Fractalkine: A Survivor’s Guide
Chemokines as Antiapoptotic Mediators
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Abstract—Chemokines are a family of low-molecular-weight proteins essential to the directed migration of cells under homeostatic and pathological conditions. Fractalkine (CX3CL1) is an unusual chemokine that can act as either a soluble or membrane-bound mediator and signals through the G protein–coupled chemokine receptor CX3CR1, expressed on monocytes, natural killer cells, T cells, and smooth muscle cells. Accumulating evidence suggests that fractalkine, in addition to its role in chemotaxis and adhesion of leukocytes, supports the survival of multiple cell types during homeostasis and inflammation. This review presents the evidence obtained from several disease models implying an antiapoptotic function for fractalkine and shows how this is relevant to the pathology of atherosclerosis and other vascular diseases. We discuss whether the key role of fractalkine, unlike other chemokines, is the promotion of cell survival and whether this has implications for vascular disease. (Arterioscler Thromb Vasc Biol. 2012;32:00-00.)

Key Words: apoptosis ■ atherosclerosis ■ macrophages ■ vascular biology ■ chemokines
To determine whether this phenotype was affected by apolipoprotein E (Apoe) deficiency reduces plaque size and macrophage content in the mouse model of atherosclerosis. Thus, in liver injury, CX3CR1 appears to have a protective role that limits fibrosis. In the absence of the CX3CL1-CX3CR1 axis, infiltrating monocytes undergo apoptosis, which leads to further monocyte recruitment, a proinflammatory macrophage phenotype, and the perpetuation of the inflammatory response. This may be relevant to vascular disease because it implies that fractalkine-induced monocyte survival may have positive or negative effects depending on the disease context.

**Fractalkine in Monocyte Survival During Atherogenesis**

Using Cx3cr1<sup>gfp/gfp</sup> knock-in and Cx3cl1<sup>-/-</sup> mice, Landsman et al demonstrated that the absence of the CX3CL1-CX3CR1 axis led to reduced circulating monocyte numbers under homeostatic conditions, specifically those of the Gr1<sup>low</sup> nonclassical subset. Using adoptive transfer into irradiated recipients, the absence of CX3CR1 signaling was found to impair survival of nonclassical monocytes in the periphery over an 8-week experimental time course. This phenotype could be rescued by enforced expression of a human Bcl-2 transgene under the control of a neutrophil- and monocyte-specific promoter (MRP8). Ex vivo, fractalkine was also found to promote the survival of human monocytes. Using serum starvation or treatment with 7-β-hydroxycholesterol to induce cell death, treatment with full-length murine fractalkine reduced human monocyte apoptosis via a pertussis toxin–sensitive mechanism. The authors found that the effect of fractalkine was not confined to a particular monocyte subset, and the mechanism of antiapoptosis was not elucidated.

Previous reports have demonstrated that CX3CR1 deficiency reduces plaque size and macrophage content in the apolipoprotein E (Apoε)<sup>-/-</sup> mouse model of atherosclerosis. To determine whether this phenotype was affected by altered monocyte survival, irradiated Apoε<sup>-/-</sup> mice were injected with bone marrow cells from WT, Cx3cr1<sup>gfp/gfp</sup>, and Cx3cr1<sup>+/gfp</sup> mice and then fed a high-fat diet for 3 months.

Mice receiving Cx3cr1<sup>gfp/gfp</sup> bone marrow showed reduced circulating monocyte numbers, slower plaque development and a higher number of terminal deoxynucleotidyl transferase dUTP nick-end labeling–positive apoptotic cells in the plaque. Again, the enforced expression of human Bcl-2 in the Cx3cr1<sup>gfp/gfp</sup> donor cells reversed the phenotype, leading to plaque size and composition similar to those of mice receiving WT bone marrow. These data suggest that monocyte/macrophages within the plaque rely on CX3CR1 to convey an essential survival signal that contributes to the progression of atherosclerosis.

**Fractalkine in Monocyte Survival During Liver Inflammation**

In contrast to the study mentioned above, Karlmark et al have demonstrated that CX3CL1-induced survival of monocytes during liver injury has a protective role to limit inflammation and subsequent fibrosis. In an acute model of carbon tetrachloride–induced liver damage, Cx3cr1<sup>gfp/gfp</sup> mice showed slower resolution of inflammation and prolonged leukocyte infiltration compared with WT controls. Infiltrating cells were mainly Cd11b<sup>+</sup> F4/80<sup>+</sup> monocyte-derived macrophages, and Cx3cr1<sup>gfp/gfp</sup> monocyte/macrophages underwent apoptosis more readily during inflammation as determined by terminal deoxynucleotidyl transferase dUTP nick-end labeling staining.

In 2 models of chronic liver injury, long-term carbon tetrachloride injection and bile duct ligation, Cx3cr1<sup>gfp/gfp</sup> mice showed increased monocyte accumulation and liver fibrosis compared with WT controls. Using an adoptive transfer model, the role of CX3CR1 in restricting liver fibrosis was shown to be mediated by hematopoietic cells only. Furthermore, hepatocytes and hepatic stellate cells were shown to produce CX3CL1 in the carbon tetrachloride model, providing a plausible mechanism for a local origin of the survival signal. Finally, in the absence of CX3CR1, macrophages infiltrating the liver were found to have a more proinflammatory phenotype, with increased expression of tumor necrosis factor-α and inducible nitric oxide synthase.

Thus, in liver injury, CX3CR1 appears to have a protective role that limits fibrosis. In the absence of the CX3CL1-CX3CR1 axis, infiltrating monocytes undergo apoptosis, which leads to further monocyte recruitment, a proinflammatory macrophage phenotype, and the perpetuation of the inflammatory response. This may be relevant to vascular disease because it implies that fractalkine-induced monocyte survival may have positive or negative effects depending on the disease context.

**CX3CR1 in T-Cell Survival During Asthma**

CX3CR1 has been shown to have a critical role in airway inflammation via its effect on T-cell survival. In mice sensitized and challenged with a range of allergens, Cx3cr1<sup>gfp/gfp</sup> knock-in mice showed reduced infiltration of inflammatory cells into the lung, less mucus production, and better lung function compared with WT Cx3cr1<sup>+/+</sup> littermate controls. This effect was due to CX3CR1 expression on CD4<sup>+</sup> Th2 cells, which was shown to be critical to the development of airway disease. CX3CR1 was shown to be
Fractalkine in Diabetes: A Role for Survival?

A recent study has shown that fractalkine is an adipokine that is produced by adipocytes and stromal vascular cells in response to systemic inflammation in a human endotoxemia model and is found at higher levels in the subcutaneous adipose tissue of obese subjects. These authors also demonstrated that patients with type 2 diabetes have higher plasma CX3CL1 levels compared with nondiabetic subjects. It is known that a high glucose concentration, as seen in type 2 diabetes, promotes the expression of fractalkine by smooth muscle cells and endothelial cells in vitro, which may then enhance monocyte adhesion and potentially promote atherogenesis. It is known that a high glucose concentration, as seen in type 2 diabetes, promotes the expression of fractalkine by smooth muscle cells and endothelial cells in vitro, which may then enhance monocyte adhesion and potentially promote atherogenesis.

Epidemiological Evidence for a Key Function of CX3CR1

CX3CR1 has 2 nonsynonymous single-nucleotide polymorphisms in the coding region of the receptor (Val249Ile and Thr280Met) that have a documented association with coronary artery disease and cerebrovascular disease. In a recent study, the Met280 allele was also associated with metabolic traits including increased waist circumference, greater insulin resistance, and lower levels of adiponectin.

The functional effect of the polymorphisms on CX3CR1 has not been fully explored, although in 2 separate studies, cells expressing the Met280 mutant were found to have impaired adhesion to cell-surface fractalkine. The reason for this discrepancy is unclear because similar assays were used in both studies. A subsequent study has demonstrated no differential signaling in Chinese hamster ovary cells transfected with the mutant versions of the receptor, whereas a recent report suggests that CX3CR1 is capable of clustering, with the Ile249/Met280 variant adopting a subtly different conformation from WT CX3CR1 multimers. A number of independent epidemiological studies suggest that CX3CR1 polymorphism is associated with the development of vascular disease, but the mechanism remains unclear. It remains to be determined whether the survival function of fractalkine is affected by receptor polymorphism.

Remaining Questions

Positive Versus Negative Effects of Survival in Atherosclerosis

The studies described above demonstrate that cell survival induced by fractalkine may have detrimental effects, eg, in lesion progression in the Apoe−/− mouse model of atherosclerosis, or beneficial functions, as seen in liver injury models where monocyte survival aids the resolution of inflammation. In atherogenesis, apoptosis of macrophages and vascular smooth muscle cells can occur at all stages of lesion progression and may have positive or negative effects depending on the stage of plaque development. Macrophage apoptosis in early lesions seems to prevent plaque progression by inhibiting cellular accumulation within the vessel wall (reviewed in ). In late-stage atherogenesis, however, the degree of macrophage apoptosis overwhelms the clearance machinery required to remove dead cells and leads to the development of a necrotic core and plaque instability. In human plaques, fractalkine expression can be detected at all stages of lesion development, though it is most abundant in advanced plaques. Thus, because fractalkine promotes monocyte/macrophage survival within the plaque, this could...
promote both initial lesion formation while also limiting necrotic core generation, depending on the stage of disease.

In the Apoe−/− mouse model, hypercholesterolemia is associated with monocytosis, which is proportional to lesion size and contributes directly to atherogenesis.34,35 Patients with coronary artery disease also have elevated levels of nonclassical monocytes in blood.56 Because fractalkine also promotes survival of peripheral monocytes, this is an additional mechanism by which the CX3CL1-CX3CR1 axis could contribute to atherogenesis. Finally, fractalkine also promotes smooth muscle cell survival and proliferation,14 which aid plaque stabilization17 but also lead to restenosis following arterial injury.38 This chemokine clearly has diverse roles in vascular pathology.

Mouse Versus Human CX3CR1

An important consideration is whether the survival role of fractalkine demonstrated in murine disease models is also applicable in humans. A study by Davis et al suggests that there are important differences in signaling downstream of mouse versus human CX3CR1.29 In transfected cells expressing human CX3CR1, fractalkine induces activation of extracellular signal–regulated kinase and Akt via phosphoinositide 3-kinase. In contrast, murine CX3CR1 is unable to couple to these pathways. Substitution of a single residue (proline 326) in the C terminus of the receptor with the human equivalent residue (serine) enables murine CX3CR1 to signal in the same way as the human receptor. Because both extracellular signal–regulated kinase and Akt are crucial mediators of survival signals downstream of G protein–coupled receptors, this may have important functional consequences in vivo.

Shed Versus Membrane-Bound Fractalkine

A recent study suggests that different functional effects are mediated via membrane-bound versus shed fractalkine in vivo, though both forms of fractalkine have signaling capacity.4 It is known that metalloproteases can generate shed versions of fractalkine that retain or lack the mucin-like stalk responsible for tethering fractalkine to the cell surface.5 Kim et al generated Cx3cl1−/− mice with transgenic expression of either WT CX3CL1 (including the mucin stalk—see Figure 1) or an isoform (105Δ—chemokine domain fractalkine) that undergoes obligatory shedding from the cell surface and lacks the mucin stalk.5 As shown previously, the authors demonstrated that Cx3cl1−/− mice had reduced circulating levels of nonclassical monocytes. Expression of the WT CX3CL1 transgene rescued this phenotype, whereas the shed CX3CL1105Δ variant did not. Thus, in mice it appears that either membrane-bound or shed full-length fractalkine, including the mucin stalk, but not chemokine domain fractalkine is required for monocyte survival. It remains to be seen whether the same is true for humans—in their study, Landsman et al used recombinant full-length murine fractalkine to rescue human monocytes from apoptosis induced by serum-starvation.16 The only other chemokine known to be expressed as a membrane-bound molecule, CXCL16,39 may also have differential effects depending on whether or not the stalk is present. Petit et al demonstrated that a point mutation of the CXCL16 receptor CXCR6 (Glu274Gln) abrogated binding of soluble (chemokine domain) CXCL16 but had no effect on adhesion mediated by full-length membrane-bound CXCL16.40 The authors suggest that the mucin stalk in the full-length molecule causes the chemokine domain to adopt a different conformation compared with soluble chemokine domain CXCL16. These 2 forms may then stabilize different receptor conformations that induce different functional effects on cells expressing the CXCR6 receptor.

Paucity of Mechanism

Despite several studies identifying fractalkine as an important cell survival mediator in leukocytes, none have addressed the mechanism involved. Two studies have demonstrated that transgenic expression of the Bcl-2 gene can rescue the cell survival defect observed in Cx3cr1−/− mice.16,20 However, this merely shows that cell death is induced via an apoptotic pathway and can be subverted by enforced high-level expression of an antiapoptotic factor.

In other cell types, specifically microglia and smooth muscle cells, the pathways involved in fractalkine-mediated survival have been investigated. Bohme et al showed that in rat microglia, Fas-induced apoptosis could be blocked by fractalkine via a phosphoinositide 3-kinase–dependent activation of Akt and subsequent phosphorylation and inactivation of the proapoptotic BAD protein.41 These authors also demonstrated that fractalkine induced expression of the antiapoptotic protein BCL-XL and reduced expression of the proapoptotic proteins BAX and BID (active p15 form) via a separate pathway. The activation of phosphoinositide 3-kinase, Akt, and BAD phosphorylation were subsequently confirmed by another study in rat aortic smooth muscle cells.42 These authors also demonstrated that fractalkine induced inhibitory phosphorylation of glycogen synthase kinase-3 via Akt. Using human coronary artery smooth muscle cells, studies from our laboratory showed that fractalkine-induced antiapoptosis is mediated by cross-talk to the epidermal growth factor receptor.14 Fractalkine signaling induced shedding of epiregulin, which acted in an autocrine/paracrine manner to activate the epidermal growth factor receptor, leading to phosphoinositide 3-kinase activation and Akt phosphorylation. The signaling pathways involved in fractalkine-mediated survival in smooth muscle cells and microglia are summarized in Figure 3.

An understanding of exactly how fractalkine blocks apoptosis induction in leukocytes and whether the pathway described above is involved may suggest novel avenues for therapeutic targeting.

Fractalkine as a Target for Therapeutic Intervention

The evidence presented in this review, both from animal studies and human epidemiology, implies that fractalkine has a central role in the development of vascular disease. Two important aspects to consider when assessing fractalkine as a therapeutic target are the potential redundancy in the chemokine system and the disparate functional effects of fractalkine at different stages in cardiovascular disease development. Strong evidence suggests that fractalkine has unique functions when compared with other chemokines, yet deletion of...
the mechanism and timing of fractalkine’s actions may be informative in choosing which vascular pathology could be therapeutically targeted.

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None.

References


Summary

This article has reviewed the evidence that fractalkine plays a key role as an antiapoptotic mediator in multiple cell types involved in vascular disease. The use of animals deficient in CX3CR1 in multiple models of disease has shown fractalkine to have a nonredundant role in cell survival under homeostatic and inflammatory conditions. It remains to be seen whether fractalkine is critical for survival of human inflammatory cells and whether the known polymorphisms in CX3CR1 affect the survival function of fractalkine. Finally, several lines of evidence suggest that fractalkine may be a useful therapeutic target. A more detailed understanding of


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