Implications of chemokines, chemokine receptors, and inflammatory lipids in atherosclerosis

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Abstract

Chemokines are a diverse group of molecules with important implications for the development of solid tissues and normal function of the immune system. However, change of the conditions for such a complex system can have important and dangerous consequences leading to diseases. The specific implications of the various chemokines in diseases have been elucidated in the last few years, prompting hope of manipulating this system for therapy or prevention of diseases. On the other hand, inflammatory lipids are biologically active molecules with crucial impacts on the function of various cell types, including immune cells in health and disease. Here, we describe how these lipids affect the chemokine system and how they interact with chemokines to shape chronic inflammation in the case of atherosclerosis.


Atherosclerosis

The development of an atherosclerotic lesion occurs in multiple steps. First, activation of endothelial cells leads to adhesion of leukocytes and activated platelets with increased permeability of plasma lipids [1]. A lesion develops that is characterized by a core region surrounded by smooth muscles and a matrix rich with collagens. Continuous deposition of extracellular proteins, in part as a result of stimulation by cytokines and growth factors secreted from leukocytes, results in narrowing the arteries. As the plaque size increases, oxygen levels diminish, leading at the same time to a central necrosis and neovascularization within the plaque, which may account for leakage of blood components. Eventually, secretion of proteases and cytokines by cells in the affected lesion leads to matrix degradation with plaque eruption and triggering of the coagulation cascade with devastating consequences.

Adhesion of monocytes to the endothelial wall of the artery is a prerequisite for development of atherosclerosis, although not the first step in the process. More than 30 years ago, atherosclerotic lesions were shown to contain monocytes as their main cellular components [2], and monocytes are present already at the stage of fatty streaks [3]. Depletion of monocytes from the circulation reduced plaque formation [4], and it was later shown that CD11b⁺ monocytes are critical for the development of atherosclerotic lesions [5]. The vital importance of monocyte recruitment for plaque development is shown by the close correlation between monocyte content and the surface area of the plaques [6], the abundance of leukocytes in atherosclerotic lesions [1], and the fact that monocyte counts are risk factors for coronary heart disease [7]. Although monocytes are vital in progression of the disease [4], established plaques are resistant to reduction in monocyte numbers [5], suggesting that they may not be as important in the chronic stage of the disease.

Whereas a wide range of stimuli induces adhesion of monocytes to the endothelial wall on the venous side of capillaries [8], only proatherosclerotic stimuli, including cigarette smoke, ox-LDL, or cytokines, such IL-1β and TNF-α, as well as angiotensin II, are thought to induce adhesion to arterial endothelium. Atherosclerosis predominantly develops at anatomical spots in the arterial vasculature, notable for disturbed flow, which results in decreased shear stress on the endothelial cells and the development of an atherogenic endothelial cell phenotype [9]. Still, the shear stress in arteries remains larger than in the veins. A feature facilitating adhesion under such circumstances may be the expression of abnormal von Willendbrand factor [10]. Rolling or patrolling was observed by Gr⁻/Ly6C⁺ but not inflammatory Gr1⁺Ly6C⁺ monocytes in arterioles of mice [11]. The path of patrolling monocytes seems to be...
independent of the direction of blood flow, and so, they may well participate in the early development of atherosclerosis. The mechanism for this patrolling is still largely unknown, although it may involve the chemokine receptor CX₃CR1 [12]. This is interesting for the development of the disease, as CX₃CR1 is also important for survival of monocytes [13].

Further progress of atherosclerosis includes the development of foam cells, namely necrotic, oxLDL-filled macrophages that eventually die as a result of apoptosis [14, 15]. The SRs, SR-A1 and CD36, are regarded most important for oxLDL uptake [16]. This is highlighted by the protection from atherosclerosis when CD36 is deleted in ApoE⁻/⁻ mice [17] and by trapping of macrophages in the arterial intima by CD36 signaling in response to oxLDL [18].

An indication of bioactive lipid contribution in the process of monocyte recruitment came from studies of ApoE⁻/⁻ hypercholesterolemia mice, in which there were no changes in the number of the Gr1⁺Ly6⁰ cells, whereas the Gr1⁺Ly6C⁺ cells were abundant and observed to adhere to activated endothelium, infiltrate lesions, and become lesional macrophages [19]. These subsets correspond to human CD14⁺CD16⁻ and CD14⁻CD16⁺, respectively.

Figure 1. Role of chemokines/chemokine receptors on monocytes in the development of atherosclerosis. 1. CCR2 is increased on blood monocytes in hypercholesterolemia and decreased upon treatment with statins. Its expression promotes the release of monocytes from the bone marrow. CXCR2 similarly contributes to plaque development via the recruitment of monocytes to the blood. The deletion of CCR5 results in decreasing circulating monocytes and protection against atherosclerosis. 2. Patrolling of the endothelium is mediated via CX₃CR1. Arrest of monocytes on the activated endothelium is mediated via CCR5 and CXCR2. 3. CCR1 and CCR5 are crucial for recruitment of monocytes into atherosclerotic vessels. Loss of CCR2 traps the cells inside the plaques, whereas the induction of CCR7 enables evasion. 4. SRs, CD36 and SR-1A, induce a foam cell phenotype, whereas the SR CXCL16 seems to have the opposite effect, as its activity is atheroprotective. 5. Increasing concentrations of CXCL10 are associated with vulnerable plaques and increased risk of atherothrombosis. The effects of lipids and statins on various activities of chemokines and chemokine receptors are also shown. ↑, Up-regulation; ↓, down-regulation.

**CHEMOKINE RECEPTORS IN ATHEROSCLEROSIS**

Chemokines are small signaling proteins that play important roles in health and disease by orchestrating the infiltration of leukocytes [20]. These molecules are divided into four subfamilies based on the position of the C residue in the amino terminal end of the molecules; these are known as CXC or α, CC or β, C or γ, and CX₃C or δ chemokines. Chemokines and their receptors are also classified based on their functions as inflammatory chemokines/inflammatory chemokine receptors or as housekeeping molecules that are involved in the circulation and homing of cells under physiological conditions [21]. All chemokine receptors activate heterotrimeric G proteins and intracellular signaling pathways [22].

Several chemokine receptors are involved in the development of atherosclerosis (Fig. 1, and see Table 1). In this respect and important in the case of atherosclerosis, the CD14⁺CD16⁻ cells are CCR2⁺CCR5⁻, whereas the CD14⁺CD16⁺ are CCR2⁻CCR5⁺, suggesting that certain monocyte subsets are unresponsive to CCL2/MCP-1-driven recruitment into atherosclerotic plaque, which is normally dependent on the CCL2/CCR2 axis [23]. However, this process is a lot more...
complicated and involves several chemokines (see Table 1 summarization and reviewed in ref. [24]) and adhesion molecules [8]. The combined importance of these two protein families is highlighted by the fact that hyperlipidemia is correlated with increased expression of cell adhesion molecules on endothelial and mononuclear cells [25]. With the emphasis of the importance of such close contact between leukocytes and tissue-resident cells, crosstalk between monocytes expressing CX3CR1 and smooth muscle cells expressing CX3CL1/fraktalkine augments the expression of proatherogenic molecules, such as TNF-α, IL-1β, IL-6, CX3CR1, MMP-2 and MMP-9 [26], demonstrating how patrolling monocytes are activated. CX3CR1 is expressed at high levels in DCs during atherosclerosis and is pertinent to their development [27]. In addition to the release of various cytokines, induction of CX3CR1 on monocytes and its ligand CX3CL1/fraktalkine on smooth muscle cells acts to attract and adhere monocytes, reflecting the earliest stage of plaque development [28]. Epidemiological data contribute further to strengthen the association, as a defective receptor variant reduces the risk of cardiovascular disease, and mice lacking CX3CR1 are at reduced risk of vascular lesions when fed a Western diet [29–33]. Similarly, CCR2 acts to attract monocytes to the inflamed sites [34, 35], and down-regulation of this receptor serves to trap cells from further migration toward other sites [36].

CCR6 deletion results in 30–40% reduced lesion area and 44% reduced number of macrophages, which was suggested to be mediated by an effect on the number of circulating monocytes [37]. A recent study confirmed these observations and tied CCR6 to the recruitment of Gr-1hi and Gr-1low monocytes, as well as their adherence to the endothelium [38]. Furthermore, CCR5 and CXCR2 are also important for the arrest of monocytes on activated endothelium [39–41], whereas the importance of CCR4 was shown when the DC, producing one of its ligand, namely CCL17, was found in atherosclerotic lesions [42]. Upon deletion of CCL17 in this model, atherosclerosis was reduced as a result of increased numbers of Tregs. On the other hand, CXCL16, a SR for oxLDL and a chemoattractant for Th1 cells, induces foam cells in a protective way, and its deletion was shown to accelerate atherosclerotic development [43].

At a later stage, induction of CCR7 is a critical step in the clinically important process of plaque regression, as its up-regulation in foam cells prompted their departure from plaques [44–46]. In T cells however, CCR7 acts to induce atherosclerosis, and its deletion results in reduced plaque development, which was associated with impaired entry and exit of T cells [47]. In this model, priming CCR7-positive T cells with DCs that were exposed to oxLDL reconstituted the disease, highlighting the importance of the plaque microenvironment for the progression of the disease. Deletion of CXCL10/IFN-inducible protein 10, which attracts CXCR3-positive effector T cells to the plaques, also reduced lesion size significantly, while at the same time, revealing the existence of naturally occurring forkhead box p3-expressing Tregs secreting IL-10 and TGF-β1 [48].

The combined inhibition of CCL2/MCP-1, CCR2, CX3CR1, and CCR5 abrogated Ly6Ghi and Ly6Clo monocyteosis and thus, almost abolished atherosclerosis. This effect was also observed for each of the receptors independently [29, 32, 49–52]. CX3CR1 is important when considering the paradigm of patrolling monocytes but is also a result of its importance for adhesion to the endothelium, as well as for survival [11, 13, 53]. In CCR5−/− mice, plaque size and macrophage contents are reduced [52], whereas CCR2 induces monocyte release from the bone marrow [54], as emphasized by their reduced number in the CCR2−/− mouse [50]. Furthermore, it is important for monocyte transmigration and neointimal hyperplasia [55]. The number of accumulated cells translates into pathology, as absence of the CCR2 ligand CCL2/MCP-1 is cardioprotective [56], whereas CCL2/MCP-1 and the CCR5 ligand CCL5/RANTES are independent risk factors for short-term mortality in patients with acute coronary syndrome [57]. Table 1 summarizes these observations.

A recent study identified CXCL1/GRO-α signaling through the CXCR2 receptor as important for mobilizing classical monocytes in the mouse hypercholesterolemia model, whereas CCR1, CCR5, and CX3CR1 but not CCR2 are crucial for their recruitment into atherosclerotic vessels [24]. Hence, CXCL1/GRO-α is crucial for mobilizing monocytes from the bone marrow, whereas CCR1 is important for their recruitment into sites of atherosclerotic development. Of note, this is contrary to a previous study showing athero progression in CCR1−/− mice [58]. As the disease progresses, CXCL12/SDF-1α signaling through CXCR4 is vital for the recruitment of smooth muscle progenitor cells and the formation of neointima hyperplasia [59, 60], as well as increased inflammation as a result of IL-2 and IFN-γ secretion from T cells because of LPC-induced up-regulation of CXCR4 in T cells [61].

CROSSTALK BETWEEN CHEMOKINES AND INFLAMMATORY LIPIDS IN ATHEROSCLEROSIS

The importance of hyperlipidemia as a risk factor for cardiovascular disease has been well known since the Framingham study in the 1960s, as published by Thomas et al. [62]. To demonstrate further the instrumental role of LDLs, it was shown that although humans, on average, have LDL levels in the range of 130–160 mg/dl, other animals that do not develop atherosclerosis have levels of 50 mg/dl or lower [63]. A comparison was made between the Japanese and American populations in the 1960s. In this study, it was observed that Japanese population levels of LDLs were only ~100 mg/ml, and their mortality from cardiovascular disease was just 10% when compared with their counterparts in the United States [64]. Importantly, the current guidelines on treating blood cholesterol, published by the American College of Cardiology and the American Heart Association, identify the risk reduction for atherosclerotic cardiovascular disease upon statin treatment as outweighing the risk of adverse effects for patients with a baseline LDL cholesterol of ≥70 mmol/l within certain criteria [65].

A high-fat diet induced monocyteosis [19], which is an independent risk factor for coronary artery disease [7]. It was shown that the expansion was limited to the CD115+ Gr1− sub-
set of monocytes, but in another study, it was shown that CD11c$^+$ monocytes were expanded and that CD11c was important for firm adhesion to the endothelium via VCAM-1 and E-selectin [66]. Circulating Gr1$^+$ monocytes in atherosclerotic mice have higher levels of CCR2 [49], which is likely to be a result of hypercholesterolemia [67, 68]. Accordingly, monocytic CCR2 expression is reduced in man, mouse, and rat by statins [69]. Statins are drugs that lower serum cholesterol, and treatment with pravastatin decreased leukocyte counts [70]. Thus, reduction in monocyte numbers was correlated with reduced plaque size [70]. Research in the field of “lipid-lowering treatment” using statins is a success, as it translates into survival. Present research in the field aims to understand the underlying mechanisms, as the mere inhibition of the 3-hydroxy-3-methylglutaryl-CoA reductase, which is the limiting step in cholesterol synthesis, does not account for all of the effects of statins. In the context of lipids and chemokines, a number of studies examining statins have provided clues as to how the detrimental effects of LDL are counteracted by statins. The crosstalk between lipids and chemokines/chemokine receptors and the role that LDL and statins play in atherosclerosis are shown (see Table 2).

DC migration into LNs was impaired in dyslipidemia, suppressing immunologic priming [71]. Impaired migration results from inhibitory signals generated by PAF or oxLDL, which may mimic the effect of PAF. However, DC migration and priming were restored by HDL or HDL-associated PAF acetyl hydrolase, which mediates inactivation of PAF and oxLDL [71]. The maturation of DCs in the presence of oxLDL favors T cell stimulation, as the expression of HLA-DR, CD80, and CD86, important for presenting antigens to T cells, was increased by oxLDL [72]. The fact that a low (10 M) level of oxLDL had no effect in this regard, whereas the 50 M did, suggests that the levels of oxLDL, present in the blood of severe hyperlipidemia, may not be sufficient to affect the phenotype or function of leukocytes, whereas higher levels inside the plaques may do so. However, DCs lost most of their T cell stimulatory capacity with a significantly lower expression of maturation markers, such as CD40, CD83, CD86, and HLA-DR, upon exposure to statins [73]. Hence, the net effect of oxLDL on DCs is to induce their sequestration in the periphery where

<table>
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<tr>
<th>Chemokine Receptors in Atherosclerosis</th>
<th>Reference</th>
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<tr>
<td>CX3CR1</td>
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<td>[23]</td>
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they increase local inflammation, while statins counteract this effect.

HODE is a lipid derived from LA, which is the most abundant fatty acid in LDLs and atherosclerotic plaques. Several isomers of HODE are formed by an enzymatic process involving either 15-LOX, resulting in the formation of 13-HODE [74], or COX [75], in which a mixture of the R- and S-enantiomers of 13-HODE and the isomer 9-HODE is formed upon oxidation of LA by COX [75]. In a nonenzymatic process, HODE is produced from LDLs upon lipid peroxidation [76], which led to the suggestion that the levels of HODEs in blood may be used as a marker for in vivo hydrogen-donor activity of antioxidants. Only recently were the concentrations of LA metabolites determined in rat plasma by using a mass spectrometric method [77].

The receptors for oxidized lipids and especially the HODEs are a matter of much debate. PPAR-γ and GPR132 both seem to be of importance, and there are reports of TR4 involvement [78]. GPR132 is a receptor for oxidized fatty acids, and 9-HODE is the most potent ligand [79–81]. A GPR132-mediated proinflammatory effect of 9-HODE in skin was demonstrated by Hattori et al. [82], and genetic studies in animals support a role for GPR132 in the pathogenesis of atherosclerosis [83–87]. On the other hand, HODEs have been reported to decrease platelet adhesion to endothelial cells [88, 89]. Hence, it is not known whether the general effect of GPR132 on human vascular cells is favorable or unfavorable. This may be a result of differential effects imposed by different ligands, as shown in studies of 9-HODE and 13-HODE in skin.

Whereas 9-HODE has proinflammatory action mediated through GPR132 [82], 13-HODE accumulates in the ceramides and phospholipids of the skin [90, 91], exerting anti-inflammatory activity. An indication of the importance of these lipids in establishing physiological conditions is their decreased levels in hyperproliferating skin [92], whereas topical application of 13-HODE reversed increased proliferation [93].

Accumulation of HODEs in human atherosclerotic lesions was described more than two decades ago [94]. HODEs have later been shown to be the most abundant oxidative products in plaques—present in all advanced lesions although in different quantities [95, 96]. Increased 13-HODE levels were reported in the circulation of patients with hypertension, presumably reflecting increased oxidative stress [97]. On the other hand, they may relate to macrophage accumulation with related increased expression of macrophage 15-LOX-1 early in the development of atherosclerosis [98, 99]. Disruption of 12/15-LOX (the mouse orthologue of 15-LOX-1), decreased lesion progression, whereas overexpression of the enzyme was associated with increased lesion size [98, 99]. It was also proposed that as 13-HODE is produced by the action of 15-LOX-1, it leads to increased reverse cholesterol transport through a mechanism involving PPAR-α [99], with protection at the early stages of the disease as the consequence. 15-LOX-1, which is absent from normal vascular intima, produced almost exclusively 13-HODE from LA [100]. Importantly, whereas enzymatic generation of 13-HODE is predominated in early human lesions, it was shown that nonenzymatic HODE generation predominated in advanced human lesions, producing approximately equal mixtures of 9-HODE and 13-HODE [100].

A proapoptotic effect of HODEs has been reported in nonvascular [101, 102] as well as in monocytes [103, 104]. In the studies of monocytes, Jostarndt et al. [104] reported a CD36 increase, as well as induced monocyte apoptosis by ox-LDL and 13-HODE, but did not investigate the role of 9-HODE. However, Hampel et al. [103] observed inhibition of proliferation, G0/1-phase accumulation, and enhanced apoptosis, but only proliferation was blocked by the PPAR-γ antagonist GW9662. There is also considerable evidence that HODEs activate PPAR-γ in macrophages and monocytes, leading to increased expression of CD36 [105, 106] and SR-A [106], suggesting that HODE signaling through PPAR-γ may be instrumental in the induction of foam cells and increased lesion size through enhanced scavenging of oxidized lipids and at the same time, induction of apoptosis. Of note, in TR4 knockout, decreased macrophage expression of CD36 and reduced foam cell formation were demonstrated, suggesting that these effects may be mediated partly via TR4 [78].

oxLDL was instrumental in impairing the distinct up-regulation of IL-6, IL-12, and TNF-α, as well as costimulatory molecules upon activation of TLRs [107]. This is in favor of a Th2-type response, which might relate to increased atherosclerotic burden, as deletion of the Th2 cytokine IL-4 leads to decreased lesion formation [108]. Paradoxically, endocytosis and the basal secretion of the cytokines IL-6, CXCL8/IL-8, IL-12, and TNF-α were inhibited by statins [109]. The latter effect is only true for iDCs, whereas LPS-stimulated DCs increased the secretion of these proinflammatory cytokines upon statin treatment [109], which is contrary to the inhibition evoked by ox-LDL in TLR-stimulated, maturing DCs. At the same time, the ability of DCs to cluster with T cells and induce T cell proliferation was decreased.

Statins affect DCs differentially according to their maturation state. This “maturation stage-dependent effect” suggests that phenotypic changes important for lipid signaling in the atherosclerotic microenvironment occur during the course of maturation from monocytes to mDCs. Also, interference by PPAR-γ with maturation of myeloid cells upon TLR activation highlights that not only lipid-lowering treatment but also lipid-induced receptor signaling may contribute to the anti-inflammatory effect. This hypothesis was tested in a knockout model of PPAR-γ, which resulted in increased atherosclerosis [110]. Hence, dissecting how oxidation products in the milieu might affect these cells functionally, as well as phenotypically, will be an important task that needs to be tackled. In particular, the understanding of how the sequential appearance of 13-HODE and later 9-HODE in the atherosclerotic plaques may contribute to shape the local inflammation and through what receptors these lipids exert their actions should be investigated in further details.

Statins and LDL-related lipids also affect the expression of chemokine receptors. Statin treatment decreased the expression of CCR4, CCL2/MCP-1, and the mRNA for CCR1, CCR2, and CCR5, thus inducing an antiatherosclerotic phenotype in endothelial cells and macrophages [111]. Statins also induced up-regulation of CCR7, the ligand for CCL19/MIP-3β and...
CCL21/MIP-3α, and enhanced the ability of macrophages to emigrate from the plaques [46]. Reduction in CCR2 expression initiated by oxidized lipids [36], although intuitively comparable with the effect impacted by statins, serves a completely different purpose. LDL, which is found in the bloodstream, is an inducer of CCR2 expression in monocytes, thus recruiting them from the bone marrow. When oxLDL, which is found primarily inside the plaques, down-regulates CCR2, monocytes are consequently trapped inside the lesion. At the same time, SRs [36] and CX3CR1 [28], are up-regulated, resulting in retention of cells expressing these receptors (Table 2). To date, there are not many reports describing the regulation of chemokines and their receptors by the oxidative products of LA in monocytes, iDCs, or mDCs or in other cell types of the innate immune system.

**TABLE 2. Effects of LDL-Related Lipids and Statins on Chemokine Receptor Expression**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Effect</th>
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<td>Hypercholesterolemia</td>
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<tr>
<td>Statins</td>
<td>Monocytes</td>
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<tr>
<td>oxLDL</td>
<td>Monocytes</td>
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<tr>
<td>oxLDL</td>
<td>iDCs</td>
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<tr>
<td>LPC</td>
<td>T cells</td>
<td>+CXCR4</td>
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<td>oxLDL</td>
<td>Monocytes, macrophages</td>
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<td>Monocytes</td>
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<td>oxLDL</td>
<td>iDCs</td>
<td>+CX3CR1</td>
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<tr>
<td>Statins</td>
<td>DCs</td>
<td>Inhibition of oxLDL-induced maturation</td>
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<tr>
<td>Statins</td>
<td>Endothelial cells, macrophages</td>
<td>−CCR1, −CCR2, −CCR4, −CCR5, −MCP-1, −MIP-1α, −MIP-1β</td>
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<tr>
<td>Statins</td>
<td>Macrophages</td>
<td>+CCR7 leading to plaque regression</td>
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+, Increased; −, decreased.

**EFFECTS OF INFLAMMATORY LIPIDS ON INNATE IMMUNE CELLS**

In lieu of the above and other observations, we examined the effects of 9S-HODE, 9R-HODE, 13R-HODE, and LPC on the chemotaxis NK cells and on cytokine and chemokine secretion by these cells [112]. The levels of several cytokines and chemokines, including IL-1β, IL-4, IL-6, IL-10, IL-12, IL-17a, IFN-γ, TNF-α, TGF-β1, CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β, were measured. Surprisingly, we observed increased secretion of IFN-γ induced by 9R-HODE but decreased release by 9S-HODE [112]. These differential effects call for closer investigations into mechanisms underlying differences in cellular response to these lipids, as well as the importance of a cellular maturation stage. To see if the differences in regulation of IFN-γ by the lipids were related to differences in receptor specificity, we examined the ability of the lipids to induce mobilization of intracellular calcium. Addition of 9S-HODE prior to the addition of LPC inhibited >50% of the effect of LPC, whereas addition of LPC prior to the addition of 9S-HODE completely abrogated the effect of the latter lipid. Also, there was a complete reciprocal desensitization between 9R-HODE and LPC on the influx of intracellular Ca2+. Hence, it can be concluded that at least two different signaling pathways exist for these oxidized lipids, which may explain many of the conflicting observations [112].

Currently, we are investigating the maturation potential imposed by lysosphingolipids and oxidized lipids on myeloid cells. Preliminary results indicate that culturing monocytes with low concentrations of different HODEs during monocyte maturation does not change the phenotype of these cells, and there are no changes in the maturation markers of iDCs or mDCs. Regarding the chemokine receptors, we observed tendencies in increased expression of CCR9 and CX3CR4 after incubating monocytes with 9S-HODE, 9R-HODE, and 13R-HODE (unpublished observations). However, there is no such effect on DCs, suggesting that the maturation stage of the target cells is of importance for the potential effect of the oxidized lipids.

Taken together, the antiatherogenic effect of statins and pro- or antiatherogenic effects of LDL-related lipids can differ among DCs of different maturation stages. However, two observations suggest that there may be unidentified mechanisms other than lowering cholesterol levels underlying the clinical effects of statins: (1) clinical improvement is seen after 6 months, whereas the earliest observation of plaque regression is observed after 12 months [113], and (2) the disproportionate clinical impact compared with the degree of plaque regression. Observations of increased levels of S1P [114] and increased expression of S1PR1 in patients undergoing statin treatment combined with the ability to retard development of atherosclerosis by the S1PR agonist FTY720 strongly suggest the involvement of S1P [115].

SIP is a multifunctional lipid present at concentrations up to the micro-molar range in the serum. It is derived from the plasma membrane of many different cells and regulates many cell responses in health and disease [116]. Some results point toward the involvement of the SIP/S1PR system as an effector for some of the effects of statin treatment, although evidence of this relation is still scarce [117]. In addition, indications that the cardioprotective effect of HDL is mediated by S1P [118] and the ability of FTY720, which acts on various S1PRs,
to retard the development of atherosclerosis [119] emphasize the role that S1P provides toward immune modulation.

A different mechanism for lipid-chemokine interaction is represented by S1P. Although chemokine receptor expression may be increased by S1PR agonism [120], S1P-mediated effects rely on factors other than simply affecting the chemokine system. An example is migration stimulated by FTY720, which may depend on CCR7 and its ligands CCL19 and CCL21 [121, 122]. Furthermore, enhancement of T cell homing by FTY720 depends on CCR2, CCR5, CCR7, and CXCR4 [123], whereas complete lymphocyte sequestration seems to be independent, at least of CCR7 and CXCR5 [124]. Function of CXCR4 is also affected by the S1P system, as overexpression of S1PR1 leads to decreased expression of this molecule, with an approximate tenfold-reduced migration toward CXCL12/SDF-1α [125]. However, even without affecting the expression level, agonists of the S1PRs also sensitize signaling through CXCR4 [126–128], and the S1P gradient seems to be instrumental in the release of CXCL12/SDF-1α [129]. Furthermore, the combined activities of S1P and CXCL12/SDF-1α are important for transendothelial migration [130].

We also investigated the ability of S1P to affect NK cells, which are notable for their crosstalk and killing of DCs, and observed a decrease in NK cell lysis of DCs when NK cells were pretreated with S1P. This may reflect the increased expression of HLA-E and HLA-I on the surface of DCs treated with S1P [112, 132].

Direct evidence of regulating chemokines and their receptors by S1PR ligands was provided from studying leukocytes in partly necrectomized rats treated with high doses of FTY720. The expression of CCR1, CCR2, and CCR5 was reduced significantly, as was the secretion of CCL2/MCP-1, CCL5/RANTES, IFN-γ, TNF-α, IL-6, and IL-12 [120]. It seems plausible that this may reflect a similar mechanism of down-regulating CXCR4 upon overexpression of S1PR1 [125]. Table 3 summarizes these findings.

### FUTURE PROSPECTS AND CONCLUDING REMARKS

In light of the proposed link between S1P and the effects of statins, it is intriguing that the S1PR agonist FTY720 seems to mimic some of the statin effects on the chemokine system by down-regulating CCR1, CCR2, and CCR5. S1P and oxidized lipids regulate IFN-γ release, indicating their opposing effects on inflammation, as S1P down-regulates the proinflammatory cytokine IFN-γ, whereas it is up-regulated by 9R-HODE. Furthermore, work regarding the roles of inflammatory lipids and chemokines is needed before we can fully understand the complex interactions among these systems and their implications in atherosclerosis. Figure 1 depicts our view of how chemokines and their receptors may affect the development of atherosclerosis.

Although there is currently no other therapy other than statins that provides acceptable reduction of atherosclerotic cardiovascular disease risk compared with the potential adverse effects [65], a number of chemokine-directed compounds are in the pipeline [133]. It is therefore reasonable
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REFERENCES


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