment to native atherogenic lesions. Activated platelets deposit chemokines, such as RANTES or PF4, on the endothelial cell (EC) lining of early atherosclerotic lesions, where RANTES oligomers trigger the CCR1-dependent arrest of monocytes. Endothelial fractalkine (FKN) and GRO/KC immobilized via heparan sulfate proteoglycans induce the firm adhesion of monocytes via CX3CR1 and CXCR2, respectively. MIG and IP-10 expressed and immobilized on EC mediate the arrest of T cells via CXCR3. Smooth muscle cells (SMC) display the chemokines FKN and CXCL16 and secrete MCP-1 as a soluble molecule. MCP-1 (via CCR2) sheds soluble FKN (via CX3CR1) and RANTES (via CCR5) or CXCL16 mediate the subsequent subintimal immigration of monocytes or T cells. Extravasated monocytes can transform into macrophages or differentiate into dendritic cells (DC) expressing CCR7, its ligands ELC, MDC, and TARC, which can recruit CCR4-bearing T cells of the Th2 type. Upregulated in EC and macrophages, MIF has been proposed to contribute to macrophage accumulation.

...prominent role of CCR2 in advanced lesions...40 (F. Mach, personal communication). Nonredundant roles of CCR2 and CXCR3 are also indicated by the more pronounced protection against overall lesion formation in CCR2<sup>−/−</sup> and CXCR3<sup>−/−</sup> apoE<sup>−/−</sup> triple knockout as compared with apoE<sup>−/−</sup> mice or deletion of either receptor in apoE<sup>−/−</sup> mice alone...40 (F. Mach, personal communication). Similarly, it is conceivable from in vitro studies that immobilized SDF-1α may participate in the recruitment of memory T cells on activated EC in flow...45,50 because it may occur in growing lesions.

Fractalkine as a structurally distinct chemokine fused to a transmembrane mucin stalk...51 represents another candidate engaged in atherogenic leukocyte recruitment. Although the transmembrane protein acts as an efficient adhesion molecule capturing monocytes and T cells on activated endothelium under flow conditions by an integrin-independent mechanism...52,53 cleavage by specific metalloproteases, such as ADAM17, of the mucin stalk can produce a soluble form of fractalkine with chemoattractant activity for these cells...54,55 Migration but not arrest induced by fractalkine, although both mediated through CX3CR1, is sensitive to pertussis toxin, supporting the concept that specialized functions in arrest and migration require distinct signal transduction pathways...51–53 Evidence for distinct modes of function depending on its form of presentation may further be derived from the intraluminal localization of fractalkine. When expressed by lesional SMC, fractalkine may be prone to cleavage by metalloproteases into the promigratory soluble form, as insinuated by adjacent macrophage infiltrates.25 In contrast, transmembrane fractalkine on the endothelial surface may be less accessible to metalloproteases and thus predestined to mediate arrest of monocytes (Figure 1). Studies addressing the functions of fractalkine by using noncleavable and membrane-anchored mutants and overexpression of cleaving enzymes, eg, tumor necrosis factor-converting enzyme, are underway. Recently, a novel role for membrane-bound fractalkine has been described in platelet activation and adhesion...56 which may sustain recruitment mechanisms involving the delivery of platelet-derived chemokines as discussed.

As another transmembrane chemokine, CXCL16, which is expressed in dendritic cells, macrophages, and aortic SMC, has been detected in human and murine atherosclerotic lesions...27,28 CXCL16 has been shown to function as a scavenger receptor for oxidized LDL and can be upregulated in monocytes by interferon-γ...27,28,57 Similar to fractalkine, CXCL16 acts as a chemoattractant and adhesion molecule for T cells and natural killer cells expressing its receptor CXCR6 without requiring signal transduction for integrin activation...58,59 The adhesive function of CXCL16 can be enhanced by blocking its shedding from the cell surface. Whereas constitutive shedding can be mediated by the metalloprotease ADAM10, induced shedding, which is presumably more important in the lesion, involves ADAM17.57,60 By analogy, it may be postulated that the recruitment function of transmembrane and soluble CXCL16 for CXCR6-positive T cells resembles the role of fractalkine in monocyte/macrophage recruitment in native atherosclerotic lesions (Figure 1). The functional specialization of these chemokines in atherogenic recruitment summarized and depicted in Figure 1 puts the apparent redundancy into perspective and confers an enormous potential for selective, additive, and possibly synergistic modes of interference with this deleterious process.

**Importance of Platelet Chemokines in Atherogenic Recruitment of Mononuclear Cells**

Platelets represent another source of chemokines and their precursors, which may be involved in the process of atherogenic recruitment. From their α-granules, platelets secrete not only CXC chemokines, such as PF4 (CXCL4) or ENA-78, and precursors for the CXCR2 ligand NAP-2 (CXCL7), such...
as CTAP-III or β-thromboglobulin, but also CC chemokines, such as MIP-1β or RANTES.61 Initially, RANTES expression has been detected in macrophages of human atherosclerotic plaques and arteries with transplant atherosclerosis, and has been implicated in allograft rejection.17,18,62 Recently, the presence of RANTES has been revealed on the luminal surface of carotid arteries with early atherosclerotic endothelium or on neointimal lesions after arterial injury in apoE−/− mice.63 Subsequently, it could be demonstrated that activated platelets can deliver RANTES and PF4 to the endothelial lining of early atherosclerotic and neointimal lesions (Figure 1), as well as to the surface of monocytes via a mechanism involving platelet P-selectin.64,65 The deposition and immobilization of platelet-derived RANTES has been shown to trigger enhanced recruitment of monocytes on activated aortic endothelium but not on surface-adherent platelets.63,64 This may be related to the fact that platelets can also secrete the proteoglycan chondroitin sulfate, which can block the presentation and arrest function of RANTES on cell surfaces, eg, on platelets.66 Notably, a blockade of RANTES with the Met-RANTES receptor antagonist inhibited not only RANTES-mediated arrest in vitro but also neointimal macrophage infiltration and hyperplasia after arterial injury in hyperlipidemic mice.64 The important observation that the intermittent injection of activated, but not P-selectin–deficient, platelets exacerbated lesion formation in apoE−/− mice strongly suggests that mechanisms of P-selectin–mediated chemokine delivery are also relevant to the in vivo pathogenesis of native atherosclerosis.65 The concept that the deposition of RANTES may be an important mechanism underlying the involvement of platelets in native lesion formation is corroborated by findings that the long-term treatment with Met-RANTES reduced atherosclerotic lesion formation in apoE−/− mice.67 Alternatively, this could be explained by a blockade of RANTES produced in mononuclear cells infiltrating the lesions or by modulating other chemokine receptors, eg, by decreasing CCR2 mRNA.67

The hypothesis that beyond specific modes of chemokine presentation the functional specialization may be determined by the intrinsic characteristics of a given receptor has been further tested in a model in which RANTES immobilized on activated endothelium triggers the arrest of leukocytes.63,64 The use of selective receptor antagonists demonstrated that CCR1 but not CCR5 mediates RANTES-induced arrest of monocytes, activated T cells, and Th1 cells expressing different levels of CCR1 and CCR5. In contrast, CCR5 supported spreading along the endothelium, whereas both CCR1 and CCR5 contributed to transendothelial chemotaxis of these cells triggered by RANTES.68 This revealed that the engagement of different receptors by the same chemokine ligand can produce dramatically distinct functions, further extending the selectivity of specialization. In a study using RANTES mutants to define the structural features for its functions in leukocyte recruitment, oligomerization of RANTES was found to be crucial for CCR1-mediated arrest in flow, likely by bridging between surface-bound RANTES and CCR1, but not for CCR5-mediated transmigration.69 In contrast, proteoglycan binding of RANTES was essential for arrest and transmigration. The dichotomy of receptor-specific functions may rely on distinct structural prerequisites, ie, monomers sufficed for chemotaxis but not arrest.

Regarding a functional role of PF4 or β-thromboglobulin secreted by platelets, little is known so far. Previous studies have shown a correlation of PF4 and thromboglobulin plasma levels as platelet activation markers and independent risk factors for carotid atherosclerosis, ie, wall thickness.70 Preliminary data indicate that β-thromboglobulin, depending on its proteolytic conversion to NAP-2, can induce monocyte arrest when presented on activated endothelium, whereas PF4 secreted or deposited by activated platelets may synergistically enhance RANTES-triggered monocyte arrest (C. Weber et al, unpublished data, 2004). This may be caused by heterodimerization with RANTES, alterations of receptor specificity, or enhanced binding to proteoglycans. Alternatively, PF4 effects may be mediated via a spliced variant of CXCR3 described as a novel functional receptor.71 Beyond direct contributions to monocyte recruitment, chemokines such as SDF-1α, MDC, or fractalkine have conversely been involved in platelet activation, which not only may cause aggregation and adhesion but also may sustain degranulation and deposition of platelet chemokines.24,56,72,73 Chemokine-mediated platelet activation, however, strongly depends on the presence of low levels of primary agonists such as ADP or thrombin.56,73 Thus, in comparison to stimulation with these mediators, platelet-activating chemokines rather appear to exert an adjuvant function in amplifying platelet activation and aggregation, or in promoting platelet function under inflammatory conditions.

Different Functions of MCP-1/CCR2 in Neointima Formation and Native Atherosclerosis

Murine models of atherosclerosis have demonstrated the central role of the MCP-1/CCR2 axis in monocyte recruitment and lesion formation.29–31,48 In the context of hyperlipidemia, an induction of MCP-1 expression in SMC12 and an upregulation of CCR2 on monocytes74 have been described, which may account for the over-recruitment on monocytes into the vessel wall.13,75 In hyperlipidemic apoE−/− mice, wire-induced injury of the carotid artery caused a rapid upregulation of MCP-1 levels in serum and in the vessel wall, and MCP-1 staining was detectable in medial SMC but also in platelets adherent to the denuded vessel wall,76 and its inhibition diminished neointimal hyperplasia and macrophage infiltration.77,78 In vitro studies revealed that MCP-1 binds to the surface of adherent platelets and triggers monocyte arrest in flow.78 Hence, this pathway may be relevant for an excessive response to vascular injury. By contrast, a role of MCP-1/CCR2 in macrophage recruitment after arterial injury is less well-established in normolipidemia. Although monocyte accumulation was reduced after arterial cuff placement or stent placement,78,79 neointimal SMC content was decreased and macrophage content was unaffected in CCR2−/− mice or MCP-1 antibody-treated rats after endothelial denudation.80,81 Thus, the function of the MCP-1/CCR2 axis in vascular repair and monocyte recruitment appears to differ between normolipidemic and hyperlipidemic models.
MIF as a Cytokine With Chemokine-Like Function Regulates Plaque Composition

It has recently been proposed to group mediators with similar functional patterns, which cannot be structurally classified into the known chemokine subfamilies, as a family termed chemokine-like function chemokines. Among other members, eg, leukotrienes or fMLP, known to signal via G-protein–coupled receptors, this group includes MIF, a pleiotropic inflammatory T cell and macrophage cytokine, which is involved in immune-mediated diseases, eg, septic shock and chronic inflammation. Although a membrane receptor for MIF is yet to be identified, MIF displays a remarkable homology in its 3-dimensional crystal structure with chemokines, in particular with a dimer of IL-8 and with MIP-3α. Despite a lack of N-terminal cysteines, in support of a chemokine-like function, MIF has been found to desensitize the chemotactic activity of MCP-1 in monocytes and to stimulate cell migration. The pathogenic role of MIF in local tissue inflammation has been attributed to monocyte and T cell recruitment in a model of glomerulonephritis. An upregulation of MIF has been observed in EC, SMC, and macrophages during progression of atherosclerosis in humans and in hypercholesterolemic rabbits. Recent reports have helped to clarify the contribution of MIF to macrophage accumulation and its function in atherosclerotic disease.

The role of MIF in neointimal lesion formation was studied after wire injury of carotid arteries in apoE null mice. MIF expression was upregulated in SMC early after endothelial denudation but predominantly found in EC and macrophage-derived foam cells at later stages. Neutralizing MIF markedly reduced neointimal macrophage content and inhibited transformation into foam cells. Conversely, the content of SMC and collagen in the neointima was increased, amounting to a slight reduction in neointimal area. This reflects a remarkable shift in the cellular composition of neointimal plaques toward a stabilized phenotype. In a study of arterial injury in LDLR null mice, blocking MIF inhibited neointimal hyperplasia and macrophage infiltration, as well as SMC proliferation, confirming an important role of MIF in plaque formation. The genetic deletion of MIF in LDLR null mice has also been
shown to reduce lipid deposition and intimal thickening in the aorta. This retardation of native atherogenesis was accompanied by a decrease in lesional cell proliferation, protease expression, and activity. Although differences in these models may be caused by incomplete blockade by antibody treatment or related to the degree of injury, all data concur by establishing a novel pathway in unstable lesion formation by MIF.

In vitro flow assays revealed that a short-term incubation of aortic EC with MIF triggers monocyte arrest under flow conditions and that monocyte arrest induced by oxidized LDL is mediated by endothelial MIF. This observation supports a model in which MIF directly affects endothelial-monocyte interactions by a novel mechanism resembling the function of immobilized chemokines in native atherogenesis and after injury (Figures 1 and 2). Preliminary data suggest that the induction of arrest is mediated via G-protein–coupled signaling possibly involving chemokine receptors (C. Weber et al., unpublished data, 2004). Thus, the contribution of MIF to athrogenesis may at least in part be caused by a chemokine-like function.

**Crucial Role of SDF-1α in the Neointimal Recruitment of SMC Progenitor Cells**

The CXC chemokine SDF-1α is essential for stem cell mobilization, bone marrow engraftment, and homing, as well as organ system vascularization. It is also expressed in human atherosclerotic plaques and effectively activates platelets in vitro. Because bone marrow–derived cells have been shown to contribute to neointimal SMC content in native atherosclerosis or after arterial injury, and because circulating SMC progenitors have been found in human blood, this was highly suggestive of a participation of SDF-1α in human atherothrombotic disease and the response to vascular trauma. SDF-1α plasma levels were transiently elevated after wire injury of carotid arteries in apoE−/− mice, mediating a marked expansion of sca-1+ lineage peripheral blood progenitor cells. The systemic injection of these cells after injury led to their SDF-1α–dependent recruitment into the lesions, where they adopt an SMC-like phenotype, whereas neutralizing SDF-1α markedly reduced the neointimal area and the relative content of SMC but not macrophages. Thus, SDF-1α plays an instrumental role in neointima formation after injury in apoE−/− mice, attributable to a recruitment of circulating SMC progenitors. Notably, the extent of SDF-1α expression and concomitant recruitment of bone marrow–derived progenitor cells appeared to correlate with the degree of arterial trauma, ie, it was prominently detectable after wire injury but not after cuff placement or ligation.

After arterial wire injury, SDF-1α is highly expressed in medial SMC and only later in a subset of neointimal SMC. This is compatible with the concept that resident medial SMC initially establish SDF-1α expression, migrate into the intima, and constitute a subpopulation of SDF-1α–producing neointimal SMC. Known to share phenotypic characteristics with stromal cells, lesional SMC may provide a niche for immigrating progenitor cells by secreting SDF-1α and/or other factors. Although SDF-1α expression has not been detected in blood cells, it cannot be excluded that progenitor cells recruited to neointimal lesions may serve as an alternative source of SDF-1α when adopting an SMC phenotype. Because increased proteoglycan synthesis after arterial injury may add to the neointimal extracellular matrix, SDF-1α may be released into the circulation early after endothelial denudation, but may be bound to proteoglycans in the developing neointima, shifting its contribution from mobilization toward recruitment of progenitor cells. Beyond the initial arrest of progenitor cells, which appears to be triggered by immobilized SDF-1α in concert with activated platelets adherent at the injury site (C. Weber et al., unpublished data, 2004; see Figure 2), local SDF-1α may affect the neointimal SMC differentiation and architecture, contributing to arterial remodeling. Experiments using mice with bone marrow deficient in CXCR4 or SMC-specific promoters will help to clarify the role of the SDF-1α/CXCR4 axis in neointimal recruitment and differentiation of SMC progenitors.

Interestingly, reduced SDF-1α plasma levels are associated with symptomatic coronary artery disease, suggesting an anti-inflammatory role for SDF-1α in stabilizing the phenotype of native atherosclerotic plaques. It is conceivable that the expression of SDF-1α in native lesions may regulate plaque composition by supporting a chronic influx of SMC progenitors at low levels. In apoE−/− mice, injections of bone marrow–derived progenitor cells, which also respond to SDF-1α, retarded the development of primary atherosclerotic lesions. In contrast to its adverse effects in neointima formation, SDF-1α may thus attenuate the inflammatory progression and rather promote the stabilization of native atherosclerotic lesions.

It has been shown that neointimal SMC, which become luminally exposed after endothelial denudation injury, display a pro-inflammatory phenotype with increased expression of chemokines, thus supporting enhanced recruitment of monocytes and T cells and forming a pseudo-endothelium. Namely, transmembrane fractalkine and immobilized KC triggered the arrest of monocytes, whereas surface-bound SDF-1α was involved in the arrest of activated T cells but not of monocytes (Figure 2). Similarly, increased expression of RANTES by neointimal SMC (C. Weber et al., unpublished data, 2004) may provide an additional explanation for the inhibition of neointima formation after arterial injury in apoE−/− mice. These data extend and substantiate the functional specialization of chemokines by including those expressed on lesional SMC.

**Role of Chemokines in Endothelial Recovery After Arterial Injury and Angiogenesis**

Recent evidence further indicates that modalities that accelerate the re-endothelialization of vascular lesions can reduce neointima formation, eg, after vascular injury. Among the options described to date, gene transfer of vascular endothelial growth factor and treatment with statins or troglitazone have been found to improve endothelial recovery involving progenitor cells and thereby to decrease in-stent restenosis or neointima formation after denudation injury. The intra-
venous transfusion with endothelial progenitor cells (EPC) or spleen-derived mononuclear cells has been directly demonstrated to enhance re-endothelialization and to decrease neointima formation after vascular injury, however, only in splenectomized mice without atherosclerosis. Findings that EPC exhibit a migratory response to SDF-1α,116,117 which may be involved in their lesional recruitment, make it less conceivable that this mechanism would contribute to limiting neointima formation after denudation in the context of atherosclerosis, because SDF-1α is upregulated and blocking SDF-1α markedly inhibits neointima formation after arterial injury in atherosclerotic mice. Nevertheless, neovascularization induced by SDF-1α injection or gene transfer and associated with EPC recruitment in models of myocardial regeneration116–118 should be a focus of further investigation (Figure 2).

A similar conundrum is exposed by a recent report that the intra-arterial application of bone marrow monocyte lineage CD34+/CD14+ cells resulted in their adhesion to injured endothelium after activation with MCP-1 by gene transfer in vivo or by pretreatment in vitro, thereby accelerating re-endothelialization and reducing neointima formation.119 Notably, peripheral blood monocytes did not exhibit marked MCP-1–dependent adhesion or progenitor function, which is in contrast to previous reports but in line with findings on cytokine-activated endothelium. It can be assumed that these findings are rather limited to nonatherosclerotic injury repair, because transplantation of MCP-1–expressing cells or gene transfer of MCP-1 clearly exacerbates atherosclerosis in susceptible models. Similarly, local injection of MCP-1 used to increase collateral formation after arterial occlusion via CCR2-mediated perivascular recruitment of monocytes, induced systemic, ie, aortic plaque progression in apoE−/− mice.120,121

In another interesting contrast between native atherogenesis and vascular injury, we have recently found that blockade of KC inhibits the recruitment of monocytes on early atherosclerotic endothelium46 but enhances neointima formation after injury, caused by effects on endothelial recovery.122 This supports a hypothesis that restenosis may partly result from impaired re-endothelialization. ELR CXC chemokines, especially IL-8, have been implicated in angiogenesis caused by activation of CXCRII on EC or their progenitors38,39 and also by displacing growth factors from endothelial proteoglycans. The effects of blocking KC38 may also be related to interference with similar mechanisms and are not only attributable to direct effects on EPC. Endothelial migration and wound healing after scratch injury in vitro could be delayed by blocking either KC or CXCRII and was promoted by addition of exogenous KC.123 In human atherectomy tissue of native coronary lesions, IL-8 has been identified as an important mediator of angiogenesis and may thereby promote plaque formation.123 Understanding and controlling the delicate balance in the ambivalent effects of chemokines on endothelial recovery and neovascularization as crucial features of plaque formation may hold the key to a successful therapeutic targeting.

Conclusions and Perspectives: Toward Therapeutic Targeting of Chemokines

In a synopsis, it becomes evident that the previously prevailing picture of an apparent functional redundancy of chemokines in atherogenic recruitment has to be abandoned in favor of a highly elaborate specialization and cooperation of multiple chemokines in distinct steps of the recruitment process for different mononuclear subsets and precursors of other vascular cell types. In addition, this may involve cytokines with chemokine-like functions, such as MIF, which may serve a closely related purpose. It should be noted that remarkable differences in the presentation and functional involvement of chemokines in mononuclear cell recruitment have been observed between native atherogenesis and neointima formation after injury. The framework for such a highly elaborated specialization is illustrated in Figures 1 and 2. It cannot be excluded, however, that changes in monocyte infiltration after interference with chemokine receptors may also be caused by an altered equilibrium between influx, proliferation, and apoptosis or survival rather than solely attributable to direct effects on recruitment. This warrants studies into the effects of chemokines on vascular cell homeostasis beyond the recruitment process and may be addressed by experiments using monoclonal antibodies and conditional or inducible knockout models after manifest plaques with significant monocyte infiltration have formed. In addition, this could be directed toward achieving a regression of complicated lesions. The differences in the contribution of certain chemokines, eg, SDF-1α or KC, to neointimal hyperplasia after arterial injury versus progression of native atherosclerotic plaques could be further elaborated or corroborated by the long-term inhibition of these candidates in an atherosclerosis model simultaneously undergoing arterial injury.

Concerning the use of chemokines as therapeutic targets, various chemokine receptor antagonists have been developed that prove to be effective in animal models.48,64 As opposed to targeting other cytokines expressed in atherosclerotic plaque (eg, interferon-γ, tumor necrosis factor-α, or interleukins4,124), which may exert an even more complex and pleiotropic spectrum of actions than chemokines including effects on T cell and monocyte differentiation, chemokine, and protease induction itself, the therapeutic inhibition of specific chemokine receptor functions may harbor the advantage of higher selectivity. However, clinical studies on the effectiveness and safety of such antagonists in the treatment and prevention of human atherosclerosis have been hampered by a lack of suitable surrogate markers for the disease in humans, which could initially substitute for hard end points such as myocardial infarction or death. This may be accomplished by developing advanced and molecular imaging techniques or disease-specific plasma biomarkers. Thus, it is not surprising that current clinical trials evaluating the efficacy of antagonist or antibodies, eg, against CCR1, CCR2, CXCR1, or CXCR4, are mainly restricted to rheumatoid arthritis, which avoids many of these difficulties. An improved identification of patients susceptible to treatment could help to minimize potential side effects of a systemic application. Alternatively, such problems may be alleviated.
by identification of marker molecules enabling therapeutic targeting specific for atherosclerotic or unstable lesions. Taking these caveats into consideration, the prevention of restenosis after arterial injury or stent implantation may be a more appropriate application for assessing the efficacy of chemokine antagonists, because it may allow the use of drug-eluting stents for locally confined delivery. Similarly, other strategies for interfering with chemokine activity or expression, such as transfer of viral orthologs, inhibitors of signaling, transcription factor decoys, antisense oligonucleotides, or siRNA, may be facilitated by site-directed applications. Studies using polymer-coated stents for local delivery of chemokine antagonists are underway to assess the feasibility of this approach. It will be exciting to see these issues addressed and resolved by future research.

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Chemokines: Key Regulators of Mononuclear Cell Recruitment in Atherosclerotic Vascular Disease
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