Atherogenic Lipids Induce Adhesion of Human Coronary Artery Smooth Muscle Cells to Macrophages by Up-regulating Chemokine CX3CL1 on Smooth Muscle Cells in a TNFα-NFκB-dependent Manner

Jana Barlic 1, Yuan Zhang 1, and Philip M. Murphy 2
From the Molecular Signaling Section, Laboratory of Molecular Immunology, NIAID, National Institutes of Health, Bethesda, Maryland 20892

Recent genetic evidence has implicated the adhesive chemokine CX3CL1 and its leukocyte receptor CX3CR1 in atherosclerosis. We previously proposed a mechanism involving foam cell anchorage to vascular smooth muscle cells because: 1) CX3CL1 and CX3CR1 are expressed by both cell types in mouse and human atherosclerotic lesions; 2) foam cells are reduced in lesions in cx3cr1−/−apoE−/− mice; and 3) proatherogenic lipids (oxidized low density lipoprotein [oxLDL] and oxidized linoleic acid derivatives) induce adhesion of primary human macrophages to primary human coronary artery smooth muscle cells (CASMCs) in vitro in a macrophage CX3CR1-dependent manner. Here we analyze this concept further by testing whether atherogenic lipids regulate expression and function of CX3CL1 and CX3CR1 on CASMCs. We found that both oxLDL and oxidized linoleic acid derivatives indirectly up-regulated CASMC CX3CL1 at both the protein and mRNA levels through an autocrine feedback loop involving tumor necrosis factor α production and NF-κB signaling. Oxidized lipids also up-regulated CASMC CX3CR1 but through a different mechanism. Oxidized lipid stimulation also increased adhesion of macrophages to CASMCs when CASMCs were prestimulated prior to assay, and a synergistic pro-adhesive effect was observed when both cell types were prestimulated. Selective inhibition with a CX3CL1-specific blocking antibody indicated that adhesion was strongly dependent on CX3CL1 in both cell types. These findings support the hypothesis that CX3CR1 and CX3CL1 mediate leukocyte recruitment and that this chemokine/chemokine receptor pair may be considered as a pro-inflammatory target for therapeutic intervention in atherosclerotic cardiovascular disease.

Atherosclerosis involves a complex interplay of inflammatory cells, vascular elements, and lipoproteins coordinated by adhesion molecules, cytokines, and chemokines (1, 2). Oxidation of low density lipoprotein (LDL) 3 and its accumulation in the subendothelial space are key initiating events that promote accumulation of leukocytes and other cell types that organize over time to form plaque (3). Leukocyte recruitment mechanisms are unclear; however, recent genetic data from mouse and man have implicated members of the chemokine family, a large group of leukocyte chemoattractants active at G protein-coupled receptors (4). Of these, the evidence for CX3CL1 (also known as fractalkine) and its receptor CX3CR1 is particularly strong (5–10). CX3CL1 is an atypical multimodular chemokine that exists both in membrane-tethered and shed forms. The immobilized form consists of a chemokine domain anchored to the plasma membrane through an extended mucin-like stalk, a transmembrane helix, and an intracellular domain (11). Transmembrane CX3CL1 is an adhesion molecule that mediates integrin-independent cell capture by binding to CX3CR1 on target cells (12). Following protease-mediated release of the chemokine domain (13, 14), CX3CL1 may also promote classical chemotactic responses of CX3CR1+ monocytes, platelets, NK cells, NK-T cells, T cells, and dendritic cells (15).

Two lines of cx3cr1−/− mice established on the atherosclerosis-prone apoE−/− background both have decreased lesion formation in the aorta compared with controls, with fewer macrophages infiltrating plaque in the aortic root (5, 7). A dysfunctional human CX3CR1 variant named CX3CR1 M280 has been consistently associated in multiple epidemiologic studies with reduced risk of measured disease end points, including coronary endothelial dysfunction and stenosis (8), acute coronary events (10), and progression of carotid atherosclerosis (6). Consistent with this, deletion of cx3cl1 on an apoE−/− background resulted in decreased brachiocephalic artery lesions; cx3cl1−/−/ldlr−/− mice displayed reduced lesion size in both the aortic root and the brachiocephalic artery (16).

Neither CX3CL1 nor CX3CR1 has been found in normal human arteries; however, their expression is up-regulated in

3 The abbreviations used are: LDL, low density lipoprotein; oxLDL, oxidized low density lipoprotein; SMC, smooth muscle cells; CASMC, coronary artery smooth muscle cells; PGPC, 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine; POV-PC, 1-palmitoyl-2-(5-oxovaleroyl)sn-glycero-3-phosphocholine; 9-HODE, 9-hydroxy-9Z,11E-octadecadienoic acid ester; 13-HODE, 13-hydroxy-9Z,11E-octadecadienoic acid ester; TNFα, tumor necrosis factor α; sTNFαR, soluble TNFα receptor; RNAi, RNA interference; sRNAi, stealth RNAi; IFN, interferon; IL, interleukin.
the context of coronary artery disease on both foam cells and vascular smooth muscle cells in plaque (17, 18). Moreover, CX3CL1 on smooth muscle cells (SMCs) was found to co-localize with macrophage CX3CR1 (18), consistent with close proximity of these cells in human plaque (19, 20). In atherosclerosis, both cell types are exposed to atherogenic lipids (3) that could affect gene expression and function. In this regard we have shown that oxLDL and its bioactive oxidized linoleic acid metabolites deposited in human plaque, 9-HODE (9-hydroxy-10E,12Z-octadecadienoic acid ester) and 13-HODE (13-hydroxy-9Z,11E-octadecadienoic acid ester) (21–23), specifically induce differentiation of human CX3CR1low monocytes to CX3CR1high macrophages that strongly adhere to CASMCs under static conditions in a CX3CR1-dependent manner (24).

In our present study we tested whether the same atherogenic lipids regulate CX3CR1 and CX3CL1 expression and function on CASMCs.

**EXPERIMENTAL PROCEDURES**

**Materials**—LDL and oxLDL were purchased from Intracel (Frederick, MD). 9-HODE and 13-HODE, and arachidonic acid-containing lipids, PGPC and POV-PC, were from Cayman Chemical (Ann Arbor, MI). Monoclonal antibodies included: rat anti-human CX3CR1 (MBL International Corp., Woburn, MA), mouse anti-human CD14 (BD Biosciences, San Diego, CA), and neutralizing mouse anti-human IFNγ and
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**Figure 2. OxLDL and oxidized linoleic acid components of LDL induce human macrophages to adhere to CASMCs in a CX3CL1-dependent manner.** Static adhesion of macrophages to CASMCs. A, prior to the adhesion assay, monocytes and CASMCs were stimulated with or without LDL or oxLDL as indicated for 24 h. Also prior to the adhesion assay, CASMCs were blocked with 2.5 μg/ml control rabbit IgG (rgIgG) or 2.5 μg/ml rabbit antiserum raised against CX3CL1. B, prior to the adhesion assay, monocytes and CASMCs were stimulated with or without 9-HODE or 13-HODE as indicated for 24 h, and then the adhesion assay was performed as described for A. Data in A and B represent the mean ± S.E. from three independent experiments using three different monocyte donors each with three different CASMC donors. p < 0.05 (*) and p < 0.01 (**), compared with the corresponding unblocked control value.

**Figure 3. Oxidized lipids induce production of TNFα, IFNγ and IL-1β in CASMCs.** CASMCs were cultured with or without 9-HODE (5 μg/ml), 13-HODE (5 μg/ml), LDL (25 μg/ml), or the indicated concentrations of oxLDL for 24 h. Cell culture supernatants were analyzed for the presence of the indicated cytokines by enzyme-linked immunosorbent assay. Data represent the mean ± S.E. from three independent experiments using three different donors with each condition tested in duplicate.
dsRNA or with 200 or 400 nm concentration of negative control or NF-κB-specific sRNAi. These concentrations of sRNAi have not interfered with viability (cell death ≤8%) or morphology of transfected cells. Following transfection, cells were resuspended in 2 ml of CASMC proprietary medium containing all recommended supplements (Cambrex) and then cultured with or without LDL, oxLDL, or lipids at 37 °C for 24 h.

Static Adhesion Assay—Monocytes (2.5 × 10⁶) were cultured with or without LDL, oxLDL, 9-HODE, or 13-HODE for 24 h, washed with prewarmed RPMI 1640, and loaded for 30 min with 5 μM Calcein AM at 37 °C. Cells were resuspended at 0.5 × 10⁶/100 μl then incubated at 37 °C for 60 min with sRNAi-transfected or control CASMCs (10⁶) that were either unstimulated or stimulated with LDL, oxLDL, 9-HODE, or 13-HODE for 24 h. Non-adherent cells were removed by washing 4× and end point fluorescence (unit/ml) was measured using a fluorescein filter set (absorbance 494 nm/emission 517 nm) on a FlexStation (Molecular Devices, Sunnyvale, CA). Data were corrected by subtracting the autofluorescence from the peak fluorescence in each well. For blocking experiments, CASMCs were pretreated with CX3CL1-directed Ab (2.5 μg/ml) or an equal amount of isotype-matched control IgG prior to co-cultivation with macrophages, and the adhesion assay was then carried out as outlined above.

Statistical Analysis—All conditions were performed in triplicate, and each experiment was performed in three different monocyte and CASMC donors. Values for each condition were averaged, and data are presented as means ± S.E. of mean (S.E.). The statistical significance of differences among matched groups was tested by the nonparametric Friedman two-way analysis of variance by ranks, followed by Dunn’s post-test, using the GraphPad Prism 3.0 Program (GraphPad Software, San Diego, CA). p values less than 0.05 were considered to be statistically significant.

RESULTS

Atherogenic Lipids Specifically Increase CX3CL1 and CX3CR1 Expression in CASMCs—Although neither CX3CL1 nor CX3CR1 have been observed in healthy coronary arteries (18), we found that both molecules were constitutively expressed on the surface of CASMCs cultured in vitro (Fig. 1). Expression of CX3CL1 was increased by 7–15% by unmodified LDL or two bioactive arachidonic acid-containing phospholipid components of LDL, POV-PC and PGPC, which are found in atherosclerotic plaque (25), but this did not reach statistical significance. In contrast, oxLDL and either 9-HODE or 13-HODE, two oxidized linoleic acid-derived components of oxLDL found free at high concentrations in plaque (21–23), all increased the frequency of CX3CL1⁺ and CX3CR1⁺ cells (Figs. 1, A and B). Furthermore, mean fluorescence on CX3CL1⁺ and CX3CR1⁺ cells increased significantly when CASMCs were stimulated with oxLDL or oxidized linoleic acid-containing lipids (supplemental Fig. 1). Up-regulation of both molecules was also observed at the mRNA level (Fig. 1C).

Atherogenic Lipids Enhance Macrophage Adhesion to CASMCs in a CX3CL1-dependent Manner—In vitro, monocyte adhesion to CASMCs was low if both cell types were either unstimulated or stimulated with LDL. However, monocyte-CASMC adhesion increased 4.5-, 2.9-, or 3.6-fold over control values when CASMCs, but not monocytes, were stimulated with oxLDL (Fig. 2A), 9-HODE, or 13-HODE (Fig. 2B), respectively. Reciprocally, cell adhesion increased to a similar extent, 5.7-, 3.9-, and 3.7-fold, when monocytes but not CASMCs were stimulated with oxLDL (Fig. 2A), 9-HODE, or 13-HODE (Fig. 2B), respectively. When both monocytes and CASMCs were stimulated before assay, adhesion of the two cell types increased dramatically, by 10.2-, 8.6-, or 11.4-fold, respectively. Since CASMCs constitutively express CX3CL1 in our system, pretreatment of unstimulated CASMCs with anti-CX3CL1 Ab prior to the adhesion assay partially decreased adhesion of oxLDL⁻ (Fig. 2A), 9-HODE⁻, or 13-HODE-stimulated monocytes (Fig. 2B). Furthermore, pretreatment of stimulated CASMCs with this antibody strongly and specifically reduced adhesion induced by oxLDL (Fig. 2A), 9-HODE, and 13-HODE (Fig. 2B) by 64, 52, or 60%, respectively. Preincubation of CASMCs with the isotype-matched control IgG had no effect on adhesion (Fig. 2). Thus, the results show that adhesion of macrophages to CASMCs is specific and predominantly mediated by CX3CL1.

TNFα Mediates Oxidized Lipid-induced CX3CL1 Up-regulation on CASMCs—The pro-inflammatory cytokines TNFα, IFNγ, and IL-1β are all present in human atherosclerotic lesions (26). Since in vitro stimulation with recombinant forms of each of these three cytokines has previously been shown to induce CX3CL1 expression in cultured vascular endothelial cells and aortic SMC (27, 28), we investigated whether oxidized lipid stimulation could induce production of the corresponding endogenous cytokines in CASMCs. As shown in Fig. 3, stimulation of cells with LDL did not induce TNFα production, whereas 9-HODE, 13-HODE, or increasing concentrations of oxLDL strongly up-regulated CASMC production of TNFα, IFNγ, and IL-1β.

Neutralization of endogenous cytokines did not affect constitutive expression of either CX3CL1 or CX3CR1 (Fig. 4 and supplemental Fig. 2). In contrast, neutralization of endogenous TNFα with sTNFαR (Fig. 4, A and B) interfered with oxLDL⁻, 9-HODE⁻, and 13-HODE-induced production of CASMC CX3CL1. Furthermore, antibody neutralization of IFNγ (Fig. 4, C and D), but not IL-1β (supplemental Fig. 2), inhibited in a...
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dose-dependent manner the oxidized lipid-induced increase in frequency of CX3CL1 + cells within the total CASMC population. The inhibitory effect was modest and did not quite reach statistical significance for the total CASMC population (Fig. 4, C and D) but did for the CX3CR1 negative subpopulation (p value = 0.0278 for 9-HODE and p value = 0.0432 for 13-HODE; Fig. 4E). No effect of IFNγ neutralization on CX3CL1 + cell frequency was observed for the CX3CR1 positive subpopulation (Fig. 4, C and D). Moreover, none of the three neutralizing agents interfered with oxidized lipid-induced expression of CX3CR1 on these cell types (Fig. 4 and supplemental Fig. 2). Thus, our data suggest that TNF expression of CX3CR1 on these cell types (Fig. 4 and supplemental Fig. 2) and used for all subsequent experiments. The same concentration of cell stimulus.

CX3CL1, but Not CX3CR1, Up-regulation on CASMCs Is NF-κB-dependent: Pharmacologic Analysis—TNFα, IFNγ, and IL-1β activate NF-κB (29, 30). Therefore, we investigated whether NF-κB promotes atherogenic lipid-induced CX3CL1 up-regulation on CASMCs. We also tested CX3CR1, despite the lack of a modulatory effect of these cytokines on its expression, since it remained possible that oxidized lipids could activate NF-κB directly as shown previously for monocytes and endothelial cells (31) or indirectly by inducing production of another factor able to activate NF-κB.

Pretreatment of CASMCs with either BAY11–7082, which selectively and irreversibly inhibits TNF-α-inducible phosphorylation of IκBα resulting in decreased expression of NF-κB (32), or NF-κB (AI), a cell-permeable quinazoline that acts as a highly potent inhibitor of NF-κB transcriptional activation (33, 34), had no effect on either basal CX3CL1 and CX3CR1 expression or oxidized linoleic acid-containing lipid-induced CX3CR1 up-regulation but blocked oxidized lipid-driven induction of CX3CL1 in a dose-dependent manner (Fig. 5).

Atherogenic Lipid Induction of CX3CL1 and TNFα in CASMCs Is NF-κB-dependent: Genetic Analysis—To test directly whether NF-κB up-regulates CX3CL1 expression and TNFα production in COSMC cultures exposed to atherogenic lipids, we blocked endogenous expression of NF-κB p50 with target-specific sRNAi. Transfection of CASMCs with either oligomer alone suppressed accumulation of the target mRNA in a dose-dependent manner (supplemental Fig. 3A). At 200 nM, inhibition was >95% and appeared specific since constitutive expression of CX3CL1 and CX3CR1 was unaffected (supplemental Fig. 3A). Thus, this concentration was judged as optimal and used for all subsequent experiments. The same concentration of a fluorescein-labeled dsRNA oligomer resulted in transfection of only 60% of CASMCs (supplemental Fig. 3, B and C). Thus this probe and the sRNAi appear to have distinct transfection efficiencies by the nucleofection method we used, which is consistent with information provided by the manufacturer.

sRNAi transfection did not alter steady-state CASMC CX3CL1 or CX3CR1 surface expression (Figs. 6, A and B) or TNFα production (Fig. 6C). In addition, the same stimuli increased the frequency of negative control sRNAi-transfected CX3CR1 + cells (Fig. 6, A and B). In contrast, NF-κB knockdown reversed the effects of oxLDL and oxidized linoleic acid metabolites on CX3CL1 surface expression and TNFα production but had no effect on increased CX3CR1 expression. Thus, these results indicate that NF-κB activity is crucial for atherogenic lipid-promoted CX3CL1 up-regulation and TNFα production in our system.

Atherogenic Lipid-induced Macrophage–CASMC Adhesion Is NF-κB-dependent—CASMC transfection with either negative control or NF-κB-specific sRNAi had no effect on adhesion of unstimulated or stimulated monocytes to unstimulated CASMCs (Fig. 7A). In contrast, CASMC NF-κB knockdown strongly decreased adhesion of unstimulated and stimulated monocytes to oxLDL-, 9-HODE-, or 13-HODE-stimulated CASMCs (Fig. 7, B–D), indicating that NF-κB signaling in CASMCs is important for adhesion to monocytes.

DISCUSSION

Oxidative stress and inflammation are accepted as major factors in the pathogenesis of atherosclerosis (2, 3), but how they interact to produce a plaque has not been clearly delineated. Recent data suggest that oxidized lipids may act in part by regulating production and function of chemokines and chemokine receptors expressed by cells in plaque (24, 35–38). Although chemokines and chemokine receptors involved in atherosclerosis are predominantly expressed by leukocytes (1, 39), recent data show that they are also expressed by other cell types such as SMC (40). SMC are the major non-leukocyte cell type...
detected in plaques at all stages of atherosclerosis (41, 42), yet how these cells contribute to atherogenesis remains unclear. In this regard we show that oxLDL and oxidized linoleic acid-containing lipids 9-HODE and 13-HODE, which are present at high concentrations in human atherosclerotic plaque (21–23), specifically and rapidly up-regulated CX3CL1 and its receptor CX3CR1 on CASMCs, augmenting CX3CL1-dependent macrophage-CASMC adhesion. Interestingly, oxLDL and oxidized linoleic acid lipids did not directly induce CX3CL1 but activated a TNFα-NF-κB-dependent autocrine loop. In contrast, CX3CR1 induction was independent of this pathway.

CX3CL1 and CX3CR1 have been implicated in atherogenesis by multiple lines of evidence: first, up-regulated expression of the receptor and the ligand in coronary artery disease patients (43) and detection of CX3CR1 and CX3CL1 by immunohistochemistry in human and mouse atherosclerotic plaques (1, 17, 18); second, partial resistance of cx3cr1−/−apoE−/−, cx3cl1−/−apoE−/−, and cx3cl1−/−ldlr−/− mouse strains to atherosclerosis (5, 7, 16) and association of the defective human CX3CR1 allele CX3CR1 M280 (9, 44) with decreased risk of cardiovascular disease in several independent patient cohorts (6, 8, 10), including the Framingham Heart Study Offspring Cohort (9). Our results link the oxidative and inflammatory theories of atherogenesis since they pinpoint oxLDL and its derivatives as major inducers of CX3CL1 and CX3CR1, which are both expressed in the context of inflammation (4). Since CX3CL1 may exist in either a membrane-tethered or soluble form (11, 13, 14), the data suggest that CX3CL1-CX3CR1 interaction, while potentially promoting leukocyte infiltration into lesions, may also be important for anchorage, retention, and organization of cells in plaque. The presence of at least two cell types in atherosclerotic lesions (41, 42) that express both the ligand and the receptor (17, 18) indicates that the CX3CL1-CX3CR1 axis could be important for formation of homotypic and/or heterotypic cell-cell interactions. Our results are consistent with previously published findings of CX3CL1-dependent adhesion of monocytes and promonocytic MonoMac 6 cells to human aortic SMC (28, 45). However, it is important to emphasize that the hallmark of the atherosclerotic microenvironment is the presence of oxLDL and its bioactive derivatives (3), which trigger monocyte differentiation to foamy macrophages (46, 47). In human atherosclerotic plaque, foamy macrophages are the major cell type of the monocyte-macrophage lineage that are in direct contact with SMC (19, 20). Moreover, our adhesion studies demonstrate that CX3CR1+ monocytes adhere very poorly to CX3CL1+ CASMCs when both cell types are cultured in the absence of oxidized lipids. In contrast, when either monocytes or CASMCs were stimulated with atherogenic lipids, the adhe-

A and B, analysis of CX3CL1 and CX3CR1 expression on the cell surface. A, representative population analysis. Treatments and sRNAi transfections are indicated at the top of the column and to the left of the row where each FACS plot is found, respectively. Numbers in the upper right corner of each quadrant indicate percent of total cells in that quadrant. B, summary data for results shown in A. Results presented are the mean ± S.E. of three different donors. C, cell culture supernatants of all three donors in B were analyzed for the presence of TNFα by enzyme-linked immunosorbent assay. Data represent the mean ± S.E. with each condition tested in triplicate. p < 0.05 (*) and p < 0.01 (**) compared to the corresponding oxLDL or lipid-stimulated control sRNAi value.
sion increased moderately, and cell adhesion was greatly increased when both cell types were stimulated. These results clearly indicate differences between adhesion properties of monocytes and macrophages. Most importantly, in plaque both cell types are exposed to an oxLDL-rich microenvironment (3) and are therefore unable to maintain the “athero-lipid naive” immunophenotype. Thus, our study, which measures CX3CL1-dependent adhesion of macrophages to atherogenic lipid-stimulated CASMCs, is the first that demonstrates the potential importance of this interaction in atherogenesis.

The atherogenic stimuli that promote vascular inflammation, especially oxLDL (3), have been shown to induce expression of several adhesion molecules including ICAM-1, VCAM-1, and selectins on endothelial cells (48, 49); hence, CX3CL1 is not the only adhesion molecule regulated by oxLDL. However, direct immunohistochemical analysis of human atherosclerotic plaques has demonstrated CX3CL1 expression on SMC and macrophages but not on endothelial cells (17, 18), which raises the possibility that adhesion molecules involved in atherosclerosis may be regulated in a cell type-specific manner. In this regard, oxLDL-mediated integrin-independent monocyte adhesion to endothelial cells was shown not to involve NF-κB (50). In contrast, CX3CL1-mediated macrophage-CASMC adhesion was NF-κB-dependent in our system. These results are in agreement with data demonstrating the role of NF-κB on CX3CL1 expression in rat aortic SMC (51). Furthermore, since NF-κB knock-down in oxidized lipid-treated CASMCs results in decreased adhesion compared with adhesion detected in unstimulated cells, it can be suggested that elimination of NF-κB activity may affect functions of other NF-κB-regulated adhesion molecules including ICAM-1, VCAM-1, and P-selectin (52–55).

Previous work has also identified TNFα-dependent regulation of CX3CL1 in human endothelial and smooth muscle cells (27, 28, 45). Our results are consistent with these reports and extend them to a novel oxidized lipid autocrine loop in CASMCs, providing pharmacologic and genetic evidence for NF-κB as a major regulator of TNFα and CX3CL1 expression. We also found that CASMCs in our system differentially expressed CX3CL1 and CX3CR1. This is consistent with a description of single positive and double positive CASMCs in human atherosclerotic lesions (17). The most
obvious implication of this is that each single positive sub- 
population would be unable to undergo homotypic adhesion 
in a CX3CL1/CX3CR1-dependent manner. On the basis of 
the data we suggest a model in which atherogenic lipids 
induce production of several pro-atherogenic cytokines 
(TNFα, IFNγ, and IL-1β); however, TNFα and IFNγ appear 
to mediate CX3CL1 up-regulation selectively within the 
CX3CR1 negative subpopulation of CASMCs. It is important 
to note that in our system the amounts of endogenously 
produced TNFα that significantly up-regulated CX3CL1 
were very low (1.3 ng/ml), ~10–15-fold lower than in previ- 
ous reports (28, 45).

Our data provide a mechanism to explain how CX3CL1 and 
CX3CR1 expression may be regulated in the atheromatous 
environment. They also suggest an explanation for organiza-
tion and retention of foam cells in plaque involving hetero-
ypic adhesive interactions with smooth muscle cells. Consistent 
with this, electron microscopy of atherosclerotic lesions has 
shown that many SMC are in fact in contact with foamy mac-
rophages (19, 20), and CX3CL1 and CX3CR1 have been shown 
to be expressed and to co-localize in plaque (17, 18). Thus, our 
study provides new insight into potential cellular and molecular 
mechanisms underlying the genetic link established previously 
between the CX3CL1-CX3CR1 axis and atherosclerosis. 
Furthermore, the results support consideration of CX3CL1 and 
CX3CR1, as well as chemokine-regulatory factors such as 
TNFα as potential drug targets for the prevention and treat-
ment of atherosclerosis.

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