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# Visions & Reflections

# Leukocyte recruitment in atherosclerosis: Potential targets for therapeutic approaches?

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**Abstract.** Atherosclerosis is a complex inflammatory disease involving cellular migration and interaction. Vascular injury in response to different cardiovascular risk factors enhances endothelial dysfunction, which in turn promotes the expression of inflammatory markers and transendothelial leukocyte migration. Recruitment of leukocytes from the blood stream into the vessel intima is a crucial step for the development of the disease. Recent findings have highlighted the role of chemokines, chemokine receptors, adhesion molecules, and gap junctions in this process by acting as chemoattractant, adhesive, or intercellular communication molecules. In this short review, we summarize new data concerning the different steps from leukocyte arrest to transendothelial migration and discuss potential new therapeutic approaches concerning these processes.

**Keywords.** Atherosclerosis, leukocyte recruitment, cell adhesion molecule, chemokine, gap junction, therapeutic strategy.

## Introduction

Atherosclerosis is a chronic inflammatory disease, responsible for the major causes of mortality, i.e. ischemic heart disease and cerebrovascular disease [1]. Although clinical damage appears in adult life, the pathology develops during a long period, starting already in the childhood. Beside genetic risk factors, hyperlipidemia, diabetes, hypertension, obesity, and smoking are the main cardiovascular risk factors which enhance endothelial injury [2, 3]. Atherosclerosis affects the aorta focally, as well as carotid, coronary, iliac, and femoral arteries. The lesions are characterized by lipid accumulation, connective tissue elements and immune infiltrates [4]. The inflammatory process is determinant in all stages of atherosclerosis, from the formation of the early stage lesions through disease progression and finally to clinical complications [5]. Current treatment regimens against cardiovascular disease are mainly based on 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors known as statins. These drugs improve cardiovascular outcome by their lipid-lowering, plaque-stabilizing, and anti-inflammatory effects. Versatile treatment options against the development of atherosclerosis are rare. Thus, a challenge for future research is the identification and development of promising novel cholesterol-independent therapies.

Early atherosclerosis is characterized by endothelial dysfunction due to many risk factors. Under normal conditions, endothelial cells inhibit platelet and leukocyte adhesion to the vascular surface. Risk factors may initiate an inflammatory response in the artery wall [6, 7]. Activated endothelial cells switch their molecule expression pattern and, as a consequence, present several types of leukocyte adhesion molecules at their surface and secrete chemoattractant molecules. These cellular changes cause leukocyte rolling along the vascular surface and cell adhesion at the site of activation [8]. This endothelial dysfunction occurs preferentially at sites of hemodynamic strain. Low or oscillatory shear stress reduces the production of nitric oxide (NO), increases expression of pro-inflamma-

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tory transcription factor NF-κB, enhances adhesion molecule expression and regulates the junctional molecule pattern in endothelial cells, all of which contributes to cellular transmigration at the atherosclerosis-prone site [9, 10]. *Via* secretion of cytokines, recruited leukocytes themselves amplify the ongoing chronic inflammatory process. This results in the presence of a large number of inflammatory and immune cells within atherosclerotic lesions [11]. The involvement of leukocytes during atherogenesis has been extensively studied in different murine models of atherosclerosis. Mice deficient for the apolipoprotein E (ApoE–/–) or low-density lipoprotein receptor (LDLR–/–) genes fed with a high-cholesterol diet for only a few weeks develop atherosclerotic lesions comparable to those in humans. In ApoE knockout mice deficient also for the gene for macrophage colony-stimulating factor (MCSF), the development of atherosclerotic lesions was reduced, and similar results were obtained in T cell-deficient LDLR knockout mice [12, 13]. The presence of monocyte/macrophages and T cells within atherosclerotic lesions is the result of several specific biological processes. At the site of inflammation, circulating leukocytes roll along the vessel wall via selectin-carbohydrate-mediated interactions with the endothelium. As a consequence, locally secreted chemokines mediate the arrest and transendothelial diapedesis of rolling leukocytes. This later step also involves adhesion molecules such as integrins, members of the immunoglobulin family, as well as gap junctions. Migration of circulating monocytes, T cells and, to a lesser extend, smooth muscle cells (SMCs) from the media lead to an accumulation of these cells within the vascular intima, resulting in neo-intima formation. These early chronic inflammatory processes lead to the formation of early atherosclerotic lesions, the fatty streak. Continuous recruitment of SMCs, together with proliferation of extracellular matrix components and accumulation of lipids characterize the following phases of atherosclerosis, fibrofatty and fibrous plaque formation [14]. Depending on the stability of the lesion, the plaque may rupture and induce thrombosis, leading to acute vascular events.

## Adhesion molecules

Cell adhesion molecules (CAMs) are implicated in many inflammatory diseases such as multiple sclerosis, asthma and rheumatoid arthritis. In the pathology of atherosclerosis, upregulation of adhesion molecule expression has been observed in animal models and humans [15–17]. Adhesion molecules are crucial mediators in the various steps of leukocyte recruitment. Different subsets of adhesion molecules have been shown to be responsible for rolling and arrest of circulating inflammatory cells on the vascular endothelium [18]. The implication of different adhesion molecules at each step is schematized in Figure 1. This occurs via modulation of CAM expression on the surface of both the endothelial cells and the circulating leukocytes. The molecules mediating these interactions belong to four major protein families: selectins, selectin ligands, integrins, and members of the immunoglobulin family.

Selectins, which induce leukocyte rolling and tethering, are carbohydrate-binding molecules, consisting of three subfamily members [19]. P-selectin, which is stored in Weibel-Palade bodies of endothelial cells and $\alpha$-granules in platelets, is quickly translocated to the cell surface in response to pro-inflammatory stimuli [20]. P-selectin glycoprotein-1 (PSGL-1) is the principal ligand for P-selectin, and binding of these two factors is crucial for the rolling of leukocytes at sites of inflammation [21]. E-selectin is not expressed under normal physiological conditions, but is synthesized and exported on the endothelial cell surface in inflammatory situations [22]. Finally, L-selectin is expressed on all granulocytes, monocytes, and on most lymphocytes [23]. Deficiency in E- and P-selectins leads to a strong decrease in the extent of atherosclerotic lesions (80%, 50%, and 45% after 8, 22, and 37 weeks of a high-cholesterol diet, respectively) in LDLR–/– mice [24]. *In vitro* data have shown that the concentration of soluble CAMs correlates well with the expression of these molecules on the cell surface [25]. A recent study suggests that soluble P-selectin could be useful to monitor disease progression in patients with coronary artery disease (CAD) [26]. In contrast, the role of L-selectin in atherogenesis is not fully understood. Mice deficient for L-selectin have impaired leukocyte recruitment into inflammatory sites [27]. According to the study of Eriksson et al. [28], L-selectin seems to play a role in the accumulation of circulating T cells during the disease. Recent data further suggest that a polymorphism of the L-selectin gene is implicated in human CAD [29].

Integrins and members of the immunoglobulin superfamily are also involved in atherosclerotic leukocyte recruitment since they mediate both adhesion and transmigration of leukocytes into the vessel wall. Integrins consist of a common $\beta$ subunit, which is non-covalently linked with various $\alpha$ subunits. Blocking $\beta_1$ integrin (VLA-4)/ligand interaction in LDLR–/– mice reduces progression of atherosclerotic lesions [30]. Moreover, in mice lacking CD18, the $\beta_2$ subunit, a 48% reduction of atherosclerotic lesions has been observed compared with controls [31].

Several repeating extracellular IgG-like domains, a transmembrane region and a short cytoplasmic domain characterize the members of the immunoglobulin superfamily [18]. Among these are the intercellular adhesion molecules-1, -2, -3 (ICAM-1, 2, 3), vascular cell adhesion molecule-1 (VCAM-1), and platelet endothelial cell adhesion molecule-1 (PECAM-1). The biological function of these molecules is mainly to act as integrin receptors.

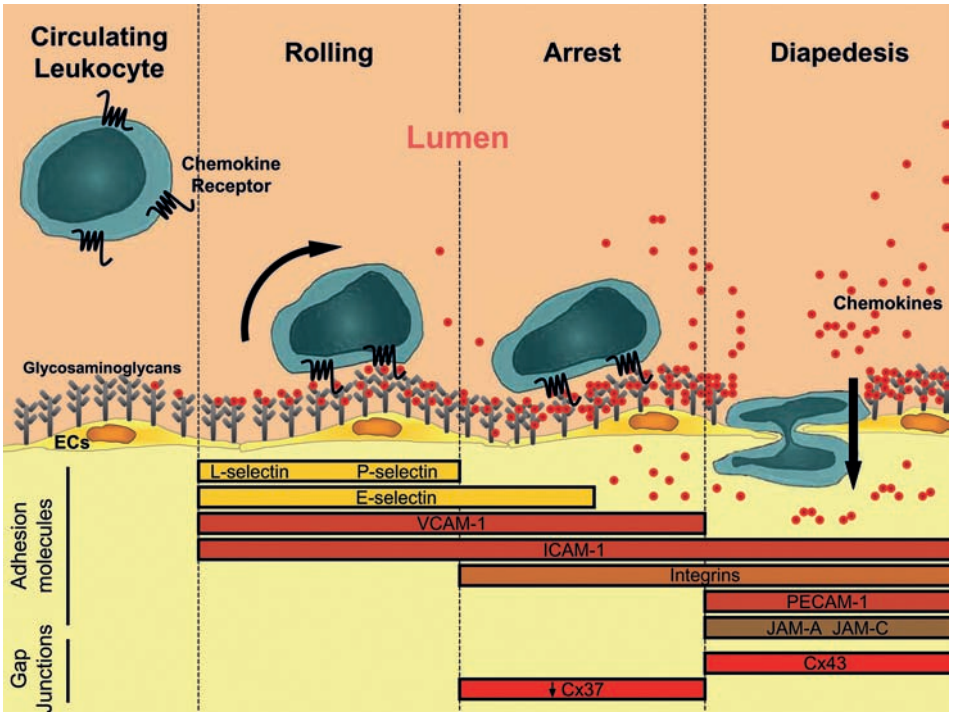


**Figure 1.** Schematic representation of different steps in leukocyte recruitment from circulating cell-to-cell transmigration through the vascular endothelium, involving chemokines/chemokine receptors, adhesion molecules and gap junction molecules. ECs, endothelial cells; VCAM-1, vascular cell adhesion molecule-1; ICAM-1 intercellular adhesion molecules-1; PECAM-1, platelet endothelial cell adhesion molecule-1; JAM-A, junctional adhesion molecule-A; JAM-C, junctional adhesion molecule-C; Cx37, connexin37; Cx43, connexin43.

Inhibition of the interaction between adhesion molecules and their ligands decreases leukocyte adhesion, diapedesis, and, in some cases, also affects leukocyte rolling. In a mouse model of atherosclerosis, ICAM-1-deficient mice have been shown to be largely protected against the development of atherosclerosis [32]. Moreover, these adhesion molecules also circulate in serum as soluble forms, as a result of proteolytic cleavage or alternative splicing. The serum levels of P-selectin, ICAM-1, and VCAM-1 are closely associated with the degree of atherosclerosis [33, 34] or different cardiovascular pathologies, such as myocardial infarction [35–37]. Thus, these soluble molecules may have potential value to predict atherosclerotic disease progression and future cardiovascular events.

Junctional adhesion molecules (JAMs) are members of the IgG superfamily and are localized in tight junctions and along the lateral membrane of epithelial cells. JAM proteins are also found in intercellular junctions of endothelial cells and on the surface of leukocytes, platelets, and erythrocytes [38, 39]. The genetic deletion of JAM-A in ApoE-deficient mice reduced neointimal hyperplasia after wire injury of carotid arteries in association with lower macrophage infiltration into the neointima [40]. To substantiate the involvement of JAM-A in leukocyte recruitment, another study showed that soluble JAM-A could effectively inhibit distinct steps of mononuclear cell recruitment on inflamed or atherosclerotic endothelium [41]. JAM-C is a counter-receptor for Mac-1, mediating leukocyte-platelet interactions as well as leukocyte transendothelial migration. Recent findings point to a potential participation of endothelial JAM-C in the enhanced inflammatory cell recruitment during atherosclerosis [42]. Thus, targeting JAMs in inflammatory disease could provide new therapeutic strategies.

## Chemokines and chemokine receptors

Chemokines are small chemoattractant proteins known to induce leukocyte migration, growth, activation and to regulate leukocyte trafficking during inflammation [43, 44]. However, this is not their only implication in cardiovascular diseases. Chemokines are also involved in the activation of platelets [45]. Thus, some chemokines seem to be implicated in neointimal hyperplasia, thrombus formation as well as thrombogenic complication of native atherosclerosis [46]. Chemokines may also play a role in keeping macrophages within the lesion. By impeding the return of macrophages from the lesion to lymph nodes, chemokines increase the cellular apoptosis within the plaque and promote the atherogenesis. Many cell types including endothelial cells, SMCs, macrophages, and leukocytes can secrete them. Approximately 50 currently identified chemokines are classified in four subfamilies (C, CC, CXC, $CX_3C$) according to the structural arrangement of N-terminal conserved cysteine residues. Chemo-

kines need to bind their coupled receptors on target cells to induce cellular changes. Their receptors constitute a superfamily of 20 members, which possess seven transmembrane loops, coupled with heterotrimeric G proteins [47]. Besides the well-known, high-affinity binding of the chemokines to their receptors, a new concept implicating another interaction has emerged. This involves a low-affinity interaction with glycosaminoglycans (GAGs) on endothelial cells in the lumen of the blood vessel which seems to be essential for the functionality of chemokines [48]. Binding of the chemokines to GAGs prolongs their retention time under vascular flow conditions and allows the formation of localized gradients at inflammatory sites [49]. Moreover, this interaction promotes the presentation of the chemokines to their receptors.

The presence of chemokines and chemokine receptors within human and animal atherosclerotic lesions is well documented [50]. Secretion of chemokines can be induced by different stimuli such as cytokines, oxidized lipids, growth factors and vascular balloon injury [51]. The expression patterns of different chemokines change according to the evolving atherosclerotic plaque. As shown in ApoE–/– mice, the progression of atherosclerotic lesion size correlates well with increased chemokine and chemokine receptor expression [52]. Monocyte chemotactic protein-1 (MCP-1/CCL2) is a powerful chemoattractant that is principally secreted by macrophages and, to a lesser extent, by activated endothelial cells and SMCs [53–55]. Gu et al. [56] have shown that MCP-1 deficiency in LDLR–/– mice leads to a significant reduction of the extent of the atherosclerotic lesion. In support of these findings, a deficiency in the MCP-1 binding receptor CCR2, which is highly expressed on monocytes, reduces the development of the disease in ApoE–/– mice [57, 58]. In these experiments, inhibition of disease progression was correlated with a decrease in relative macrophage content within the lesion. Two additional studies in experimental mouse models provide further evidence for the crucial role of MCP-1 in atherogenesis. Overexpression of MCP-1 was shown to induce local monocyte infiltration, and bone marrow transplantation of MCP-1-overexpressing cells accelerated atherosclerosis in ApoE–/– mice [59]. Gene therapy studies using an amino-terminal deletion mutant of MCP-1 that binds CCR2 and thus blocks MCP-1-mediated monocyte chemotaxis, showed a reduction of plaque progression in ApoE–/– mice [60, 61]. In humans, MCP-1 serum levels are correlated with the inflammatory activity in patients at risk of developing atherosclerosis [62]. Thus, MCP-1 serum levels may serve as a direct marker of CAD. Epidemiological studies investigated a possible role for a CCR2 polymorphism in cardiovascular disease and revealed conflicting results. Two groups suggested that a G to A gene substitution in the CCR2 gene is associated with an increased risk for myocardial infraction [63, 64]. In contrast, Gonzalez et al. [65] did not observe significant differences between patient and control groups.

Beside the fundamental role of MCP-1/CCR2 in atherogenesis, several other members of the CC chemokine subfamily are also implicated in the disease. RANTES (regulated on activation normal T cell expressed and secreted/CCL5) plays a pivotal role in the recruitment of leukocytes [66]. Endothelial cells, SMCs, macrophages and activated T cells secrete RANTES within atherosclerotic lesions [67, 68]. In addition, others and we have shown that administration of an antagonist to the chemokine RANTES, Met-RANTES, can modulate inflammatory processes ongoing during atherosclerosis development. A first study showed that injection of Met-RANTES inhibits neointima formation and macrophage infiltration in carotid arteries after wire-induced injury in ApoE–/– mice [69]. Our study showed that mice treated with Met-RANTES have fewer atherosclerotic lesions, and developing lesions are of a more stable phenotype [70]. RANTES binds to different chemokine receptors: CCR1, CCR3, and CCR5. The potential role of these receptors in atherosclerosis has been the subject of various investigations. Experiments using CCR5 deficiency in the context of atherosclerosis reported no significant differences in lesion size between ApoE–/– mice that were also deficient in CCR5 and control mice. In this study, early atherosclerotic lesion development after 16 weeks of normal chow diet was investigated. However, our own more recent findings suggest that CCR5 may play an important role in the development of more advanced atherogenic lesions. We observed reduced lesion extent in ApoE–/–CCR5–/– mice after 10 weeks of high cholesterol diet in comparison to control mice [unpublished data]. Finally, CCR5 deficiency has recently been shown to protect against neointima formation in atherosclerotic mice. The deletion of CCR5 upregulated the anti-inflammatory cytokine interleukin-10 (IL-10) and significantly reduced neointimal area after arterial injury, associated with a decrease in macrophage and T lymphocyte infiltration. In contrast, deletion of CCR1 in an experimental model of arterial injury did not affect neointimal area and cell content [71]. In experiments based on bone marrow transplantation, CCR1 disruption enhanced inflammation and atherosclerotic lesion development in LDLR–/– mice [72].

Other members of the CC chemokine subfamily which have been detected in human lesions are the macrophage inflammatory protein-1$\alpha$ and $\beta$ (MIP-1$\alpha$/CCL3 and MIP-1$\beta$/CCL4) which are expressed by activated T cells [73]. In addition to their chemoattracting properties, MIP-1$\beta$ and MCP-1 also act as pro-coagulant agents by binding to the CCR5 chemokine receptor on the surface of SMCs. They thereby induce an increase in tissue factor, the initiator of the coagulation cascade [74]. This mechanism likely plays an important role in arterial thrombosis. Fur-

thermore, MCP-4/CCL13, I-309/CCL1, pulmonary and activation-regulated chemokine (PARC/CCL18), EBI1-ligand chemokine (ELC/CCL19), and eotaxin/CCL11 have also been found to be secreted by atheroma-associated cells [75–79].

Beside the members of the CC family, various chemokines from other subfamilies have also been found to be implicated in atherosclerosis. Although most of them are primarily neutrophil chemoattractors, some CXC and CX3C chemokines and their receptors have been detected in lesions and shown to play a role in atherogenesis. Among these, one of the most important is the chemokine IL-8/CXCL8 and its corresponding receptor CXCR2, both of which are expressed in atherosclerotic lesions [80, 81]. IL-8 acts as a chemoattractant and participates in several steps of leukocyte transmigration, from the rolling to the arrest [82, 83]. Stromal cell-derived factor-1 (SDF-1/CXCL12) is a potent chemoattractor for T cells and monocytes, and can arrest circulating lymphocytes [84]. It is highly expressed in atherosclerotic lesions and thought to be involved in atherogenesis [85]. Moreover, SDF-1 is also known to be implicated in platelet activation and may therefore participate in vascular thrombosis during plaque rupture [86]. Interferon (IFN)-$\gamma$-inducible protein-10 (IP-10/CXCL10), IFN-inducible T cell $\alpha$ chemoattractant (I-TAC /CXCL11) and monokine induced by IFN-$\gamma$ (Mig/CXCL9) are three IFN-$\gamma$-inducible chemokines that are expressed by atheroma-associated cells, but are not detectable in normal vessels. Endothelial cells, SMCs and macrophages all express IP-10, whereas Mig and I-TAC are mainly expressed in endothelial cells and macrophages. The differential expression pattern of these CXC chemokines by atheroma-associated cells plays a role in the recruitment and retention of activated T lymphocytes within vascular wall lesions during atherogenesis [87]. Their chemokine receptor CXCR3 is expressed by activated T cells in atherosclerotic lesions [87]. Interestingly, we have shown in a recent study that double-knockout mice for CXCR3 and ApoE fed a Western-type diet have reduced lesion development only in early steps of atherosclerosis compared with control ApoE–/– animals. However, no difference in advanced lesion formation was observed. In contrast, mice deficient for ApoE and CCR2 genes showed reduced lesion formation only at advanced stages of atherosclerosis. Triple-knockout mice for ApoE, CCR2, and CXCR3 seem to accumulate the effects of both receptor deficiencies. They display decreased plaque size at both early and advanced lesion stages, but without additional effects [58].

New findings indicate that the chemokine CXCL16 might be implicated in lipoprotein uptake by macrophages by acting as a scavanger receptor. This chemokine is expressed in soluble and transmembrane forms, binds to the CXCR6 chemokine receptor, and guides migration of activated T cells. Moreover, it is structurally identical to the scavenger receptor SR-PSOX, which mediates uptake of oxidized LDL. The pro-inflammatory cytokine IFN-$\gamma$ enhances CXCL16 expression, as demonstrated in mice and *in vitro* in human monocytes [88]. Interestingly, a recent clinical study revealed that lower plasma levels of CXCL16 are associated with CAD, indicating a potential atheroprotective function for CXCL16 [89].

Finally, fractalkine/CXC3CL1, which is the only CX3C chemokine, has been implicated in the pathology of atherosclerosis. Fractalkine is the second chemokine expressed in both soluble and transmembrane forms. Consequently, it could combine chemoattractant functions together with adhesion molecule properties. Its transmembrane form is expressed on endothelial cells under stimulation by pro-inflammatory molecules [90]. In its soluble form, fractalkine is a potent chemoattractant for monocytes and T cells. The first evidence relating fractalkine with atherosclerosis emerged from two epidemiologic studies. A polymorphism in the fractalkine receptor was associated with a lower risk for CAD, suggesting an involvement of this receptor in cardiovascular pathologies [91, 92]. Fractalkine has also been detected in human atherosclerotic lesions [93] but not in normal arteries [94]. Additional relevant studies relating fractalkine and atherosclerosis have been performed by genetic disruption of the fractalkine receptor, CX3CR1. Double-knockout mice for CX3CR1 and ApoE developed less atherosclerosis compared with ApoE–/– controls, probably by inhibiting monocyte recruitment [95, 96]. A different set of experiments using fractalkine gene deficiency in an atherosclerotic mouse model dramatically reduced (85%) the lesion area in the brachiocephalic artery, but not in aortic roots [97]. The reduction in lesion size was associated with a reduced infiltration of monocytes.

The old understanding of the role of chemokines and their receptors, presenting apparent functional redundancy in atherosclerotic recruitment, is now changing to a more specialized interaction of chemokines to induce different specific steps of cellular recruitment. This new concept is still unsophisticated. Therefore, an important aim for the future will better characterization of the role of different chemokines and their receptors within the context of atherosclerosis.

## Gap junctions

Recent data provide evidence that gap junctions not only play a role in intercellular communication but are also implicated in leukocyte recruitment and transmigration [98, 99]. Gap junctions enable intercellular communication. They consist of two hemichannels each composed of six connexins. Three connexins, namely Cx37, Cx40 and Cx43 have been detected in vascular endothelial cells [100]. The first potential implication of connexins in

atherosclerosis was shown by Polacek et al. [101]. They found that Cx43 is strongly expressed in macrophage foam cells in human atherosclerotic carotid arteries. To extend their own findings, they demonstrated that Cx43 expression is upregulated in macrophage foam cells and downregulated in medial SMCs [102]. We demonstrated that Cx37, Cx40, and Cx43 are differentially expressed within human and mouse atherosclerotic lesions [103]. Human Cx37 genetic polymorphisms have been associated with CAD and/or myocardial infarction [104–107]. Cx37–/–, ApoE–/– mice developed more atherosclerosis than Cx37+/+, ApoE–/– control mice, indicating that Cx37 is atheroprotective [108]. Moreover, the authors observed that monocyte recruitment was enhanced in the absence of Cx37 on macrophages but not on the endothelium. In a different set of experiments, we showed that LDLR–/– mice with a reduction of Cx43 expression develop less atherosclerosis [109, 110]. Recently, Cx43 has been implicated in the monocyte/macrophage infiltration within atherosclerotic lesions in a model of carotid balloon injury in LDLR–/– mice [111]. These results and the fact that monocyte/macrophage but not lymphocyte diapedesis is significantly reduced by decreasing Cx43 [112, 113] and a lack of Cx37 increases monocyte recruitment suggest that modification of intercellular communication via gap junctions is likely to contribute to the process of atherogenesis. Development of novel strategies inhibiting Cx43 expression or maybe increasing Cx37 on monocyte/macrophages could be potential effective therapies.

## Future therapeutic strategies

Atherosclerosis involves both immune and inflammatory processes. Endothelial dysfunction due to different risk factors triggers inflammatory cell recruitment into the intima. Activated monocyte/macrophages and T cells secrete pro-inflammatory molecules that enhance new leukocyte arrest and diapedesis at the lesion site. To stop this chronic inflammatory process, new therapeutic strategies have to be investigated. Beyond reducing risk factors through improved life habits and cholesterol-lowering drugs, anti-inflammatory treatments seem to be the most promising therapies. In particular, impeding cellular recruitment at sites of vascular inflammation could be a future effective treatment. However, the benefit of such approaches is controversial. There are different limitations of immunosuppressive treatments that reduce leukocyte recruitment. First, clinical manifestations of atherosclerosis appear at advanced stages of the disease. Thus, therapies must be validated on pre-established lesions. Second, treatments impeding leukocyte adhesion or/and migration should specifically target atherosclerotic lesions without affecting the general host defence. Third, given the long development of the disease, anti-inflammatory treatments should not be administrated over the long term, to avoid potential adverse side-effects. To overcome these limitations, a system of local drug delivery could be considered. Contrary to systemic treatment, local drug administration avoids adverse side-effects, including immunosuppression. This kind of treatment could conceivably be applied by percutaneous coronary intervention. Another promising technology to selectively target specific cell types could be viral gene delivery. For example, genes encoding inhibitors of adhesion molecule or chemokine expression could be selectively delivered to activated endothelium.

Adhesion molecule/ligand interactions may be a useful therapeutic target for several reasons. Adhesion molecules are expressed in pro-atherogenic conditions and are essential for leukocyte rolling and arrest. However, adhesion molecules are principally involved in fatty streak formation. They may therefore be inappropriate targets for treatment at the advanced atherosclerotic lesion stage, but could be very useful to prevent the initiation of lesion development. Considerable progress has been realized in the development of several inhibitors of adhesion molecule expression. However, it is difficult to extrapolate these potential therapies to clinical use because animal models do not necessarily present a similar pathology to humans. Thus, further investigations to test a potential therapeutic use of these inhibitors are warranted. In addition to a potential therapeutic benefit, blood serum levels of soluble adhesion molecules could be used as a cardiovascular risk marker.

The latest findings in animal models suggest that blocking chemokine/receptor interactions may serve as a suitable approach to treat atherosclerosis. With a better knowledge of chemokine effects on specific cell subtypes, blocking chemokine interaction could be of therapeutic use to reduce inflammatory cell recruitment within atherosclerotic lesions and impede chronic inflammatory processes. Using antagonists or analogous molecules to modulate the recruitment of a specific cellular subtype of leukocyte within the lesion may be imaginable as a potential treatment. By selectively inhibiting the diapedesis of pro-inflammatory leukocytes and enhancing the recruitment of anti-inflammatory cells like regulatory T cells, it is conceivable to reverse the chronic inflammatory process and to promote a more stable lesion that is less subject to rupture. Indeed, recent findings showed that the transfer of regulatory T cells into atherosclerotic mice inhibits disease progression [114]. Thus, inducing regulatory T cells might represent an attractive tool for the treatment of atherosclerosis. With a better knowledge of underlying mechanisms, we could imagine a multiple treatment strategy that inhibits the recruitment of pro-inflammatory leukocytes and, in parallel, enhances the infiltration of regulatory T cells to lesion sites. The regulatory T cells might counteract the process of chronic

inflammation, thereby restoring a balance between pro- and anti-inflammatory mediators.

Studies demonstrating the involvement of connexins in atherosclerosis may also open new therapeutic strategies affecting paracrine cell-cell interactions and junctional intercellular communication to reduce the evolution of cardiovascular disease. Modulating the expression pattern or functional activity of connexins, for example with Cx-specific blocking peptides or antisense mRNA, might serve as a new therapeutic approach to reduce the progression of atherosclerosis.

Compounds that inhibit leukocyte recruitment could be particularly interesting for the treatment of ischemia/reperfusion disease. Myocardial ischemia is the major consequence of atherosclerosis. Animal models of sustained ischemia have shown exacerbation of myocardial injury early during reperfusion, mediated in large part by inflammatory processes. A massive neutrophil and monocyte/macrophage recruitment occurs during reperfusion. Reducing this cell infiltration via selective inhibitors could be an effective therapy to reduce reperfusion damage. Importantly, contrary to atherogenesis, infiltration of inflammatory cells within ischemic tissues is a short-term process. Thus, a major clinical improvement might be achieved by short-term treatment, without potential adverse effects of a systemic long-term therapy.

Further *in vitro* and *in vivo* investigations are needed to establish the potential clinical use of molecules inhibiting leukocyte recruitment. Recent findings in this field may help to identify new drugs against atherogenesis or ischemia/reperfusion and other inflammatory diseases, such as rheumatoid arthritis or multiple sclerosis.


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