



Chemokines: established and novel targets in atherosclerosis

Rory R. Koenen^{1,2}, Christian Weber^{1,2,3*}

Keywords: atherosclerosis; chemokine; inflammation; leukocyte; therapeutic

DOI 10.1002/emmm.201100183

Received August 03, 2011 / Revised September 09, 2011 / Accepted September 28, 2011

Introduction

Since their cloning and isolation in the late 1980s, chemokines have been established as crucial players in directing the movements and activity of leukocytes during immune surveillance and disease (Charo & Ransohoff, 2006; Richmond, 2011). After more than two decades, novel aspects of the chemokine system continue to emerge and its redundancy and versatility are still incompletely understood. Initially identified as small chemoattractant **cytokines**, which can stimulate the directed migration of neutrophils or monocytes, their known functions currently cover vast areas such as **angiogenesis**, embryonic development and cell homeostasis (Keeley et al, 2011; Raman et al, 2011) and their role in the pathogenesis of many inflammatory and autoimmune diseases is undisputed. One prominent example is atherosclerosis, a widespread chronic disease, which is characterized by the formation of lipid-rich lesions, called plaques, in the wall of larger arteries. These plaques develop from initial endothelial dysfunction, for example at sites of irregular blood flow, which leads to an increased uptake and subendothelial accumulation of cholesterol-rich plasma lipoproteins, namely **low-density lipoprotein** (LDL; Simionescu, 2009). Within the vessel wall, crystallization of cholesterol and the (oxidative) modification of LDL result in cell-activating pro-inflammatory species (Düwell et al, 2010;

In their role as small chemotactic cytokines, chemokines are crucial mediators and regulators of leukocyte trafficking during immune surveillance and inflammation. Their involvement in the development and progression of inflammatory diseases has been subject of intense investigation. Concordantly, the chemokine system has been explored in search for therapeutic targets to prevent or treat inflammatory disorders, such as atherosclerosis. Targeting the chemokine system offers various entry points for a causative treatment of this widespread and chronic illness. Although this approach has encountered some setbacks, several innovative compounds are currently in an advanced stage of development. In this review, the current standing of this dynamic field is highlighted and the potential advantages and drawbacks of particular strategies are discussed.

Navab et al, 2004), and uncontrolled phagocytosis of modified LDL (oxLDL) particles by macrophages results in the formation of foam cells, which secrete various factors that propagate inflammation. This causes endothelial cells to express adhesion molecules such as P-selectin or vascular cell adhesion molecule-1 promoting continuous leukocyte recruitment and influx, which leads to the progression of initial vascular lesions to full-blown atherosclerotic plaques. Although in clinical practice the complications of atherosclerosis are controlled by pharmacologic and surgical interventions, a true causative treatment for this chronic illness is still lacking. Being orchestrators of leukocyte trafficking, many chemokines are involved in directing leukocytes to sites of vascular inflammation and may thus represent attractive targets for drug therapy. In this review, a current state-of-the-art of the diverse roles of chemokines and their receptors in atherosclerosis is provided and the prospects of chemokine inhibition as therapeutic option are discussed.

Chemokine structure and mechanisms of action

As small, compact and stable proteins, chemokines are well amenable to structural characterization. They can be expressed

(1) Institute for Cardiovascular Prevention, Ludwig-Maximilians-University of Munich, Munich, Germany

(2) Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands

(3) Munich Heart Alliance, Munich, Germany

*Corresponding author: Tel: +49 89 5160 4350, Fax: +49 89 5160 4352; E-mail: kreislaufinstitut@med.uni-muenchen.de

and purified from *Escherichia coli* (*E. coli*) cells in high yields, opening the possibility for metabolic labelling with stable isotopes ^{15}N and ^{13}C for nuclear magnetic resonance (NMR) studies. Solution and crystal structures of chemokines were resolved in the early 1990s, revealing characteristic structural similarities between all members of the family (Clowse & Gronenborn, 1995). The monomeric structure of chemokines is highlighted by the 'chemokine fold', which consists of a poorly resolving disordered amino (N)-terminus followed by three antiparallel beta-strands arranged in a beta-sheet and stabilized by a carboxy-terminal alpha-helix. The spacing of two conserved cysteine residues at the N-terminus determines the nomenclature and classification of the chemokine family members; in C-, CC-, CXC- and CX₃C-chemokines. This cysteine spacing apparently determines the quaternary structure. Whereas, monomeric chemokine structures are all similar, CC- and CXC- chemokines considerably differ in their dimeric structures. Chemokine monomers of the CXC-type associate to form globular dimers by extending their three antiparallel beta-strands to a central 6-stranded beta-sheet, while CC-type chemokine dimers rather form by pairing of the N-termini and have an elongated shape. Some chemokines have the propensity to form higher-order multimers. For example, a recent study has revealed that the CC-chemokine macrophage inflammatory protein-1 (MIP-1 α and - β and CCL3 and CCL4, respectively), is able to form extended helical polymers with a molecular weight of up to 3000 kDa, consisting of no less than 50 MIP-1 monomers (Ren et al, 2010). This polymerization of MIP-1 was found to affect its *in vivo* cell-recruiting functions in a sense that MIP-1 mutants with reduced polymerization were

less effective in a mouse model of peritoneal cell recruitment. This might be explained by the observation that a MIP-1 polymer is more resistant to proteolytic degradation, for example by insulin degrading enzyme. Accordingly, the formation of larger protease-resistant polymers facilitates the gradual release of MIP-1 monomers over longer distances and might constitute a novel modality for chemokines to effectively promote chemotaxis. Although it is currently not known whether other chemokines can form such large polymers, the importance of higher-order oligomerization for their function *in vivo* has been well established (Campanella et al, 2006; Proudfoot et al, 2003).

There appears to be a functional link between the oligomerization of chemokines and their interaction with glycosaminoglycans (GAGs), since alterations that influence chemokine oligomerization often also affect their binding to GAGs. In fact, chemokines have been demonstrated to oligomerize on GAGs upon binding (Hoogewerf et al, 1997), leading to high local concentrations and the formation of two-dimensional 'haptotactic gradients', allowing a passing leukocyte to 'sense' the chemotactic signals presented on the endothelial surface (Rot & von Andrian, 2004). Support for this concept *in vivo* was provided by a recent study using transgenic mice with an inducible endothelial deficiency of the GAG heparan sulphate (Bao et al, 2010). Here, mice deficient in endothelial heparan sulphate showed significantly reduced migration of lymphocytes and dendritic cells (DCs) to lymph nodes, largely due to reduced presentation of the chemokines CCL19 and CCL21 on high endothelial venules. Besides presentation, GAGs have also been implied in the transport of chemokines from the basolateral

Glossary

Allogeneic

Cells or tissues from individuals of the same species, which are genetically different.

Angiogenesis

Development of blood vessels in the embryo or in an adult tissue.

Chemotaxis

Characteristic movement of somatic cells, bacteria or other organisms in response to a gradient of certain chemicals in their environment.

Citrullination

Also called deimination. Post-translational modification of arginine into the amino acid citrulline.

Cytokines

Intercellular protein mediators released by immune cells to regulate the immune response.

Dendritic cells (DCs)

Antigen-presenting immune cells found in mucosa, skin and lymphoid tissue whose main function is to activate lymphocytes and secrete chemokines. Messengers between innate and adaptive immunity.

Glycosylation

Enzymatic process, which attaches glycans to lipids, proteins or other organic molecules.

Haptotactic gradients

Molecular gradient of surface-bound adhesive molecules, which mediates directed cell migration or outgrowth.

High endothelial venules

Specialized post-capillary venous swellings found in lymphoid tissue, which enable circulating lymphocytes to directly enter a lymph node.

Humanization

Endow with human characteristics. Humanized antibodies are antibodies derived from non-human species whose amino acid sequence has been modified to increase their similarity to antibodies produced in humans.

Hypercholesterolemia

Presence of high cholesterol levels in the blood.

Immunological synapse

Also called immune synapse. The interface between a lymphocyte and an antigen-presenting cell.

Low-density lipoprotein (LDL)

Bad cholesterol, high circulating levels have been shown to correlate with atherosclerosis.

Nuclear magnetic resonance (NMR)

Physical phenomenon in which protons resonate in response to a magnetic field. This phenomenon is exploited to obtain subatomic and structural information of molecules.

Proteolytic

Relating to proteolysis. Proteolysis is the directed degradation of proteins through proteases or intramolecular digestion.

to the apical side of cells (Wang et al, 2005), complementing the role of the duffy antigen receptor for chemokines (DARC; Pruenster et al, 2009). The regions in the primary structure of chemokines that are responsible for binding to GAGs are well defined and mostly involve clusters of basic positively charged amino acids that undergo electrostatic interactions with the negatively charged carbohydrate and sulphate moieties on GAGs (Salanga & Handel, 2011). Modification of these basic residues greatly reduces the cell-recruiting function of chemokines *in vivo* (Proudfoot et al, 2003). On the other hand, the introduction or presence of additional basic residues or domains leads to a strong increase in affinity to GAGs, which is exemplified by an engineered anti-inflammatory variant of monocyte chemoattractant protein-1 (MCP-1/CCL2; Piccinini et al, 2010) or a splice variant of stromal cell-derived factor-1 (SDF-1/CXCL12; Laguri et al, 2007).

Under physiologic conditions, chemokines may undergo a large variety of post-translational modifications, many of which influence chemokine functions. The best characterized example is proteolytic cleavage by serine- and metalloproteases, but **glycosylation** and the recently described **citruination** of the arginine residues in chemokines have also been shown to have notable effects on receptor binding and signalling (Mortier et al, 2011).

Chemokine signalling is initiated by binding to and activation of G protein-coupled seven-transmembrane receptors (Fig 1). Their classification is based on their specificity for the chemokine family members, for example CC-chemokine receptors (CCR) preferentially bind to CC-chemokines and CXCR analogously bind to CXC-chemokines. Activation of chemokine receptors occurs in a two-step fashion, in which the globular part of the chemokine ligand binds to the extracellular loops of the receptor and the flexible chemokine N-terminus subsequently enters a defined pocket between the transmembrane helices (Montecarlo & Charo, 1996). An interesting feature of chemokine receptors is their ability to form homo- and heterodimers, although the functional consequences of such interactions have been diverse (Kramp et al, 2011). For example, an enrichment of CCR5 and CXCR4 was observed in the **immunological synapse** between B and T cells, and the stimulation of T-cell responses relied on the presence of both receptors, which were found in a complex on T-cell membranes (Contento et al, 2008). However, in a similar study, a negative binding cooperativity was observed between the ligands for CXCR4, CCR2 and CCR5 (Sohy et al, 2009). The affinity of CXCR4 for its ligand CXCL12 was significantly reduced in the presence of CCR2 or CCR5 ligands, both in transfected cells as

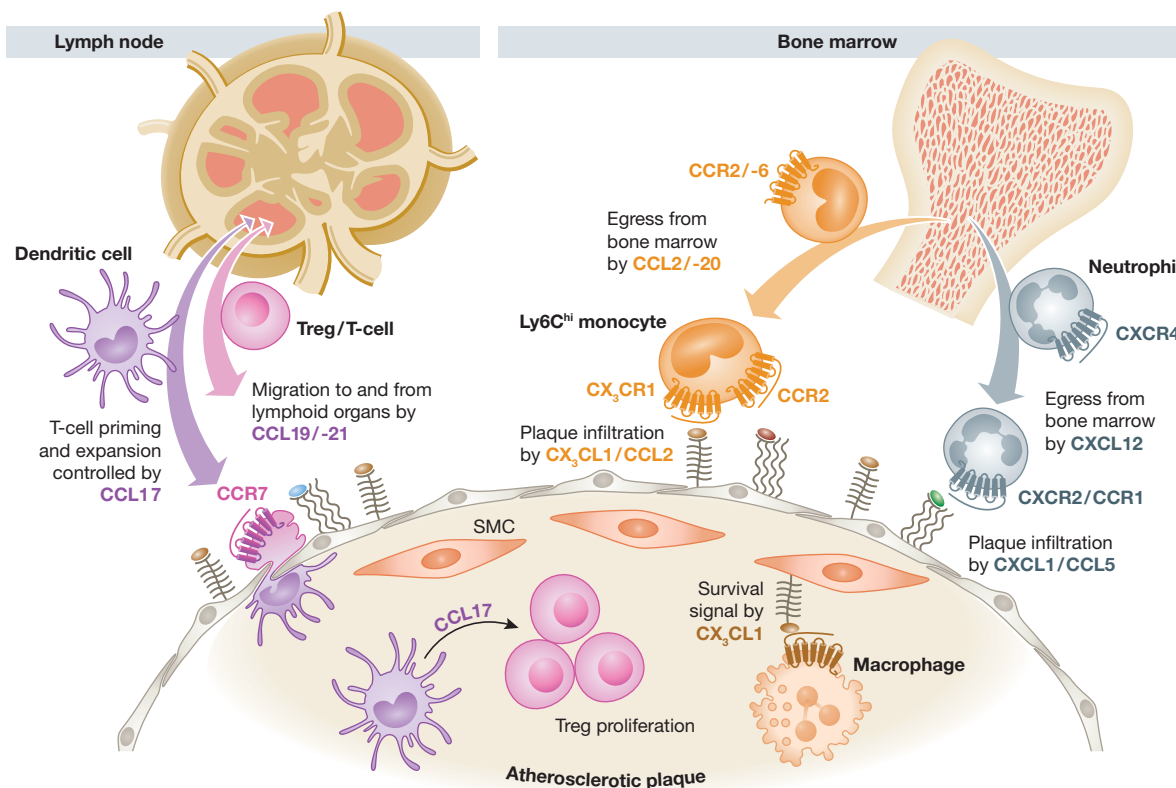


Figure 1. Schematic representation of novel chemokine players in atherosclerosis and their mechanism of action towards their target cells. Neutrophils and Ly6C^{hi} monocytes emigrate from the bone marrow through action of chemokines CXCL12 or CCL2/-20, respectively, and are recruited to atherosclerotic plaques by chemokines (e.g. CXCL1 or CX₃CL1) presented on endothelial cells of the inflamed vessel wall. Inside the plaque, the CX₃CL1-CX₃CR1 axis promotes Ly6C^{hi} monocyte survival. Dendritic cells expressing CCL17 may control the expansion of Treg in plaques and lymph nodes. Migration to and from lymphoid organs, as well as T cell priming are mediated by signalling between CCL19/-21 and CCR7 on T cells and dendritic cells.

well as in naive CD4⁺ T lymphocytes. In a mouse air pouch model of leukocyte recruitment, addition of the CCR5 antagonist TAK779 strongly reduced the migration of leukocytes towards CXCL12 (Sohy et al, 2009). Although these studies indicate that chemokine receptors readily form heterodimers on the cell surface, the functional consequences of such interaction may vary depending on the physiologic context.

In studies with chemokine receptor-expressing cells, many chemokines were found to bind to multiple chemokine receptors and *vice versa*; observations that prompted the conceptual introduction of 'redundancy' in the field of chemokine biology (Lukacs et al, 1999; Mantovani, 1999). However, since these types of experiments do not take the spatial and temporal pattern of chemokine and chemokine receptor expression in normal physiology into account, the actual redundancy in this system *in vivo* appears overestimated (Koenen & Weber, 2010; Schall & Proudfoot, 2011). Considering that most leukocyte subsets are characterized by a combination of chemokine receptors, one can envision that in a given (patho-)physiologic context, a particular palette of chemokines is generated and presented on the endothelial surface of the vessel wall by a specific repertoire of GAGs. This palette of chemokines is able to attract the leukocyte subtype with the corresponding combination of chemokine receptors, thereby, enabling a 'tailor-made' immune response. Thus, the chemokine family can rather be perceived as an extensive and multifaceted system for conveying information.

Established and novel chemokine players in atherosclerosis

Given the function of chemokines as potent leukocyte attractants, their involvement in vascular inflammatory disease is conceivable and the role of many chemokines has been investigated in the context of atherosclerosis. Pioneering studies have placed the main focus on the identification of key chemokines promoting atherosclerotic disease (Boisvert et al, 1998; Boring et al, 1998; Lesnik et al, 2003). Later studies aimed to establish a connection between the actions of chemokines and the involvement of individual leukocyte types (Boisvert et al, 2006; Heller et al, 2006; Veillard et al, 2005), while contemporary work tends to unravel the function and dynamics of leukocyte subsets by characterizing their chemokine usage and receptor profile (Luchtefeld et al, 2010; Tacke et al, 2007; Weber et al, 2011). Chemokines or chemokine receptors that classically play a role in atherosclerosis are, for example CXCR3 and its ligands, which are active on effector T cells, CCR2 and CX₃CR1 and their respective ligands CCL2 and CX₃CL1, which attract monocytes, and CCR5 with its ligand CCL5, which is involved in the recruitment of both T cells and monocytes. The role of these chemokines in atherosclerotic disease has been extensively reviewed (Zernecke & Weber, 2010). Recent studies, however, have implicated surprising novel players from the chemokine system in atherosclerosis. In addition, established players were found to act on cell types hitherto not appreciated for their contribution to atherogenesis or to exert previously

unknown functions. With advances in the definition of monocyte and lymphocyte subsets, chemokine-receptor axes were identified that specifically act on specific subsets.

Chemokine receptor usage has been intensely studied with respect to monocyte subsets. In mice, two main subpopulations can be identified: the classical or 'inflammatory' monocytes characterized by high expression of the surface markers Ly6C (also called Gr1) and L-selectin (CD62L), high expression of CCR2 and intermediate expression of CX₃CR1 and non-classical or 'resident' monocytes, which are Ly6C^{low}, CCR2⁻, CX₃CR1^{hi} and CD62L⁻ (Gautier et al, 2009; Tacke et al, 2007). During feeding of apolipoprotein E-deficient (ApoE^{-/-}) mice with a high-fat diet, the numbers of circulating classical Ly6C^{hi} monocytes rose significantly (Swirski et al, 2007). These cells preferentially infiltrated atherosclerotic lesions and differentiated into macrophages, suggesting that the classical subset dominates in driving atherosclerosis. The classical Ly6C^{hi} monocytes appear to utilize CX₃CR1 and CCR2 as major receptors to enter atherosclerotic lesions, while the non-classical Ly6C^{low} monocytes mainly depended on CCR5 to enter plaques, despite a high expression of CX₃CR1 (Tacke et al, 2007). Interestingly, the CX₃CL1-CX₃CR1 axis was found to mediate growth factor-like signals in Ly6C^{low} monocytes, rather than cell-recruiting cues (Landsman et al, 2009). In the absence of CX₃CR1, Ly6C^{low} monocyte levels were significantly reduced. This could be explained by increased cell death due to a lack of CX₃CL1 binding to CX₃CR1, which served as a specific survival signal for this subset. Transfer of CX₃CR1-deficient bone marrow into ApoE^{-/-} mice led to a reduction in atherosclerosis, compared to mice transplanted with CX₃CR1-containing bone marrow. This reduction in atherosclerosis was characterized by a marked increase of apoptotic cells in the plaques of the chimeric CX₃CR1^{-/-}ApoE^{-/-} mice and could be counteracted by overexpression of Bcl-2, a molecule that prevented cell death in CX₃CR1-deficient monocytes. Thus, it can be concluded that cell survival conferred by CX₃CR1 constitutes its pro-atherogenic potential, rather than cell recruitment (Landsman et al, 2009).

Similarly, CCR2 functions are not restricted to cell recruitment. Besides mediating infiltration of classical monocytes into plaques, CCR2 specifically controls egress of Ly6C^{hi} monocytes from the bone marrow during bacterial infection (Serbina & Pamer, 2006) or hyperlipidemia (Tsou et al, 2007). Thus, the classical monocyte subset is retained in the bone marrow of CCR2^{-/-} mice and the resulting lower blood cell count might also contribute to the reduction of atherosclerosis that is associated with CCR2 deficiency (Boring et al, 1998). Further to CCR2, a recent study has identified CCR6 and its ligand CCL20 as relevant mediators of Ly6C^{hi} monocyte trafficking (Wan et al, 2011). Deficiency in CCR6 specifically reduced Ly6C^{hi} monocyte counts in ApoE^{-/-} mice during high-fat diet and intravenous injection of CCL20 caused blood monocytoysis, indicating that CCR6 is involved in the regulation of Ly6C^{hi} subset release from the bone marrow. Like CCL2, CCL20 induced monocyte chemotaxis, which was CCR6-dependent, as CCR6^{-/-} monocytes were non-responsive (Wan et al, 2011).

Besides monocytes/macrophages, T lymphocytes are involved in the development of atherosclerotic lesions and exist in many different subtypes, which may exert pro-atherogenic or anti-atherogenic functions (Weber et al, 2008). For example, several studies highlight the importance of CXCR3 for the recruitment of CD4⁺ effector T cells (e.g. T helper 1 cells) into atherosclerotic lesions and blockade or deletion of CXCR3 or its ligand CXCL10 reduced plaque formation in mice (Heller et al, 2006; van Wanrooij et al, 2008; Veillard et al, 2005). However, knock-down of the CXCR3-CXCL10 axis not only reduced pro-atherogenic CD4⁺ T-cell counts in the lesions, but also increased the presence of CD4⁺ FoxP3⁺ CD25⁺ regulatory T cells (Treg), which are considered to play a beneficial role in atherosclerotic disease (Ait-Oufella et al, 2006; Mor et al, 2007). Thus, CXCR3 may represent a switch between a pro-inflammatory effector T cell- and an anti-inflammatory Treg-mediated immune response. A particularly potent subset of effector T cells carries CXCR6, the receptor for the transmembrane chemokine CXCL16, which possesses both chemokine and oxLDL-scavenger activity (Ludwig & Weber, 2007). These CXCR6⁺ T cells have been shown to express high amounts of interferon- γ (IFN- γ) upon activation (Calabresi et al, 2002), which is an important cytokine in atherogenesis. Genetic deletion of CXCR6 strongly attenuated atherosclerotic lesion formation in Apoe^{-/-} mice, owing to a strong reduction of CXCR6⁺ T cells in the aortas and secondary lymphoid organs of CXCR6^{-/-} mice (Galkina et al, 2007). The production of IFN- γ in CXCR6^{-/-} aortas was decreased accordingly, which led to a decreased accumulation of macrophages and, thereby, providing an explanation for the reduction of plaque formation (Galkina et al, 2007). Of note, whereas, CXCL16 is up-regulated during atherogenic conditions (Postea et al, 2008), genetic deletion of CXCL16 in hyperlipidemic mice increased atherosclerotic plaque formation (Aslanian & Charo, 2006). This was explained by the loss of the oxLDL-scavenging function of CXCL16, which may be more influential than the chemokine function in atherogenesis.

Beyond the role of single mononuclear cell subsets, recent studies focused on the interplay between antigen-presenting DC and T cells in the context of atherosclerosis and it is not surprising that chemokines have been implicated as important modulators herein (Luchtfeld et al, 2010; Weber et al, 2011). For example an attenuation of atherosclerotic plaque formation was observed in hyperlipidemic mice with a genetic deletion of CCR7 (Luchtfeld et al, 2010), a chemokine receptor, which is important for the trafficking of T cells and DC to and from lymphatic organs (Forster et al, 2008). This decrease of plaque formation was associated with elevated numbers of T cells and cells expressing the DC marker CD11c in the aortic roots of CCR7-deficient mice. The increased numbers of CCR7^{-/-} T cells in the atherosclerotic lesions were explained by enhanced retention of these cells within the plaques, as CCR7 signalling serves to guide cells to distal lymph nodes. Interestingly, the migration of CCR7^{-/-} T cells into the aorta was also significantly impaired compared with wild-type T cells, suggesting that CCR7 ligands might be responsible for guiding T cells to sites of inflammation. The impaired migration of CCR7-deficient T cells is paralleled by reduced homing of DC to secondary lymphoid

organs, resulting in reduced priming of T cells by antigen-loaded DC. In the context of atherosclerosis, oxLDL is regarded as a major antigen that induces local and systemic immune reactions (Hansson & Hermansson, 2011). In a key experiment, CCR7-deficient or wild-type T cells were cocultured with oxLDL-pulsed DC and subsequently injected into hyperlipidemic CCR7^{-/-} mice. Unlike CCR7-deficient T cells, wild-type T cells primed by oxLDL-pulsed DC could revert the decrease in atherosclerotic lesion formation in CCR7^{-/-} mice. This suggests that both impaired T cell priming in the secondary lymphoid organs and defective migration of CCR7-deficient T cells are responsible for the reduction in atherosclerosis in CCR7^{-/-} mice (Luchtfeld et al, 2010). The findings in this study might conflict with previous work that has suggested a role for CCR7 in a model of rapid plaque regression, wherein plaque-containing aortic segments from hyperlipidemic Apoe^{-/-} mice were transplanted into normolipidemic wild-type mice (Trojan et al, 2006). Gene expression analysis of regressing plaques revealed an up-regulation of CCR7 mRNA and regression of plaques was inhibited by blocking antibodies against CCR7 ligands CCL19 and CCL21, indicating that CCR7-driven egress of mononuclear cells from plaques may be responsible for plaque regression in this model. This would mean that CCR7 is atheroprotective rather than pro-atherogenic. However, in a recent study implementing a novel mouse model of gene therapy-induced plaque regression, no involvement of CCR7 during regression of plaques could be observed (Potteaux et al, 2011). Instead, it was hypothesized that an attenuation of mononuclear cell influx after viral transfer of the Apoe-gene was the mechanism underlying plaque regression, rather than a (CCR7-driven) egress of inflammatory cells from the plaque.

One aspect that has unfortunately not been taken into account in the studies discussed above is the role of CCR7 functions in Treg, which also use CCR7 to home to lymphoid tissue and down-regulate immune responses relevant to atherosclerosis (Schneider et al, 2007). Recent work identified a functional link between DC and Treg in atherosclerosis, that is controlled through the chemokine CCL17 (Weber et al, 2011). In hyperlipidemic mice, whole-body or bone marrow-specific deficiency of CCL17 reduced atherosclerotic lesion formation, accompanied by a decreased number of macrophages and CD3⁺ T cells in the plaques. Whereas, CCL17 was found to be involved in the recruitment of CD4⁺ T cells into sites of inflammation, an accumulation of Treg in the lymph nodes and aortas of CCL17^{-/-} mice was nevertheless observed, which was absent in CCL17^{+/+} control animals. This accumulation could be explained by the control of Treg homeostasis through a CCL17-expressing DC subset in the lesions and lymph nodes of hyperlipidemic mice. Cocultivation of CD4⁺ T cells with CCL17^{-/-} DC enhanced expansion of Treg and this was accompanied by lower numbers of apoptotic Treg compared to coculture with CCL17^{+/+} DC, indicating that CCL17 also serves as a suppressive signal for Treg proliferation. Reconstitution experiments revealed that injection of T cells instructed by CCL17-expressing DC was sufficient to induce atherosclerotic lesion formation in CCL17-deficient Apoe^{-/-} mice that had been depleted of CD4⁺ T cells. Finally, we could demonstrate that treatment of Apoe-deficient

Table 1. Established and novel chemokine players, functions and cellular targets in atherosclerosis

Chemokine(s)	Receptor(s)	Effect on cell type	References
CXCL9,-10,-11	CXCR3	Recruitment of effector T cells and Treg to plaques	Heller et al (2006) Veillard et al (2005)
CCL2	CCR2	Recruitment of Ly6C ^{hi} monocytes to plaques Emigration of Ly6C ^{hi} monocytes from BM	Tacke et al (2007)
CX ₃ CL1	CX ₃ CR1	Recruitment of Ly6C ^{hi} monocytes to plaques Survival of Ly6C ^{lo} monocytes in plaques	Tacke et al (2007)
CCL5	CCR5	Recruitment of Ly6C ^{hi} monocytes to plaques	Tacke et al (2007)
CCL20	CCR6	Recruitment of Ly6C ^{hi} monocytes to plaques Emigration of Ly6C ^{hi} monocytes from BM	Wan et al (2011)
CXCL16	CXCR6	Recruitment of IFN γ ⁺ T cells to plaques Scavenging of oxLDL (CXCL16)	Galkina et al (2007) Aslanian & Charo (2006)
CCL19,-21	CCR7	Trafficking of T cells and DC to SLO Recruitment of T cells and Treg to plaques	Schneider et al (2007) Luchtefeld et al (2010)
CCL17	CCR4	Homeostasis and recruitment of Treg in/to plaques and SLO	Weber et al (2011)
CCL5	CCR1	Recruitment of neutrophils to arterial injury	Drechsler et al (2010)
CXCL1	CXCR2	Recruitment of neutrophils to arterial injury	Drechsler et al (2010)

BM, bone marrow; IFN γ , interferon gamma; SLO, secondary lymphoid organ.

mice with a blocking antibody against CCL17 effectively reduced atherosclerotic plaque formation, highlighting the importance of CCL17 for Treg maintenance by DC in the context of vascular inflammation and identifying this chemokine as potential therapeutic target for the treatment of atherosclerosis (Weber et al, 2011).

Beyond mononuclear cells, additional blood cell types are involved in the initiation and progression of vascular inflammation, most notably neutrophils and platelets (Soehnlein & Lindbom, 2010; von Hundelshausen et al, 2009). **Hypercholesterolemia** induces the efflux of neutrophils from the bone marrow and increased circulating neutrophil counts accelerate atherosclerosis, particularly in early phases of disease development (Drechsler et al, 2010; Gomes et al, 2010; Zerneck et al, 2008). While the CXCL12–CXCR4 axis is responsible for the retention of neutrophils in the bone marrow (Martin et al, 2003), CXCL1–CXCR2 and CCR1, -2 and -5 were shown to be responsible for the mobilization and infiltration of neutrophils into sites of arterial inflammation, respectively (Drechsler et al, 2010). Further to a role in mononuclear cell recruitment, CXCR2 and CCR1, -2 and -5 might thus promote atherosclerosis by mediating the trafficking of inflammatory neutrophils to newly developing plaques. Since platelets contribute to the initiation of arterial inflammation through expression of cytokines such as CD40 ligand (CD40L) or interleukin-1 and by deposition of functionally synergistic chemokines such as CCL5 and CXCL4 onto the inflamed vessel wall (von Hundelshausen et al, 2005, 2009), they may cooperate with neutrophils to drive the progression of early atherosclerotic lesions. In addition, CD40L on platelets is important for atheroprotection by mediating endothelial and macrophage CCL2 expression and promoting platelet–leukocyte and platelet–endothelium interactions (Lievens et al, 2010). Platelets also influence the functions of T lymphocytes, for example by enhancing adhesion of T cells to collagen-coated surfaces *in vitro* and arterial thrombi *in vivo* (Hu et al, 2010). In cocultures with CD4⁺ T cells, platelets inhibited the proliferation but promoted the differentiation of these cells

into T helper type 1, type 17 and Treg phenotypes, an effect dependent on platelet–lymphocyte communication through direct cell–cell contact and signalling via CCL5 and CXCL4 (Gerdes et al, 2011). Thus, platelets may modulate the course of inflammatory vascular disease on multiple levels. An overview of the chemokines involved in atherosclerosis is given in Table 1 and the novel mechanisms of chemokine control of atherosclerotic plaque progression are schematically represented in Fig 1.

Traditional chemokine-based therapeutics for treating cardiovascular disease

The involvement of the chemokine system in the vast amount of inflammatory diseases has prompted researchers in both commercial and non-profit organizations to investigate the possibility of pharmacologic intervention. This seems feasible, since 50% of the drugs on the market are targeted against G protein-coupled receptors, which also encompass chemokine receptors. Having defined pockets for chemokine binding and activation, chemokine receptors are well amenable to the design of small molecular inhibitors. Numerous small-molecular inhibitors have been designed, through which direct and full blockade can be achieved. One of the first, TAK779 (Takeda) was originally conceived as an inhibitor for human immunodeficiency virus (HIV) entry into host cells. TAK779 potently blocks CCR5 in humans and additionally inhibits CXCR3 in mice (Gao et al, 2003). Administration of TAK779 in mice effectively reduced atherosclerotic lesion formation due to a decrease of T helper type 1 cells in the lesions (van Wanrooij et al, 2005). This finding might imply that blockade of CCR5 might also decrease the risk for atherosclerosis in HIV-infected individuals, who are at increased risk for cardiovascular events. However, this study unfortunately did not reveal whether the observed beneficial effects were due to the blockade of CCR5 or CXCR3 or both by TAK779. In later work, the reduction of atherosclerosis

with the specific CXCR3 antagonist NBI-74330 (Amgen) resulted in less effector T cells in the draining lymph nodes and led to an increased amount of Treg in lymph nodes and plaques (van Wanrooij et al, 2008). Thus, through the blockade of CXCR3, the migration of effector T cells can be reduced to the benefit of Treg, leading to a net reduction of the inflammatory response. Interestingly, the CXCR2 antagonist SB-517785-M (GlaxoSmithKline) was found to reduce angiotensin II-induced leukocyte recruitment in rat arterioles (Nabah et al, 2007). Through its potential to inhibit the influx of neutrophils, this compound might be beneficial to prevent the initial phase of atherogenesis (Drechsler et al, 2010), implying that inhibition of CXCR2 might exert benefits for the prevention of atherosclerotic disease in patients at risk. A chemokine that has historically been in focus as therapeutic target for the treatment of cardiovascular disease is CCL2. In mice, pharmacologic blockade of the CCL2-CCR2 axis has shown variable success. For example, a blocking antibody against CCL2 and CCL12 reduced plaque formation and inflammatory leukocyte infiltration in Apoe^{-/-} mice (Lutgens et al, 2005). However, no reduction in atherosclerosis was observed in mice treated with INCB-3344, a potent CCR2 antagonist, although this antagonist reduced the number of circulating inflammatory Ly6C^{hi} monocytes (Aiello et al, 2010). A small molecular inhibitor of CCR2, CCX140 (ChemoCentryx) has recently completed a phase 2 clinical trial for type 2 diabetes mellitus. This antagonist might also show beneficial effects in patients with cardiovascular disease, but relevant clinical studies are not yet available (Charo & Taub, 2011). While these results collectively indicate that inhibition of chemokine receptors in principle hold promise for the prevention and treatment of cardiovascular disease, some caveats remain. Whereas, blockade of CXCR4 with the small-molecule antagonist AMD3465 (AnorMED) decreased neointima formation after arterial injury in mice (Karshovska et al, 2008), long-term treatment with AMD3465 increased diet-induced atherosclerosis by mobilizing neutrophils from the bone marrow and increasing their recruitment to plaques (Zernecke et al, 2008).

Another established way to achieve inhibition of the chemokine axis is through the use of blocking antibodies. Due to their high specificity and their often favourable plasma half-life, antibodies are generally suitable agents for therapeutic intervention. However, the necessity for administration by injection, their high production costs and the risk for allergic reactions or the generation of an adverse immune response are drawbacks of the antibody approach, although the latter can be prevented by [humanization](#) procedures. Nevertheless, for cytokines such as tumour-necrosis factor (TNF)- α , antibody-based therapeutics have already been approved for the treatment of autoimmune diseases over a decade ago. Also for chemokines and their receptors, various antibody-based drug candidates have entered clinical trials, although this has not led to notable breakthroughs so far. For example, the humanized anti-CXCL8 antibody ABX-IL8 (Abgenix) has showed disappointing results in clinical trials for the treatment of rheumatoid arthritis, psoriasis and COPD (Wells et al, 2006). In addition, the humanized monoclonal antibodies ABN-912

(Novartis) and MLN1202 (Millenium), which are directed against CCL2 and CCR2, respectively, were also inactive when clinically assessed for their benefit in rheumatoid arthritis (Schall & Proudfoot, 2011). In a phase II clinical trial, however, MLN1202 has been shown to reduce serum C-reactive protein levels in patients at risk for cardiovascular disease (Gilbert et al, 2011), suggesting that CCR2 blockade reduces inflammation for this condition and might supplement current treatment options, namely statin therapy. Other antibodies directed against the chemokine system with therapeutic potential that are currently in clinical trials is the CCR5-specific HGS004 (HGS Inc.) as anti-HIV agent and the CXCR3-specific MDX-1100 (Bristol-Myers-Squibb) for treatment of rheumatoid arthritis (Table 2).

Novel classes of chemokine blockers

Alternatively to the more conventional approaches of small molecular antagonists and antibodies, a number of innovative chemokine antagonists have been developed. Additionally, interesting chemokine-neutralizing proteins with therapeutic potential have been isolated from parasites. The application of novel compounds might be feasible, for example in the case of heterogeneity in the target chemokine or chemokine receptor, for example due to post-translational modifications that modify the affinity of the target for an antibody or small molecule antagonist.

Owing to their compact and robust structures and the relative ease of producing them by recombinant methods, chemokines themselves have been subject to structural modification, which resulted in potent antagonists (Koenen & Weber, 2010). Prototypic for modified chemokines is an N-methionylated variant of CCL5 (Met-RANTES) with antagonistic properties identified by serendipity after expression of CCL5 in *E. coli* (Proudfoot et al, 1996). The administration of Met-RANTES has been shown to exert beneficial effects in various mouse models of inflammatory disease (Ajuebor et al, 2001; Berres et al, 2010; Song et al, 2002), including atherosclerosis and neointima formation after arterial injury (Schober et al, 2002; Veillard et al, 2004). Notably, CCL5 can be considered a key determinant of disease severity, since blockade of CCL5 by antibodies or engineered antagonists has shown beneficial results in various mouse atherosclerosis and heart ischemia-reperfusion models (Braunersreuther et al, 2010, 2008; Montecucco et al, 2011). In general, N-terminal modifications of chemokines led to variants with reduced activity and antagonistic potential, a characteristic that has been exploited in recent studies that varied the N-terminal amino acids of CCL5 and CX₃CL1 in a phage display library, yielding interesting novel antagonists (Dorgham et al, 2009; Gaertner et al, 2008). These new antagonists have not been investigated in atherosclerosis models, however. In an analogous manner, N-terminal truncated mutants of CCL2 and CXCL8 effectively bound but failed to activate their receptors (Gong & Clark-Lewis, 1995; Moser et al, 1993). Besides the N-terminus, the GAG-binding domain and the amino acids responsible for oligomerization are interesting targets for mutagenesis and their modification has also resulted in

Table 2. Chemokine–receptor antagonists with therapeutic potential for cardiovascular disease

Compound	Target	Study outcome	References
TAK779	CCR5/CXCR3	Reduction of atherosclerosis in mice	van Wanrooij et al (2005)
NBI-74330	CXCR3	Reduction of atherosclerosis in mice	van Wanrooij et al (2008)
SB-517785-M	CXCR2	Reduced arteriolar leukocyte arrest in rats	Navab et al (2004)
MLN1202	CCR2	Reduction of plasma CRP levels in patients	Gilbert et al (2011)
CCX140	CCR2	Phase II clinical trial completed ^{a,b}	Charo & Taub (2011)
INCB-3344	CCR2	No reduction of atherosclerosis in mice	Aiello et al (2010)
ABX-IL8	CXCL8	Poor results in a Phase II clinical trial ^b	Mahler et al (2004)
ABN-912	CCL2	Ineffective in a Phase II clinical trial ^b	Haringman et al (2006)
HGS004	CCR5	Phase I clinical trial completed ^b	Lalezari et al (2008)
MDX-1100	CXCR3	Phase II clinical trial completed ^b	Yellin et al (2009)
AMD3465	CXCR4	Reduction of neointima formation, increase of atherosclerosis in mice	Karshovska et al (2008), Zernecke et al (2008)
Met-RANTES	CCR1, -5	Reduction of neointima formation and atherosclerosis in mice	Schober et al (2002), Veillard et al (2004)
[⁴⁴ AANA ⁴⁷]-CCL5	CCL5	Reduction of atherosclerosis and ischemia–reperfusion injury in mice	Braunersreuther et al (2010, 2008)
PA508	CCL2	Reduction of neointima formation and ischemia–reperfusion injury in mice	Liehn et al (2010)
M-T7	Chemokines ^c	Inhibition of transplant vasculopathy in mice	Dai et al (2010)
CKBP	Chemokines ^d	Not yet assessed	Smith et al (2005)
Evasin-1, -3, -4	Chemokines ^e	Reduction of ischemia–reperfusion injury in mice (Evasin-3)	Montecucco et al (2010)
MKEY	CCL5/CXCL4 ^f	Reduction of atherosclerosis in mice	Koenen et al (2009)
Anti-CCL17-Ab	CCL17	Reduction of atherosclerosis in mice	Weber et al (2011)

CRP, C-reactive protein; Ab, antibody.

^aResults from animal models not disclosed by company (ChemoCentryx).

^bClinical trials were performed for diseases other than atherosclerosis.

^cM-T7 inhibits chemokine binding to GAGs.

^dCKBP inhibits various chemokines of the CC-, CXC- and CX₃C-classes.

^eEvasins inhibit various chemokines of the CC- and CXC-classes.

^fMKEY inhibits the heterophilic interaction between CCL5 and CXCL4.

chemokine variants with antagonistic potential. This is exemplified by a mutation of proline 8 into alanine in CCL2, which resulted in an obligate monomeric form that retained *in vitro* activity towards CCR2 but nevertheless acted as an antagonist *in vivo*, possibly by displacing endogenous CCL2 from GAGs on the vessel wall (Handel et al, 2008). Likewise, mutation of the GAG-binding sites in CCL5, CCL7 and CXCL12 yielded chemokine mutants that were competent in receptor activation *in vitro* but possessed anti-inflammatory dominant-negative properties in animal models of inflammation, including atherosclerosis (Ali et al, 2010; Braunersreuther et al, 2008; O'Boyle et al, 2009). It is intriguing how chemokine mutants with reduced oligomerization or binding to GAGs might nevertheless act as inhibitors for their wild-type counterparts. For chemokines that form higher-order oligomers, such as CCL5, the mutant CCL5 might pair with endogenous CCL5 to form biologically inactive heteromers. Yet, for chemokines that maximally form dimers, such as CCL2 and CXCL12, the mechanism of action of their mutants is less clearly defined. One explanation might be desensitization of their cognate receptors by the mutant chemokines, which may still act as partial agonists *in vivo*.

By combining gain-of-function with loss-of-function modifications, a new class of chemokine blockers with increased potential to bind to GAGs but without affinity for their cognate receptors has been engineered, such as the recently described PA508 (Piccinini et al, 2010). This novel chemokine antagonist

is a modified form of CCL2 with an engineered additional GAG binding site and mutation of residues critical for CCR2 binding and activation. This resulted in strongly increased binding to GAGs, yet decreased affinity for CCR2. In our recent study in mice, administration of PA508 resulted in reduced neointima formation after arterial injury, due to a reduced infiltration of monocytes in the lesions (Liehn et al, 2010). In a model of myocardial ischemia and reperfusion, PA508 reduced myocardial damage and improved cardiac function by decreasing the immigration of monocytes and beneficially altering scar composition. Thus, by competing with endogenous CCL2, PA508 attenuates the inflammatory response after arterial and myocardial injury and is able to reduce inflammatory cell influx. Similar results were achieved with proteins derived from pox or herpes virus, which were able to inhibit the binding of chemokines to GAGs on the vessel wall (Dai et al, 2010). In an *allogeneic* vascular transplant remodelling model in mice, a single injection of pox virus M-T7 protein, which inhibited chemokine binding to GAGs, strongly reduced transplant neointima formation in wild-type and CCL2^{-/-} transplants, but not in ndst-1^{-/-} transplants, indicating that the inhibitory effects of M-T7 were specific for chemokine–GAG interactions. In addition to proteins that modulate the presentation of chemokines on the vessel wall, GAGs themselves might remove chemokines from vascular endothelial cells. This has been exemplified by a study showing that the administration of heparin in patients led to an increase in plasma concentrations

of the CXCR3-ligands CXCL9, -10 and -11 (Ranjbaran et al, 2006). Further investigation revealed that heparin displaced these chemokines from the endothelial GAGs, leading to a reduced transendothelial migration of T cells *in vitro* and recruitment of allogeneic lymphocytes in a mouse arterial allograft transplantation model. The above studies indicate that interference with chemokine binding to GAGs might present an attractive approach for the treatment of vascular inflammatory disease.

Other chemokine-binding proteins from pathogens have been cloned and expressed, and these molecules represent potent anti-inflammatory agents in animal models. For example, a 36 kDa protein secreted from eggs of the nematode *Schistosoma mansoni* (secreted chemokine binding protein, CKBP) was found to inactivate chemokines across the CC-, CXC- and CX₃C-families and reduced leukocyte recruitment in several mouse models of inflammation (Smith et al, 2005). In addition, the saliva of ticks contains proteins that attenuate immune reactions. Among these proteins are the recently discovered Evasins, a group of compact chemokine-inactivating proteins with high selectivity for members of the different chemokine subclasses (Deruaz et al, 2008). Evasin-1 and -4 bind to CC-chemokines (e.g. CCL3, -4 and -5), whereas, Evasin-3 selectively binds to the CXC-chemokines CXCL1 and CXCL8 (CXCL2 in the mouse). The 3D structure of Evasins-1 and -3 has been determined and both are very compact and characterized by unique structural folds, dissimilar to other (e.g. viral) CKBPs (Deruaz et al, 2008; Dias et al, 2009). In co-crystallization studies, Evasin-1 was shown to form a 1:1 complex with CCL3, in which the flexible N-terminus of the chemokine is embraced by the C-terminus of Evasin-1 (Dias et al, 2009). The resulting immobilization of the CCL3 N-terminus by Evasin-1 might be the mechanism underlying the potent chemokine inhibition. The therapeutic potential of Evasin-3 was explored in models of heart ischemia-reperfusion in the mouse (Montecucco et al, 2010). Here, a single administration of Evasin-3 shortly after the onset of ischemia was sufficient to reduce post-ischemic infarct size compared to vehicle-treated animals. This reduction of cardiac tissue damage was accompanied by lower concentrations of circulating troponin I and a decreased infiltration of neutrophils at the site of injury, which is the primary cell type attracted by CXCL1 and CXCL2. Thus, these studies suggest that chemokine-neutralizing proteins from pathogens might hold potential as anti-inflammatory agents for the treatment of cardiovascular disease.

Finally, innovative strategies for manipulating the chemokine system have been conceived that might hold promise as future pharmaceuticals. The therapeutic use of antibodies for instance might be replaced by high-affinity blockers constructed on a protein scaffold in the future (Binz et al, 2005). Such scaffolds might be more effective to produce and considering a potential redundancy in the chemokine system, they could also be equipped with binding sites to multiple targets. Another possibility is the disruption of pro-atherogenic heteromers of the platelet-derived chemokines CCL5 and CXCL4. Since CCL5 and CXCL4 form a heteromeric complex with an increased potential to attract monocytes to sites of inflammation (von

Hundelshausen et al, 2005), we hypothesized that the selective dissociation of this complex might be an interesting anti-inflammatory approach. A synthetic peptide termed MKEY, which was designed for this purpose, resulted in a significant decrease in atherosclerotic lesion formation in hyperlipidemic mice, due to a reduction of lesional macrophage accumulation (Koenen et al, 2009). The efficiency of this MKEY peptide required the presence of both CCL5 and CXCL4, indicating the specificity of this antagonist. Moreover, unlike the CCR1/5 antagonist Met-RANTES, host defense functions, for example during herpes simplex type-2 infection were preserved after treatment with MKEY, indicating that normal immune functions are unaffected during the disruption of CCL5–CXCL4 heteromerization. Another potential strategy is altering immune cell homeostasis through the inhibition of chemokines with homeostatic functions. The feasibility of this approach was recently demonstrated by our study showing that through the inhibition of CCL17 by a blocking antibody in Apoe^{-/-} mice, an expansion of beneficial Treg in lymph nodes and aortas was achieved, which resulted in a significant attenuation of atherosclerotic lesion formation (Weber et al, 2011). Thus, the modulation of lymphocyte homeostasis through the functional manipulation of CCL17 harbours opportunities for the design and application of novel therapeutics for the treatment of atherosclerotic vascular disease. A summary of the antagonists with potential benefit for the treatment of cardiovascular disease is listed in Table 2.

Concluding remarks

It is undisputed that the chemokine system plays an important role in the pathophysiology of cardiovascular disease. However, although research in this field is extensive, only 2 chemokine receptor antagonists have currently achieved FDA approval: the CCR5 antagonist Maraviroc for the treatment of HIV and the low molecular weight CXCR4 antagonist Plerixafor (AMD3100) for aiding stem cell mobilization. This highlights the still existing challenges in developing suitable compounds directed against the chemokine system in the context of inflammatory disease. A compound may fail due to numerous reasons and most problems that are encountered during the development of a drug are unforeseen and surface particularly during the clinical evaluation in human subjects, emphasizing the differences between animal models and the course of human life. Unexpected adverse drug interactions, lack of efficiency or heterogeneity in a patient population are only a selection of problems that may arise. In addition, some scientists argue that the search for chemokine-based therapeutics is hampered by the redundancy in the chemokine system (Horuk, 2009), while others advocate for a better conceived target selection and the improvement of drug dosage regimens (Schall & Proudfoot, 2011). Even if this results in effective agents that target the chemokine system, potential safety issues with respect to host defense have to be considered. This is emphasized by studies in mice, demonstrating an impaired mobilization of monocytes from the bone marrow in the absence of CCR2 after microbial

Pending issues

It would be of exceptional interest to investigate the spatial and temporal expression patterns of chemokines during the development of inflammatory (atherosclerotic) diseases. This would also allow better target and dosage selection for the design of effective therapeutics.

Different agonists that bind to the same chemokine receptor are known to elicit different cellular responses. The mechanisms behind this phenomenon are largely unknown.

Novel and specific drug targets can be revealed through the identification of heterophilic chemokine–chemokine or chemokine receptor–receptor interactions.

Through the identification of cell types that have previously been underappreciated to play a role in the pathophysiology of atherosclerosis, novel entry points for drug design can be created.

infection (Serbina & Pamer, 2006) and recent work revealing a narrow therapeutic bandwidth between beneficial and defense-impairing treatment with Met-RANTES in a model of experimental periodontitis (Repeke et al, 2011). Thus, novel and innovative approaches are still needed to tackle the diverse problems that are currently present in the development of chemokine system-based therapeutics. The selective inhibition of chemokine–chemokine and chemokine–GAG interactions, the use of newly identified chemokine-inhibiting proteins from ticks or viruses, or the modulation of lymphocyte homeostasis, for example by the blockade of CCL17 might open fresh perspectives for the therapeutic targeting of the chemokine system in atherosclerosis and other inflammatory diseases. However, these novel approaches each harbour their inherent drawbacks, such as pharmacologic barriers for synthetic peptide-based compounds, specificity issues for chemokine–GAG-based therapeutics or immunologic safety issues for chemokine-binding proteins and compounds blocking homeostatic chemokines. Thus, even though novel innovative approaches are under development, the way towards truly effective therapeutics based on the chemokine system may still remain curved and bumpy.

Acknowledgements

Financial support for this work was provided by the Deutsche Forschungsgemeinschaft (DFG FOR809).

Conflict of interest statement: RRK and CW are shareholders of Carolus Therapeutics, Inc.

For more information

Information about clinical trials:

<http://clinicaltrials.gov/>

Information about chemokine receptor nomenclature:

www.iuphar-db.org

Information about the authors:

<http://www.kreislaufinstitut.de>

http://www.klinikum.uni-muenchen.de/de/Kliniken_Abteilungen_Institute/Abteilungen_Institute/Inst_f_Prophylaxe_u_Ep_d_Kreislaufkrankheiten/index.html

References

- Aiello RJ, Perry BD, Bourassa PA, Robertson A, Weng W, Knight DR, Smith AH, Frederick KS, Kalgutkar A, Gladue RP (2010) CCR2 receptor blockade alters blood monocyte subpopulations but does not affect atherosclerotic lesions in apoE(–/–) mice. *Atherosclerosis* 208: 370–375
- Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J, Merval R, Esposito B, Cohen JL, Fisson S, et al (2006) Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med* 12: 178–180
- Ajuebor MN, Hogaboam CM, Kunkel SL, Proudfoot AE, Wallace JL (2001) The chemokine RANTES is a crucial mediator of the progression from acute to chronic colitis in the rat. *J Immunol* 166: 552–558
- Ali S, O'Boyle G, Hepplewhite P, Tyler JR, Robertson H, Kirby JA (2010) Therapy with nonglycosaminoglycan-binding mutant CCL7: a novel strategy to limit allograft inflammation. *Am J Transplant* 10: 47–58
- Aslanian AM, Charo IF (2006) Targeted disruption of the scavenger receptor and chemokine CXCL16 accelerates atherosclerosis. *Circulation* 114: 583–590
- Bao X, Moseman EA, Saito H, Petryniak B, Thiriot A, Hatakeyama S, Ito Y, Kawashima H, Yamaguchi Y, Lowe JB, et al (2010) Endothelial heparan sulfate controls chemokine presentation in recruitment of lymphocytes and dendritic cells to lymph nodes. *Immunity* 33: 817–829
- Berres ML, Koenen RR, Rueland A, Zaldivar MM, Heinrichs D, Sahin H, Schmitz P, Streetz KL, Berg T, Gassler N, et al (2010) Antagonism of the chemokine Ccl5 ameliorates experimental liver fibrosis in mice. *J Clin Invest* 120: 4129–4140
- Binz HK, Amstutz P, Pluckthun A (2005) Engineering novel binding proteins from nonimmunoglobulin domains. *Nat Biotechnol* 23: 1257–1268
- Boisvert WA, Santiago R, Curtiss LK, Terkeltaub RA (1998) A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J Clin Invest* 101: 353–363
- Boisvert WA, Rose DM, Johnson KA, Fuentes ME, Lira SA, Curtiss LK, Terkeltaub RA (2006) Up-regulated expression of the CXCR2 ligand KC/GRO- α in atherosclerotic lesions plays a central role in macrophage accumulation and lesion progression. *Am J Pathol* 168: 1385–1395
- Boring L, Gosling J, Cleary M, Charo IF (1998) Decreased lesion formation in CCR2–/– mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature* 394: 894–897
- Braunersreuther V, Steffens S, Arnaud C, Pelli G, Burger F, Proudfoot A, Mach F (2008) A novel RANTES antagonist prevents progression of established atherosclerotic lesions in mice. *Arterioscler Thromb Vasc Biol* 28: 1090–1096
- Braunersreuther V, Pellieux C, Pelli G, Burger F, Steffens S, Montessuit C, Weber C, Proudfoot A, Mach F, Arnaud C (2010) Chemokine CCL5/RANTES inhibition reduces myocardial reperfusion injury in atherosclerotic mice. *J Mol Cell Cardiol* 48: 789–798
- Calabresi PA, Yun SH, Allie R, Whartenby KA (2002) Chemokine receptor expression on MBP-reactive T cells: CXCR6 is a marker of IFN γ -producing effector cells. *J Neuroimmunol* 127: 96–105
- Campanella GS, Grimm J, Manice LA, Colvin RA, Medoff BD, Wojtkiewicz GR, Weissleder R, Luster AD (2006) Oligomerization of CXCL10 is necessary for endothelial cell presentation and *in vivo* activity. *J Immunol* 177: 6991–6998
- Charo IF, Ransohoff RM (2006) The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* 354: 610–621
- Charo IF, Taub R (2011) Anti-inflammatory therapeutics for the treatment of atherosclerosis. *Nat Rev Drug Discov* 10: 365–376
- Clore GM, Gronenborn AM (1995) Three-dimensional structures of alpha and beta chemokines. *FASEB J* 9: 57–62
- Contento RL, Molon B, Boularan C, Pozzan T, Manes S, Marullo S, Viola A (2008) CXCR4–CCR5: a couple modulating T cell functions. *Proc Natl Acad Sci USA* 105: 10101–10106
- Dai E, Liu LY, Wang H, McIvor D, Sun YM, Macaulay C, King E, Munuswamy-Ramanujam G, Barteck MY, Williams J, et al (2010) Inhibition of chemokine–

- glycosaminoglycan interactions in donor tissue reduces mouse allograft vasculopathy and transplant rejection. *PLoS ONE* 5: e10510
- Deruaz M, Frauenschuh A, Alessandri AL, Dias JM, Coelho FM, Russo RC, Ferreira BR, Graham CJ, Shaw JP, Wells TN, *et al* (2008) Ticks produce highly selective chemokine binding proteins with antiinflammatory activity. *J Exp Med* 205: 2019-2031
- Dias JM, Losberger C, Deruaz M, Power CA, Proudfoot AE, Shaw JP (2009) Structural basis of chemokine sequestration by a tick chemokine binding protein: the crystal structure of the complex between Evasin-1 and CCL3. *PLoS ONE* 4: e8514
- Dorgham K, Ghadiri A, Hermand P, Rodero M, Poupel L, Iga M, Hartley O, Gorochov G, Combadiere C, Deterre P (2009) An engineered CX3CR1 antagonist endowed with anti-inflammatory activity. *J Leukoc Biol* 86: 903-911
- Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O (2010) Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. *Circulation* 122: 1837-1845
- Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nunez G, Schnurr M, *et al* (2010) NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 464: 1357-1361
- Forster R, Davalos-Misslitz AC, Rot A (2008) CCR7 and its ligands: balancing immunity and tolerance. *Nat Rev Immunol* 8: 362-371
- Gaertner H, Cerini F, Escola JM, Kuenzi G, Melotti A, Offord R, Rossitto-Borlat I, Nedellec R, Salkowitz J, Gorochov G, *et al* (2008) Highly potent, fully recombinant anti-HIV chemokines: reengineering a low-cost microbicide. *Proc Natl Acad Sci USA* 105: 17706-17711
- Galkina E, Harry BL, Ludwig A, Liehn EA, Sanders JM, Bruce A, Weber C, Ley K (2007) CXCR6 promotes atherosclerosis by supporting T-cell homing, interferon-gamma production, and macrophage accumulation in the aortic wall. *Circulation* 116: 1801-1811
- Gao P, Zhou XY, Yashiro-Ohtani Y, Yang YF, Sugimoto N, Ono S, Nakanishi T, Obika S, Imanishi T, Egawa T, *et al* (2003) The unique target specificity of a nonpeptide chemokine receptor antagonist: selective blockade of two Th1 chemokine receptors CCR5 and CXCR3. *J Leukoc Biol* 73: 273-280
- Gautier EL, Jakubzick C, Randolph GJ (2009) Regulation of the migration and survival of monocyte subsets by chemokine receptors and its relevance to atherosclerosis. *Arterioscler Thromb Vasc Biol* 29: 1412-1418
- Gerdes N, Zhu L, Ersoy M, Hermansson A, Hjendahl P, Hu H, Hansson GK, Li N (2011) Platelets regulate CD4+ T-cell differentiation via multiple chemokines in humans. *Thromb Haemost* 106: 353-362
- Gilbert J, Lekstrom-Himes J, Donaldson D, Lee Y, Hu M, Xu J, Wyatt T, Davidson M (2011) Effect of CC chemokine receptor 2 CCR2 blockade on serum C-reactive protein in individuals at atherosclerotic risk and with a single nucleotide polymorphism of the monocyte chemoattractant protein-1 promoter region. *Am J Cardiol* 107: 906-911
- Gomes AL, Carvalho T, Serpa J, Torre C, Dias S (2010) Hypercholesterolemia promotes bone marrow cell mobilization by perturbing the SDF-1: CXCR4 axis. *Blood* 115: 3886-3894
- Gong JH, Clark-Lewis I (1995) Antagonists of monocyte chemoattractant protein 1 identified by modification of functionally critical NH2-terminal residues. *J Exp Med* 181: 631-640
- Handel TM, Johnson Z, Rodrigues DH, Dos Santos AC, Cirillo R, Muzio V, Riva S, Mack M, Deruaz M, Borlat F, *et al* (2008) An engineered monomer of CCL2 has anti-inflammatory properties emphasizing the importance of oligomerization for chemokine activity *in vivo*. *J Leukoc Biol* 84: 1101-1108
- Hansson GK, Hermansson A (2011) The immune system in atherosclerosis. *Nat Immunol* 12: 204-212
- Haringman JJ, Gerlag DM, Smeets TJ, Baeten D, van den Bosch F, Bresnahan B, Breedveld FC, Dinant HJ, Legay F, Gram H, *et al* (2006) A randomized controlled trial with an anti-CCL2 (anti-monocyte chemoattractant protein 1) monoclonal antibody in patients with rheumatoid arthritis. *Arthritis Rheum* 54: 2387-2392
- Heller EA, Liu E, Tager AM, Yuan Q, Lin AY, Ahluwalia N, Jones K, Koehn SL, Lok VM, Aikawa E, *et al* (2006) Chemokine CXCL10 promotes atherogenesis by modulating the local balance of effector and regulatory T cells. *Circulation* 113: 2301-2312
- Hoogewerf AJ, Kuschert GS, Proudfoot AE, Borlat F, Clark-Lewis I, Power CA, Wells TN (1997) Glycosaminoglycans mediate cell surface oligomerization of chemokines. *Biochemistry (Mosc)* 36: 13570-13578
- Horuk R (2009) Chemokine receptor antagonists: overcoming developmental hurdles. *Nat Rev Drug Discov* 8: 23-33
- Hu H, Zhu L, Huang Z, Ji Q, Chatterjee M, Zhang W, Li N (2010) Platelets enhance lymphocyte adhesion and infiltration into arterial thrombus. *Thromb Haemost* 104: 1184-1192
- Karshovska E, Zagorac D, Zerneck A, Weber C, Schober A (2008) A small molecule CXCR4 antagonist inhibits neointima formation and smooth muscle progenitor cell mobilization after arterial injury. *J Thromb Haemost* 6: 1812-1815
- Keeley EC, Mehrad B, Strieter RM (2011) Chemokines as mediators of tumor angiogenesis and neovascularization. *Exp Cell Res* 317: 685-690
- Koenen RR, Weber C (2010) Therapeutic targeting of chemokine interactions in atherosclerosis. *Nat Rev Drug Discov* 9: 141-153
- Koenen RR, von Hundelshausen P, Nesselmeier IV, Zerneck A, Liehn EA, Sarabi A, Kramp BK, Piccinini AM, Paludan SR, Kowalska MA, *et al* (2009) Disrupting functional interactions between platelet chemokines inhibits atherosclerosis in hyperlipidemic mice. *Nat Med* 15: 97-103
- Kramp BK, Sarabi A, Koenen RR, Weber C (2011) Heterophilic chemokine receptor interactions in chemokine signaling and biology. *Exp Cell Res* 317: 655-663
- Laguri C, Sadir R, Rueda P, Baleux F, Gans P, Arenzana-Seisdedos F, Lortat-Jacob H (2007) The novel CXCL12gamma isoform encodes an unstructured cationic domain which regulates bioactivity and interaction with both glycosaminoglycans and CXCR4. *PLoS ONE* 2: e1110
- Lalezari J, Yadavalli GK, Para M, Richmond G, DeJesus E, Brown SJ, Cai W, Chen C, Zhong J, Novello LA, *et al* (2008) Safety, pharmacokinetics, and antiviral activity of HGS004, a novel fully human IgG4 monoclonal antibody against CCR5, in HIV-1-infected patients. *J Infect Dis* 197: 721-727
- Landsman L, Bar-On L, Zerneck A, Kim KW, Krauthgamer R, Shagdarsuren E, Lira SA, Weissman IL, Weber C, Jung S (2009) CX3CR1 is required for monocyte homeostasis and atherogenesis by promoting cell survival. *Blood* 113: 963-972
- Lesnik P, Haskell CA, Charo IF (2003) Decreased atherosclerosis in CX3CR1-/- mice reveals a role for fractalkine in atherogenesis. *J Clin Invest* 111: 333-340
- Liehn EA, Piccinini AM, Koenen RR, Soehnlein O, Adage T, Fatu R, Curaj A, Popescu A, Zerneck A, Kungl AJ, *et al* (2010) A new monocyte chemotactic protein-1/chemokine CC motif ligand-2 competitor limiting neointima formation and myocardial ischemia/reperfusion injury in mice. *J Am Coll Cardiol* 56: 1847-1857
- Lievens D, Zerneck A, Seijkens T, Soehnlein O, Beckers L, Munnix IC, Wijnands E, Goossens P, van Kruchten R, Thevissen L, *et al* (2010) Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. *Blood* 116: 4317-4327
- Luchtefeld M, Grothausen C, Galglick A, Jagavelu K, Schuett H, Tietge UJ, Pabst O, Grote K, Drexler H, Forster R, *et al* (2010) Chemokine receptor 7 knockout attenuates atherosclerotic plaque development. *Circulation* 122: 1621-1628
- Ludwig A, Weber C (2007) Transmembrane chemokines: versatile 'special agents' in vascular inflammation. *Thromb Haemost* 97: 694-703
- Lukacs NW, Oliveira SH, Hogaboam CM (1999) Chemokines and asthma: Redundancy of function or a coordinated effort? *J Clin Invest* 104: 995-999
- Lutgens E, Faber B, Schapira K, Evelo CT, van Haften R, Heeneman S, Cleutjens KB, Bijnens AP, Beckers L, Porter JG *et al* (2005) Gene profiling in atherosclerosis reveals a key role for small inducible cytokines: validation using a novel monocyte chemoattractant protein monoclonal antibody. *Circulation* 111: 3443-3452
- Mahler DA, Huang S, Tabrizi M, Bell GM (2004) Efficacy and safety of a monoclonal antibody recognizing interleukin-8 in COPD: a pilot study. *Chest* 126: 926-934

- Mantovani A (1999) The chemokine system: redundancy for robust outputs. *Immunol Today* 20: 254-257
- Martin C, Burdon PC, Bridger G, Gutierrez-Ramos JC, Williams TJ, Rankin SM (2003) Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity* 19: 583-593
- Montecarlo FS, Charo IF (1996) The amino-terminal extracellular domain of the MCP-1 receptor, but not the RANTES/MIP-1 α receptor, confers chemokine selectivity. Evidence for a two-step mechanism for MCP-1 receptor activation. *J Biol Chem* 271: 19084-19092
- Montecucco F, Lenglet S, Braunersreuther V, Pelli G, Pellioux C, Montessuit C, Lerch R, Deruaz M, Proudfoot AE, Mach F (2010) Single administration of the CXCR2 chemokine-binding protein Evasin-3 during ischemia prevents myocardial reperfusion injury in mice. *Arterioscler Thromb Vasc Biol* 30: 1371-1377
- Montecucco F, Braunersreuther V, Lenglet S, Delattre BM, Pelli G, Buatois V, Guilhot F, Galan K, Vuilleumier N, Ferlin W, et al (2011) CC chemokine CCL5 plays a central role impacting infarct size and post-infarction heart failure in mice. *Eur Heart J* DOI: 10.1093/eurheartj/ehr1127
- Mor A, Planer D, Luboshits G, Afek A, Metzger S, Chajek-Shaul T, Keren G, George J (2007) Role of naturally occurring CD4⁺ CD25⁺ regulatory T cells in experimental atherosclerosis. *Arterioscler Thromb Vasc Biol* 27: 893-900
- Mortier A, Gouwy M, Van Damme J, Proost P (2011) Effect of posttranslational processing on the *in vitro* and *in vivo* activity of chemokines. *Exp Cell Res* 317: 642-654
- Moser B, Dewald B, Barella L, Schumacher C, Baggiolini M, Clark-Lewis I (1993) Interleukin-8 antagonists generated by N-terminal modification. *J Biol Chem* 268: 7125-7128
- Nabah YNA, Losada M, Estelles R, Mateo T, Company C, Piqueras L, Lopez-Gines C, Sarau H, Cortijo J, Morcillo EJ, et al (2007) CXCR2 blockade impairs angiotensin II induced CC chemokine synthesis and mononuclear leukocyte infiltration. *Arterioscler Thromb Vasc Biol* 27: 2370-2376
- Navab M, Ananthramiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fonarow GC, Vahabzadeh K, Hama S, Hough G, Kamranpour N, et al (2004) The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. *J Lipid Res* 45: 993-1007
- O'Boyle G, Mellor P, Kirby JA, Ali S (2009) Anti-inflammatory therapy by intravenous delivery of non-heparan sulfate-binding CXCL12. *FASEB J* 23: 3906-3916
- Piccinini AM, Knebl K, Rek A, Wildner G, Diedrichs-Moehring M, Kungl AJ (2010) Rationally evolving MCP-1/CCL2 into a decoy protein with potent anti-inflammatory activity *in vivo*. *J Biol Chem* 285: 8782-8792
- Postea O, Koenen RR, Hristov M, Weber C, Ludwig A (2008) Homocysteine up-regulates vascular transmembrane chemokine CXCL16 and induces CXCR6⁺ lymphocyte recruitment *in vitro* and *in vivo*. *J Cell Mol Med* 12: 1700-1709
- Potteaux S, Gautier EL, Hutchison SB, van Rooijen N, Rader DJ, Thomas MJ, Sorci-Thomas MG, Randolph GJ (2011) Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of ApoE^{-/-} mice during disease regression. *J Clin Invest* 121: 2025-2036
- Proudfoot AE, Power CA, Hoogewerf AJ, Montjovent MO, Borlat F, Offord RE, Wells TN (1996) Extension of recombinant human RANTES by the retention of the initiating methionine produces a potent antagonist. *J Biol Chem* 271: 2599-2603
- Proudfoot AE, Handel TM, Johnson Z, Lau EK, LiWang P, Clark-Lewis I, Borlat F, Wells TN, Kosco-Vilbois MH (2003) Glycosaminoglycan binding and oligomerization are essential for the *in vivo* activity of certain chemokines. *Proc Natl Acad Sci USA* 100: 1885-1890
- Pruenster M, Mudde L, Bombosi P, Dimitrova S, Zsak M, Middleton J, Richmond A, Graham GJ, Segerer S, Nibbs RJ, et al (2009) The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. *Nat Immunol* 10: 101-108
- Raman D, Sobolik-Delmaire T, Richmond A (2011) Chemokines in health and disease. *Exp Cell Res* 317: 575-589
- Ranjbaran H, Wang Y, Manes TD, Yakimov AO, Akhtar S, Kluger MS, Pober JS, Tellides G (2006) Heparin displaces interferon-gamma-inducible chemokines (IP-10, I-TAC, and Mig) sequestered in the vasculature and inhibits the transendothelial migration and arterial recruitment of T cells. *Circulation* 114: 1293-1300
- Ren M, Guo Q, Guo L, Lenz M, Qian F, Koenen RR, Xu H, Schilling AB, Weber C, Ye RD, et al (2010) Polymerization of MIP-1 chemokine (CCL3 and CCL4) and clearance of MIP-1 by insulin-degrading enzyme. *EMBO J* 29: 3952-3966
- Repeke CE, Ferreira SB, Jr., Vieira AE, Silveira EM, Avila-Campos MJ, da Silva JS, Santos CF, Campanelli AP, Trombone AP, Garlet GP (2011) Dose-response Met-RANTES treatment of experimental periodontitis: a narrow edge between the disease severity attenuation and infection control. *PLoS ONE* 6: e22526
- Richmond A (2011) Chemokine research moves on. *Exp Cell Res* 317: 553-555
- Rot A, von Andrian UH (2004) Chemokines in innate and adaptive host defense: basic chemokines grammar for immune cells. *Annu Rev Immunol* 22: 891-928
- Salanga CL, Handel TM (2011) Chemokine oligomerization and interactions with receptors and glycosaminoglycans: the role of structural dynamics in function. *Exp Cell Res* 317: 590-601
- Schall TJ, Proudfoot AE (2011) Overcoming hurdles in developing successful drugs targeting chemokine receptors. *Nat Rev Immunol* 11: 355-363
- Schneider MA, Meingassner JG, Lipp M, Moore HD, Rot A (2007) CCR7 is required for the *in vivo* function of CD4⁺ CD25⁺ regulatory T cells. *J Exp Med* 204: 735-745
- Schober A, Manka D, von Hundelshausen P, Huo Y, Hanrath P, Sarembock IJ, Ley K, Weber C (2002) Deposition of platelet RANTES triggering monocyte recruitment requires P-selectin and is involved in neointima formation after arterial injury. *Circulation* 106: 1523-1529
- Serbina NV, Pamer EG (2006) Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol* 7: 311-317
- Simionescu M (2009) Cellular dysfunction in inflammatory-related vascular disorders' review series. The inflammatory process: a new dimension of a 19 century old story. *J Cell Mol Med* 13: 4291-4292
- Smith P, Fallon RE, Mangan NE, Walsh CM, Saraiva M, Sayers JR, McKenzie AN, Alami A, Fallon PG (2005) *Schistosoma mansoni* secretes a chemokine binding protein with antiinflammatory activity. *J Exp Med* 202: 1319-1325
- Soehnlein O, Lindbom L (2010) Phagocyte partnership during the onset and resolution of inflammation. *Nat Rev Immunol* 10: 427-439
- Sohy D, Yano H, de Nadai P, Urizar E, Guillibert A, Javitch JA, Parmentier M, Springael JY (2009) Hetero-oligomerization of CCR2, CCR5, and CXCR4 and the protean effects of "selective" antagonists. *J Biol Chem* 284: 31270-31279
- Song E, Zou H, Yao Y, Proudfoot A, Antus B, Liu S, Jens L, Heemann U (2002) Early application of Met-RANTES ameliorates chronic allograft nephropathy. *Kidney Int* 61: 676-685
- Swirski FK, Libby P, Aikawa E, Alcaide P, Luscinskas FW, Weissleder R, Pittet MJ (2007) Ly-6Chi monocytes dominate hypercholesterolemia-associated monocyteosis and give rise to macrophages in atheromata. *J Clin Invest* 117: 195-205
- Tacke F, Alvarez D, Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, Garin A, Liu J, Mack M, van Rooijen N, et al (2007) Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J Clin Invest* 117: 185-194
- Trogan E, Feig JE, Dogan S, Rothblat GH, Angeli V, Tacke F, Randolph GJ, Fisher EA (2006) Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice. *Proc Natl Acad Sci USA* 103: 3781-3786
- Tsou CL, Peters W, Si Y, Slaymaker S, Aslanian AM, Weisberg SP, Mack M, Charo IF (2007) Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest* 117: 902-909
- van Wanrooij EJ, Happe H, Hauer AD, de Vos P, Imanishi T, Fujiwara H, van Berkel TJ, Kuiper J (2005) HIV entry inhibitor TAK-779 attenuates

- atherogenesis in low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 25: 2642-2647
- van Wanrooij EJ, de Jager SC, van Es T, de Vos P, Birch HL, Owen DA, Watson RJ, Biessen EA, Chapman GA, van Berkel TJ, *et al* (2008) CXCR3 antagonist NBI-74330 attenuates atherosclerotic plaque formation in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 28: 251-257
- Veillard NR, Kwak B, Pelli G, Mulhaupt F, James RW, Proudfoot AE, Mach F (2004) Antagonism of RANTES receptors reduces atherosclerotic plaque formation in mice. *Circ Res* 94: 253-261
- Veillard NR, Steffens S, Pelli G, Lu B, Kwak BR, Gerard C, Charo IF, Mach F (2005) Differential influence of chemokine receptors CCR2 and CXCR3 in development of atherosclerosis *in vivo*. *Circulation* 112: 870-878
- von Hundelshausen P, Koenen RR, Sack M, Mause SF, Adriaens W, Proudfoot AE, Hackeng TM, Weber C (2005) Heterophilic interactions of platelet factor 4 and RANTES promote monocyte arrest on endothelium. *Blood* 105: 924-930
- von Hundelshausen P, Koenen RR, Weber C (2009) Platelet-mediated enhancement of leukocyte adhesion. *Microcirculation* 16: 84-96
- Wan W, Lim JK, Lionakis MS, Rivollier A, McDermott DH, Kelsall BL, Farber JM, Murphy PM (2011) Genetic deletion of chemokine receptor Ccr6 decreases atherogenesis in ApoE-deficient mice. *Circ Res* 109: 374-381
- Wang L, Fuster M, Sriramarao P, Esko JD (2005) Endothelial heparan sulfate deficiency impairs L-selectin- and chemokine-mediated neutrophil trafficking during inflammatory responses. *Nat Immunol* 6: 902-910
- Weber C, Zernecke A, Libby P (2008) The multifaceted contributions of leukocyte subsets to atherosclerosis: lessons from mouse models. *Nat Rev Immunol* 8: 802-815
- Weber C, Meiler S, Doring Y, Koch M, Drechsler M, Megens RT, Rowinska Z, Bidzhekov K, Fecher C, Ribechini E, *et al* (2011) CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice. *J Clin Invest* 121: 2898-2910
- Wells TN, Power CA, Shaw JP, Proudfoot AE (2006) Chemokine blockers—Therapeutics in the making? *Trends Pharmacol Sci* 27: 41-47
- Yellin M, Paliienko I, Balanescu A, Vizir V, Ter-Vartanian S, Tian J, Zhu X (2009) A phase II, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of MDX-1100, a fully human anti-CXCL10 monoclonal antibody, in combination with methotrexate (MTX) in patients with rheumatoid arthritis (RA) [abstract]. *Arthritis Rheum* 60: 414 DOI: 10.1002/art.25497
- Zernecke A, Weber C (2010) Chemokines in the vascular inflammatory response of atherosclerosis. *Cardiovasc Res* 86: 192-201
- Zernecke A, Bot I, Djalali-Talab Y, Shagdarsuren E, Bidzhekov K, Meiler S, Krohn R, Schober A, Sperandio M, Soehnlein O, *et al* (2008) Protective role of CXCR4 receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. *Circ Res* 102: 209-217