Abstract—Chemokines play important roles in atherosclerotic vascular disease. Expressed by not only cells of the vessel wall but also emigrated leukocytes, chemokines were initially discovered to direct leukocytes to sites of inflammation. However, chemokines can also exert multiple functions beyond cell recruitment. Here, we discuss novel and recently emerging aspects of chemokines and their involvement in atherosclerosis. While reviewing newly identified roles of chemokines and their receptors in monocyte and neutrophil recruitment during atherogenesis and atheroregression, we also revisit homeostatic functions of chemokines, including their roles in cell homeostasis and foam cell formation. The functional diversity of chemokines in atherosclerosis warrants a clear-cut mechanistic dissection and stage-specific assessment to better appreciate the full scope of their actions in vascular inflammation and to identify pathways that harbor the potential for a therapeutic targeting of chemokines in atherosclerosis. (Arterioscler Thromb Vasc Biol. 2014;34:742-750.)

Key Words: atherosclerosis • chemokines • inflammation • leukocytes • receptors, chemokine

Atherosclerotic vascular disease and its sequelae, such as coronary artery disease, myocardial infarction, and stroke, remain the leading cause of death and morbidity worldwide. It is now well established that a multitude of different factors contribute to its pathogenesis, including not only an imbalanced lipid metabolism but also immune responses that initiate and propagate chronic inflammation of the arterial wall. In particular, the infiltration of the vessel wall with not only monocytes but also other immune cells, such as neutrophils and T cells, has been regarded as a hallmark of atherosclerosis. The recruitment of these cells is governed by adhesion molecules together with chemokines and their receptors.

Chemokines constitute a structurally related family of chemoattractant cytokines that are classified in subgroups based on the position of the N-terminal cysteine residues (CC, CXC, CX3C, XC). Expressed by not only activated endothelial cells and smooth muscle cells but also emigrated leukocytes, chemokines were initially discovered to direct leukocytes to sites of inflammation. However, it is now clear that chemokines can also exert functions beyond cell recruitment and can also control cell homeostasis and activation. Extending our knowledge about the contribution of different chemokines to vascular inflammation,3,4 several novel players and chemokine functions have been revealed in recent years. We here provide an overview and a critical discussion on these developments in the involvement of chemokines in atherosclerosis.

Monocyte and Neutrophil Recruitment to Atherosclerotic Vessels—Where Do We Stand?

Chemokines and Their Receptors Revisited

Monocytes in blood can be divided into ≥2 distinct subsets, namely classical or inflammatory Ly-6Chigh and nonclassical or resident Ly-6Clow monocytes that display a remarkable heterogeneity in chemokine receptor expression. The developmental relationship of these monocyte subsets is still under debate. Although adoptive transfer experiments have demonstrated that Ly-6Chigh monocytes shuttle between blood and bone marrow and lose Ly-6C expression, suggesting that they give rise to Ly-6Cmonocytes, it was also demonstrated that neither a genetic defect in nor the antibody-mediated depletion of Ly-6Chigh monocytes affected the generation of Ly-6Clow monocytes.5 In contrast, a recent fate-mapping study of the murine monocyte/macrophage compartment indicated that short-lived Ly-6Chigh monocytes constitute obligatory steady-state precursors of blood-resident Ly-6Clow subsets.6 Ly-6Chigh monocytes increase in hypercholesterolemia and are the predominant cell type to infiltrate atherosclerotic lesions and become plaque macrophages.7
The deposition of lipids during hypercholesterolemia insti- gates an early endothelial cell activation and dysfunction. It has recently been shown that CXC chemokine ligand (CXCL) can be released from endothelial cells during early atherosclerosis by lysophosphatidic acid, a component of low-density lipoprotein (LDL), via its receptors lysophosphatidic acid 1 and lysophosphatidic acid 3, and contributes to the mobilization of not only classical monocytes but also neutrophils via CXC chemokine receptor (CXCR)2 in hypercholesterolemia in apolipoprotein E–deficient (Apoe−/−) mice.8–10 In addition, CXCL1 can recruit leukocytes to the vessel wall and has been implicated to mediate the progression of atherosclerosis in response to lysophosphatidic acid (Figure).10

A specific functional contribution of different monocyte subsets has recently been addressed. Experiments, in which white blood cells depleted of either classical or nonclassical monocytes were adoptively transferred into Apoe−/− mice initially showed that CCR2+ CXCR1low classical monocytes used CCR2, CCR5, and CXCR1 to enter plaques, whereas nonclassical monocytes used CCR5 for recruitment.11 More direct evidence from adoptive transfer experiments, pharmacolog- ical receptor blockade during intravital microscopy of carotid arteries, and studies correlating circulating and plaque content of classical monocytes, however, revealed that Ly-6Chigh monocytes use CCR1 and CCR5 but not CCR2 and CXCR1 to recruit to the vessel wall (Table).9

The involvement of these receptors may not in all cases translate to direct changes in atherosclerotic lesion formation in mice deficient in respective chemokines or receptors. For instance, a reduction in lesion size in Ccr2−/− Apoe−/− mice together with fewer classical monocytes in the aorta of these mice9,36 may thus primarily stem from lower circulating monocyte counts12 rather than direct defects in their recruitment. Likewise, this may also underlie atheroprotective effects in other studies showing a reduction in CCL2 expression after inflammatory stimulation, as for instance seen in mice deficient in microRNA-155.37 CX3CR1, on the contrary, may primarily provide survival signals for monocytes (Figure). Accordingly, an increased apoptosis of plaque macrophages and a reduction in plaque size are observed in Cx3cr1−/− Apoe−/− mice.32 However, besides direct interactions of monotypic chemokine receptors with their ligands expressed by cells of the vessel wall, it may also be conceivable that monocyte recruitment is mediated by indirect mechanisms not fully addressed by short-term experiments investigating monocyte recruitment in vivo. For instance, CXCR1 expressed on platelets and upregulated on this cell type in hypercholesterolemia was involved in the formation of platelet–monocyte complexes that triggered monocyte adhesion to arterial lesions, in turn supporting monocyte recruitment.34 Likewise, the cross-talk between monocytes and smooth muscle cells via CXCR1–CXCL1 was shown to augment the inflammatory response in both cell subsets.
types, leading to an increased expression of cytokines that in turn may promote adhesion molecule expression. Along these lines, pharmacological inhibition of CX3CR1 was proven to be effective to interfere with initial atherosclerotic lesion formation and evolution and shown to reduce classical monocyte adhesion and survival. Atherogenic effects seem to be less clear for the chemokine receptors CCR1 and CCR5, although they share several ligands (including CCL3 and CCL5). Although pharmacological blockade of CCL5 receptors and CCR5 deficiency are associated with reduced atherosclerotic

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Receptor</th>
<th>Functions in Atherosclerosis*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2</td>
<td>CCR2</td>
<td>Monocyte mobilization from bone marrow</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recruitment of neutrophils</td>
<td>8</td>
</tr>
<tr>
<td>CCL3</td>
<td>CCR1/5</td>
<td>CCL3 in bone marrow cells promotes atherosclerosis</td>
<td>13,14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neutrophil survival</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recruitment of neutrophils</td>
<td>14</td>
</tr>
<tr>
<td>CCL5</td>
<td>CCR1/5</td>
<td>Recruitment of classical monocytes</td>
<td>9,11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recruitment of neutrophils</td>
<td>8</td>
</tr>
<tr>
<td>CCL20</td>
<td>CCR6</td>
<td>Monocyte mobilization from bone marrow</td>
<td>15,16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recruitment of monocytes, macrophage accumulation</td>
<td>15,16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recruitment of atheroprotective B cells</td>
<td>17</td>
</tr>
<tr>
<td>CCL19/21</td>
<td>CCR7</td>
<td>Macrophages egress during lesion regression after aortic transplantation</td>
<td>18–20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No effects on lesion formation or macrophage migration</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atheroprotective, inhibition of T-cell recruitment</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atheroprotection, recruitment of T cells</td>
<td>23</td>
</tr>
<tr>
<td>CXCL1</td>
<td>CXCR2</td>
<td>Mobilization of classical monocytes and neutrophils</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recruitment of neutrophils</td>
<td>8</td>
</tr>
<tr>
<td>CXCL4–CCL5 heteromers</td>
<td></td>
<td>Monocyte recruitment</td>
<td>25</td>
</tr>
<tr>
<td>CXCL5</td>
<td></td>
<td>Atheroprotective, blockage of foam cell formation</td>
<td>26</td>
</tr>
<tr>
<td>CCL17</td>
<td>CCR4</td>
<td>Recruitment of T cells, limits Treg maintenance</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>But atherosclerosis independent of bone marrow CCR4</td>
<td>27</td>
</tr>
<tr>
<td>MIF</td>
<td>CXCR2/4</td>
<td>Monocyte recruitment, macrophage and T-cell accumulation</td>
<td>28</td>
</tr>
<tr>
<td>CXCL12</td>
<td>CXCR4/7</td>
<td>Atheroprotection</td>
<td>16,29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stabilization of atherosclerotic lesions</td>
<td>30,31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-inflammatory neutrophil homeostasis via CXCR4</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control of hyperlipidemia via CXCR7</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antiapoptotic in endothelial and smooth muscle cells</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mobilization of smooth muscle progenitor cells</td>
<td>31</td>
</tr>
<tr>
<td>CX3CL1</td>
<td>CX3CR1</td>
<td>Monocyte survival</td>
<td>32,33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Formation of platelet–monocyte complexes and monocyte recruitment</td>
<td>32–34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cross-talk between monocytes and smooth muscle</td>
<td>35</td>
</tr>
</tbody>
</table>

CXCL indicates CXC chemokine ligand; CXCR, CXC chemokine receptor; MIF, migration inhibitory factor; and Treg, regulatory T cell.

*All chemokines/receptors promote atherosclerosis, unless indicated otherwise.
lesion size, CCR1 deficiency (somatic or in bone marrow) was shown to exacerbate plaque formation.\cite{3,4,15,38,40} Given the expression of these chemokine receptors on a vast array of different cell types, differences may relate to their cell-specific involvement in disease. For instance, neutrophils use the chemokine receptors CCR1, CCR2, CCR5, and CXCR2 for infiltrating larger arteries in atherosclerosis\cite{8} and contribute to atherosclerotic lesion formation predominantly during early phases.\cite{41} In addition, a functional interaction of chemokines (Figure) or its site-specific deposition may affect monocyte recruitment and plaque growth.\cite{3,4} Thus, a complex interplay of different cell types and functions may explain the contribution of individual chemokines/receptors in atherosclerosis.

**CCL20/CCR6 as a New Player in Monocyte Mobilization and Recruitment in Atherosclerosis**

Detection of CCL20 in atherosclerotic carotid arteries and coronary plaques and an increased concentration of this chemokine in the circulation of hypercholesterolemic patients\cite{11} prompted investigations of this chemokine/receptor pair in atherosclerosis. Interestingly, genetic deletion of CCR6 in both Apoe\textsuperscript{−/−} and LDL receptor–deficient (Ldlr\textsuperscript{−/−}) mice entailed a substantial reduction in atherosclerotic lesion formation in the aortic root and aorta, together with a marked reduction in macrophage accumulation within lesions.\cite{42,43} Similarly, transplantation of Ccr6\textsuperscript{−/−} bone marrow into Apoe\textsuperscript{−/−} mice attenuated atherosclerotic lesion burden,\cite{42} indicating that CCR6 in hematopoietic cells exerts proatherosclerotic functions. CCR6 can be expressed by different cell types, including monocytes, where this receptor is upregulated by inflammatory cytokines.\cite{41} Indeed, it has now been revealed that the CCL20/CCR6 axis affects atherogenic monocyte accumulation at different levels (Table). First, monocyte counts and in particular Ly-6Chigh monocytes are decreased in blood but increased in bone marrow of both Ccr6\textsuperscript{−/−} Apoe\textsuperscript{−/−} and Ccr6\textsuperscript{−/−} Ldlr\textsuperscript{−/−} mice compared with respective controls.\cite{42,44} whereas injection of recombinant CCL20 induced monocytosis,\cite{42} indicating that CCL20/CCR6 function to mobilize monocytes from the bone marrow. CCR6 was furthermore revealed to trigger classical chemotactic responses in monocytes.\cite{42} Finally, CCL20 can be immobilized on the endothelial cell surface to support CCR6-mediated monocyte adhesion.\cite{43} These findings highlight CCL20/CCR6 as an important novel chemokine/receptor in monocyte recruitment in atherosclerosis (Figure). Interestingly, expression of CCL20 is reduced in the aorta in Ccr6\textsuperscript{−/−} Apoe\textsuperscript{−/−} mice, suggesting that CCR6/CCL20 may in addition form a local positive feedback loop in the vessel wall. Namely, monocytes/macrophages recruited directly by CCR6 may in turn locally secrete CCL20 and other inflammatory mediators that furthermore amplify CCL20 expression in the aorta.\cite{41}

Although CCR6 has received attention as a Th17 marker, no differences in the aortic accumulation or in systemic alterations of Th17 cell frequencies have been observed in Ldlr\textsuperscript{−/−} mice lacking Ccr6, arguing against an important role of Ccr6 in modulating Th17 cell responses or their recruitment during atherosclerosis. Moreover, no significant changes in regulatory T cells (Tregs) were detectable in Ccr6\textsuperscript{−/−} Ldlr\textsuperscript{−/−} mice, indicating that Ccr6 does not control Treg homeostasis in atherosclerosis.\cite{43} However, CCR6 was recently discovered to regulate B-cell trafficking to the aorta. Ccr6\textsuperscript{−/−} Apoe\textsuperscript{−/−} B-cell transfer to μMT Apoe\textsuperscript{−/−} mice was attenuated atherosclerotic lesion formation, not observed on the transfer of Ccr6\textsuperscript{−/−} Apoe\textsuperscript{−/−} mice, indicating that CCR6 controls B-cell–mediated atheroprotection in μMT Apoe\textsuperscript{−/−} mice.\cite{17} Given that mice globally deficient in CCR6 are protected from atherosclerosis, CCR6 thus seems to have opposing roles in different cell populations. However, proatherogenic properties of CCR6 in monocyte mobilization and recruitment clearly override atheroprotective B-cell homing functions and its redundant role in adaptive T-cell responses in atherosclerosis.

**Proatherogenic Functions of Hematopoietic CCL3**

The function of CCL3 (macrophage inflammatory protein-1α), known to be expressed in murine and human atherosclerotic plaques,\cite{3,44} and to bind both the chemokine receptors CCR1 and CCR5, was recently addressed in 2 independent studies. Although CCL3 deficiency in Ldlr\textsuperscript{−/−} mice had no effects on atherosclerotic lesion formation in the aortic sinus, chimeric Ldlr\textsuperscript{−/−} mice reconstituted with Ccl3\textsuperscript{−/−} bone marrow displayed a decrease in plaque burden in the aortic root.\cite{13,14} These protective effects were associated with lower lipid levels in blood and decreased adipose tissue mass but no changes in circulating leukocyte populations in 1 study.\cite{15} Conversely, de Jager et al.\cite{45} ascribed decreased plaque formation to a reduction in lesional neutrophil accumulation as a consequence of decreased neutrophil adhesion and recruitment into plaques. In addition, CCL3 was revealed to extend the half-life of neutrophils and to interfere with their apoptosis but not maturation or stromal release from bone marrow, leading to diminished circulating neutrophil counts in mice with CCL3-deficient bone marrow.\cite{14} In line with observations in CXCR4-deficient mice and other models showing an expansion of circulating neutrophils,\cite{30,46} blood levels of this cell population may directly correspond to their accumulation within plaques. Notably, CCL3 deficiency also mitigated migratory responses of CCR5-expressing neutrophils toward CXCL1, which may point toward a CXCL1-induced production of CCL3, in turn affecting neutrophil migration via CCR5.\cite{44} CXCL1-induced neutrophil mobilization and neutrophilia in hypercholesterolemia\cite{8} may thus also be supported by a CCL3-mediated prolongation in neutrophil survival. Findings that only hematopoietic but not global deficiency of CCL3 affects lesion formation remain to be elucidated, but suggest that CCL3 specifically secreted from leukocytes (and in particular macrophages and neutrophils\cite{31}) or site-specific effects of its production exert proatherogenic functions in atherosclerosis (Table).

**CCR7 Plays Diverging Roles in Macrophage Emigration and T-Cell Migration**

The role of CCR7 was initially tested in a model of atherosclerosis regression, in which the aortic arch of Apoe\textsuperscript{−/−} mice with established atherosclerotic lesions was transplanted into wild-type recipients. In this model, the blockade of CCR7 ligands CCL19 and CCL21 inhibited plaque regression and preserved foam cell content,\cite{13} suggesting that CCR7 mediated
the egress of macrophages during lesion regression. Similarly, a role for CCR7 in macrophage emigration from plaques was deduced from regression studies after transplantation of the aortic arch into Apoe<sup>-/-</sup> mice transgenic for human apoAI<sup>39</sup> or in Apoe<sup>-/-</sup> mice treated with statins. In a different approach, the role of CCR7 in lesion regression was addressed by treating Apoe<sup>-/-</sup> or Ccr7<sup>-/-</sup> Apoe<sup>-/-</sup> mice with adenosiviral vectors encoding apoE. Although Ccr7<sup>-/-</sup> Apoe<sup>-/-</sup> and Apoe<sup>-/-</sup> mice did not differ in atherosclerotic plaque size or macrophage content, expression of apoE induced plaque regression and macrophage loss in both mouse strains, indicating that the continuous decrease in plaque macrophage content during regression did not involve CCR7-dependent migratory macrophage egress. Contradictory to these findings, Wan et al<sup>2</sup> showed that Ccr7<sup>-/-</sup> Apoe<sup/+</sup> mice displayed a marked increase in atherosclerotic lesion size compared with Apoe<sup>-/-</sup> controls, associated with increased circulating and lesional T-cell content, and a more proficient migration of Ccr7<sup>-/-</sup> Apoe<sup>-/-</sup> T cells into mouse aortas compared with Ccr7<sup>+/+</sup> Apoe<sup>-/-</sup> counterparts. Similarly, an exacerbated plaques growth was observed in chimeric Apoe<sup>-/-</sup> mice reconstituted with Ccr7<sup>-/-</sup> Apoe<sup>-/-</sup> bone marrow, corroborating that CCR7 on bone marrow–derived cells mediated these effects. In striking contrast, deficiency of CCR7 in Ldlr<sup>-/-</sup> mice led to a reduction in atherosclerotic plaque development associated with a modest augmentation of CD11<sup>c</sup> dendritic cells (DCs) and T cells in atherosclerotic plaques. Adoptive transfer experiments in this study showed a reduced capacity of Ccr7<sup>-/-</sup> T cells to migrate into atherosclerotic aortas, and it was concluded that CCR7 controls the exit and entry of T cells into atherosclerotic lesions, constituting a mechanism essential for the generation and maintenance of an proatherogenic immune response. CCR7 may thus not only mediate macrophage egress under certain conditions, but also control T-cell recruitment in atherosclerosis with different functional outcomes depending on the atherogenic mouse model and factors that remain to be elucidated (Figure, Table).

Chemokine-Like Functions of Migration Inhibitory Factor

Adding to the various proatherogenic factors, such as oxidized LDL that can induce the expression of migration inhibitory factor (MIF) in different vessel wall cells and macrophages during atherosclerosis,<sup>46</sup> MIF can also be released by endothelial cells under conditions of hypoxia.<sup>55</sup> Besides binding cell surface CD74, MIF directly interacts with both CXCR2 and CXCR4 to induce MIF-induced monocyte recruitment to the arterial wall and macrophage and T-cell accumulation in atherosclerotic lesions (Figure, Table).<sup>28</sup> Importantly, blocking MIF (as a dual CXCR agonist) results in a regression of pre-existing atherosclerotic plaques in Apoe<sup>-/-</sup> mice, accompanied by a decrease in both macrophage and T-cell content, resulting in a more stable plaque phenotype. Recently, the structural requirements of MIF to bind to CXCR2 were addressed, and it was revealed that MIF–chemokine receptor interactions are dependent on a pseudo-(E)LR motif and an N-loop–based 2-site binding mechanisms, typical for bona fide chemokines.<sup>48,49</sup> Notably, Gremlin-1 was recently identified as an endogenous inhibitor of MIF, and administration of a recombinant fusion molecule mGremlin-1-Fc that binds to MIF substantially reduced atherosclerotic lesion formation,<sup>50</sup> indicating its potential as a novel therapeutic strategy to limit atheroprogession.

Intriguingly, MIF has also been shown to play a role in directing leukocytes that had extravasated from postcapillary venules toward NG2<sup>+</sup> pericyte-rich regions along arterioles and capillaries in the periendothelial compartment.<sup>51</sup> Interestingly, pericytes were identified as the main source of MIF in the perivascular compartment of microvessels, where it can be not only released but also immobilized on the cell surface, probably through binding to CD74 and CXCR4. Given that leukocyte immigration into plaques may in large part also depend on vasa vasmorum and the adventitia,<sup>24</sup> interstitial migration routes deployed by MIF may contribute to the MIF-mediated leukocyte recruitment to sites of inflammation in atherosclerosis.

Role of CXCL12 in the Recruitment of Progenitor Cells to Stabilize Atherosclerotic Plaques

CXCL12 and its receptor CXCR4 have been revealed to exert atheroprotective functions in atherosclerosis in Apoe<sup>-/-</sup> or Ldlr<sup>-/-</sup> mice.<sup>16</sup> Besides maintaining an anti-inflammatory leukocyte homeostasis (Table), we have been able to demonstrate that apoptotic bodies derived from endothelial cells in the context of apoptosis convey microRNA-126 as a regenerative signal to surviving cells in the vicinity to induce the production of CXCL12.<sup>30</sup> Acting as an important signal to mobilize stem cells and as an antiapoptotic factor in endothelial and smooth muscle cells, CXCL12 in turn contributes to the stabilization of atherosclerotic lesions. Importantly, CXCL12 may sustain an autoregulatory, self-amplifying feedback loop, in which CXCL12, through CXCR4, triggers the expression of CXCL12, enhancing its impact in the vasculature (Figure). In line, in a model of plaque formation after carotid ligation, repeated intravenous injections of CXCL12 were shown to enhance the expression of CXCL12. This model, it was furthermore shown that CXCL12 can induce the mobilization of smooth muscle progenitor cells, resulting in reduced macrophage content but an increased smooth muscle cell accumulation and fibrous cap thickness within lesions, corroborating plaque stabilizing functions of CXCL12 in atherosclerosis.

Heterophilic Interaction of Chemokines—Epitomized by Platelet Chemokines

The contribution of platelet chemokines in atherogenesis has been discussed. Among the chemokines present in platelets, CXCL4 (platelet factor 4) exists as a tetramer in α-granules of platelets. In addition, however, CXCL4 can also associate with CCL5 to form CC-type mixed heterodimers (presumably already within platelet granules), which can be found in circulating blood. A functional relevance of CCL5 and CXCL4 heteromerization in aggravating atherogenesis was demonstrated by developing a synthetic peptide disrupting CXCL4–CCL5 interactions, which attenuated plaque formation and atherogenic monocyte recruitment in Apoe<sup>-/-</sup> mice (Table).<sup>2</sup> These findings epitomize the concept of a functional interactome, constituted by a variety of homophilic and heterophilic chemokine interactions that integrate signals conferred by individual chemokines for the combinatorial control
of leukocyte responses.\textsuperscript{5,25,53} The paradigm of targeting functional chemokine interactions in atherosclerosis may offer advantages over direct antagonism of chemokines or their receptors, such as preservation of normal immune defense and surveillance mechanisms that may depend on distinct chemokine effects but not combined actions of these mediators, as observed in inflammation.

Adding another level of complexity, also chemokine receptors can form hetero- or oligodimers. For instance, CCR2 forms heterodimers with CCR5 and CXCR4, and CXCR1 associates with CXCR2. Because chemokine receptor heteromerization can not only affect ligand binding but also downstream receptor signaling,\textsuperscript{54,55} these interactions may be of functional relevance for atherogenesis but await to be addressed in future studies.

### Homeostatic Functions of Chemokines

#### Chemokines in Macrophage Survival and Foam Cell Formation

CX\textsubscript{X},CR1 is known to provide essential survival signals for monocytes (Table). While protecting both human monocyte subsets from cell death in vitro, \textit{Cx3cr1\textsuperscript{-/-}} Apoe\textsuperscript{-/-} mice displayed reduced numbers of circulating Ly-6C\textsuperscript{hi} monocytes,\textsuperscript{32} whereas pharmacological inhibition of CX\textsubscript{X},CR1 reduced Ly-6C\textsuperscript{hi} monocyte levels in Apoe\textsuperscript{-/-} and Ldlr\textsuperscript{-/-} mice in vivo.\textsuperscript{8} Accordingly, an increased apoptosis of plaque macrophages and a reduction in plaque size are observed in \textit{Cxc3cr1\textsuperscript{-/-}} Apoe\textsuperscript{-/-} mice.\textsuperscript{2} Similarly, pharmacological inhibition of CX\textsubscript{X},CR1 reduces plaque macrophage accumulation and lesion formation.\textsuperscript{35}

Insights into other alternative roles of chemokines in atherosclerosis are emerging. Beyond its properties as a chemokine, CXCL12, for instance, also acts as a scavenger receptor for apoptotic cells, phosphatidylserine, and oxidized LDL, possibly conveying atheroprotective functions, as implied by a reduction in atherosclerotic lesion size in \textit{Cxc16\textsuperscript{+/-}} Ldlr\textsuperscript{-/-} mice.\textsuperscript{3,56}

Although CXCL5 is upregulated in the aorta and plasma of atherosclerotic Apoe\textsuperscript{-/-} mice, blocking this chemokine using a specific monoclonal antibody did not diminish lesion size or macrophage accumulation in the brachiocephalic artery. However, an increased foam cell formation and macrophage activity and a reduction in collagen content were observed in Apoe\textsuperscript{-/-} mice on CXCL5 blockade, indicative of a less stable plaque phenotype. Interestingly, CXCL5 (via its receptor CXCR2) was found to upregulate the expression of ATP-binging cassette, subfamily A, member 1 in macrophages, in particular in macrophages of the M2 phenotype, a transporter mediating the efflux of cholesterol. CXCL5 thus plays a protective role in atherosclerosis by limiting macrophage cholesterol accumulation and foam cell formation\textsuperscript{26} (Table).

Of note, although unequivocally ascribed to exert proatherogenic functions in different disease models,\textsuperscript{3} CXCL4 has been shown to induce a unique transcriptome in monocyte-derived macrophages (proposed to be called M4 phenotype), resulting in a lower abundance of scavenger receptors but higher expression of cholesterol efflux transporters, distinct from classical M1 and M2 phenotypes and not clearly pro- or antiatherogenic.\textsuperscript{37} Whether these properties are also relevant in atherosclerosis and require CXCL4 actions independent of its heteromerization with CCL5 remains to be assessed.

#### DC-Derived CCL17 Drives Atherosclerosis by Restraining Treg Homeostasis

Besides regulating cell recruitment and macrophage phenotype, chemokines are emerging to control the interplay of different immune cells and adaptive immune responses. Given the expression of the DC chemokine CCL17 in human plaques, its function in atherosclerosis was investigated (Table). Interestingly, Apoe\textsuperscript{-/-} mice with a targeted replacement of the \textit{Ccl17} gene by the enhanced green fluorescent protein gene (\textit{Egfp}; \textit{Ccl17\textsuperscript{E/E}}) mice and thereby deficient in the DC-derived chemokine CCL17 displayed a marked attenuation of atherosclerosis. This was associated with a marked reduction in T-cell recruitment to atherosclerotic vessels; \textit{Ccl17\textsuperscript{E/E}} Apoe\textsuperscript{-/-} mice displayed fewer CD3\textsuperscript{+} T cells within lesions and adoptively transferred CD4\textsuperscript{+} T cells (but not Tregs) homed less efficiently to aortas of \textit{Ccl17\textsuperscript{E/E}} Apoe\textsuperscript{-/-} mice.\textsuperscript{27} This extends previous findings on the limited knowledge about chemokine receptors attracting T cells to sites of vascular inflammation (such as CCR1, CCR5, CXCR3, and CXCL16).\textsuperscript{4} Notably, however, we also evidenced an increased Treg accumulation in aortas of \textit{Ccl17\textsuperscript{E/E}} Apoe\textsuperscript{-/-} mice and in lymph nodes of \textit{Ccl17}-deficient mice and could reveal that CCL17+ DCs control the maintenance of Tregs (Figure). Adoptively transferred T cells showed an enhanced expansion and proliferation and an increased rate of conversion into Tregs in CCL17-deficient mice.\textsuperscript{27} Similarly, an expansion of Tregs has also been observed in a model of intestinal inflammation in \textit{Ccl17\textsuperscript{E/E}} mice.\textsuperscript{54} The chemokine receptor(s) mediating these effects remains to be conclusively addressed. Although a role of CCR4 in recruiting CD4\textsuperscript{+} T cells and Tregs has been shown in vitro,\textsuperscript{59} we could not confirm a predominant role of this receptor in vivo. Moreover, deficiency in Ccr4 did not phenocopy the effects of CCL17 deficiency in vivo, and lesion formation was unaltered in chimeric Ldlr\textsuperscript{-/-} mice reconstituted with Ccr4\textsuperscript{+/+} versus Ccr4\textsuperscript{-/-} bone marrow.\textsuperscript{27} The mechanisms enacted by CCL17 thus do not solely seem to be mediated by CCR4, implying the contribution of other receptors addressed by CCL17 or potential heteromer partners.

#### Chemokine Decoy Receptors

A group of silent or decoy receptors are able to sequester chemokines\textsuperscript{60} that may in addition be of importance in controlling chemokine functions in atherogenesis. D6, an important member of the decoy receptor family, was recently shown to sequester the chemokines CCL2 and CCL3, thereby preventing excessive infiltration of classical monocytes and neutrophils into the myocardium in a mouse model of myocardial infarction.\textsuperscript{61} The Duffy antigen receptor for chemokines can act as a sink involved in buffering and patterning inflammatory chemokine actions.\textsuperscript{62} In addition, CXCR7 identified as an alternative receptor scavenging CXCL12 does not trigger classical G-protein signaling but β-arrestin recruitment and receptor internalization to control bioavailability of extracellular CXCL12.\textsuperscript{63,65} By conditional knockout of CXCR7 and use of the synthetic agonist ligand CCX771, recent studies revealed that CXCR7 reduced atherosclerotic lesion formation, lowering hyperlipidemia and subsequent monocyteosis.\textsuperscript{29} Not expressed on leukocytes, CXCR7 in adipocytes regulated blood cholesterol by promoting very-low-density lipoprotein
uptake in adipose tissue, associated with an enhanced lipase activity and reduced angiotopetin-like protein 4 expression. In contrast, the relevance of chemokine-scavenging receptors to atherogenic mobilization and recruitment of inflammatory cells remains to be addressed.

**Virally Encoded Chemokines/Receptors**

Human viruses that have pirated the chemokine system of their hosts during evolution can encode chemokine receptors, chemokines, and chemokine-binding proteins. These virus-encoded receptors bind chemokines and can signal on binding and thus are fully operational as chemokine receptors. Many γ2-herpesvirus-encoded receptors contain a CXC chemokine receptor; cytomegalovirus encodes different 7 transmembrane receptors, among which are 3 chemokine receptor homologs; human herpes virus 6 and human herpes virus 7 encode receptors with homology to CC chemokine receptors. In addition, virus-encoded chemokines are found that may have agonistic or antagonistic properties. Moreover, virus-encoded chemokine-binding proteins that function as chemokine scavengers have been identified. Given evidence that certain viruses, for example, cytomegalovirus, may play a role in the pathogenesis of atherosclerosis and restenosis, the potential role of virally encoded chemokines/receptors in atherosclerosis warrants further investigation. Interestingly, smooth muscle cell migration was shown to depend on cytomegalovirus-encoded R33 chemokine receptor expression in cytomegalovirus-accelerated vascular transplant sclerosis, providing evidence that virally encoded chemokine receptors may indeed be functional in vascular inflammation.

**Antichemokine Drugs: Future Challenges**

Although unequivocally shown to control atherogenesis at different levels, potential therapeutic approaches targeting chemokines are scarce. Only few inhibitors of chemokines and their receptors have been tested and proven to be effective experimentally. First evidence that targeting a chemokine receptor may be feasible for the treatment of atherosclerosis stems from a study in which the CCL5 receptor antagonist Met-RANTES reduced diet-induced atherosclerosis. Similarly, also the chemokine variant [45AANA47]-RANTES that carries specific mutations in the principal CCL5/glycosaminoglycan binding site limited atherosclerotic plaque progression of established lesions by interfering with leukocyte recruitment and inducing a more stable plaque phenotype. Moreover, the HIV entry inhibitor TAK-779, functioning as an antagonist of both CCR5 and CXCR3, was shown to reduce atherosclerotic lesion size and T-cell plaque content. In addition, also CXC chemokines have been targeted experimentally. Evasin-3 that binds and neutralizes CXCL1 and CXCL2 reduces neutrophilic inflammation and potentially related plaque vulnerability in carotid atherosclerosis in mice, suggesting this therapeutic approach may represent a prevention strategy against ischemic stroke caused by carotid ath erosclerotic stenosis. Moreover, the MIF inhibitor Gremlin-1 was shown to reduce atherosclerotic lesion formation.

Although a large number of molecules targeting chemokines and chemokine receptors including neutralizing antibodies for inflammatory diseases are currently in clinical trials, only few molecules targeting chemokines and their receptors have been approved by the US Food and Drug Administration. In 2007, the CCR5 inhibitor maraviroc (for prevention of HIV) was approved; the CXCR4 antagonist mozobil (for hematopoietic stem cell mobilization) was approved in 2008. These principal successes may herald the establishment of chemokine/receptor antagonists as an anti-inflammatory therapy in atherosclerosis. Notably, maraviroc was recently shown to reduce atherosclerosis progression by interfering with inflammatory cell recruitment into plaques in mice, suggesting its potential applicability for the treatment of chronic vascular inflammation. In this study, maraviroc, in addition, modulated splenic T and B lymphocytes effector functions in an anti-inflammatory sense. Although a suppression of inflammation is regarded favorable in atherosclerosis, this finding highlights a potential problem of targeting chemokines in the setting of chronic vascular inflammation. Chemokines play fundamental roles in a plethora of different settings, such as the organization and maintenance of lymphoid organ architecture and immune cell localization to different tissues and organs during both innate and adaptive immune responses. In light of this, interfering with chemokine heteromers that specifically form under conditions of inflammation, or applying inhibitors that require binding of several chemokine receptors to exert their functions, may be promising. On this note, the synthetic stable peptide inhibitor CK5X63 that specifically disrupts proinflammatory CCL5–CXCL4 interactions could attenuate plaque formation without interfering with systemic immune responses.

To date, not the redundancy of the chemokine system but inappropriate target selection and ineffective dosing may have constituted the main barriers, as observed in other inflammatory settings. Given the ever-growing complexity and contribution of multiple chemokines that mediate similar steps in the process of atherogenesis, appropriate target selection is critical and needs to be constantly weighed against new biological information that emerges.

**Conclusions**

Considering the evidence that chemokines not only control the trafficking of a vast number of different cell populations, but also exert nonchemotactic functions beyond recruitment, it is now clear that not only cell-specific effects of distinct chemokines, their synergistic interactions but also stage-specific functions integratively govern the initiation and progression of atherosclerosis. Findings that CCL2 can bind to oxidized LDL and thereby be carried by lipoprotein(a) in human plasma may provide an additional mechanism of a tissue-or site-specific localization and presentation of chemokines, not yet examined in atherosclerosis. Conversely, chemokine receptors have been found to directly regulate lipid levels and hyperlipidemia-induced inflammation, for example, modulating very-low-density lipoprotein receptor uptake. Notably, atherosclerosis progression has recently been shown to not solely rely on a continued influx of newly recruited monocytes. Whereas monocyte recruitment and differentiation is important in early lesion formation, lesional macrophages proliferate to locally augment their numbers in plaques at later stages. Thus, it may be warranted to scrutinize whether chemokines affect macrophage proliferation in atherosclerosis.
Given their emerging role in macrophage foam cell formation and cholesterol efflux, as shown for CXCL5,26 and knowledge that trapped cholesterol in hematopoietic stem and progenitor cells leads to the expression of cytokines that contribute to their excessive proliferation,2 a role of chemokines herein could possibly be envisioned. Global effects of single chemokine receptor pairs in atherosclerosis thus demand a clear dissection into distinct functions to better understand the full scope of their actions in vascular inflammation. Accordingly, multiple mechanisms may harbo the potential for yet elusive therapeutic targeting of chemokines in atherosclerosis.

Sources of Funding
This work was supported by the Deutsche Forschungsgemeinschaft (FOR809, ZE 827/1–2 and SFB688 TPA12 and TPA22 to A. Zernecke and WE1913/11–2 to C. Weber) and European Research Council (ERC AdG #249929 to C. Weber).

Disclosures
None.

References


Chemokines in Atherosclerosis: Proceedings Resumed
Alma Zernecke and Christian Weber

*Arterioscler Thromb Vasc Biol.* 2014;34:742-750; originally published online January 16, 2014; doi: 10.1161/ATVBAHA.113.301655

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 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/34/4/742

**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/