Fractalkine in Vascular Biology
From Basic Research to Clinical Disease

Hisanori Umehara, Eda T. Bloom, Toshiro Okazaki, Yutaka Nagano, Osamu Yoshie, Toshio Imai

Abstract—Fractalkine (now also called CX3CL1) is a unique chemokine that functions not only as a chemoattractant but also as an adhesion molecule and is expressed on endothelial cells activated by proinflammatory cytokines, such as interferon-γ and tumor necrosis factor-α. The fractalkine receptor, CX3CR1, is expressed on cytotoxic effector lymphocytes, including natural killer (NK) cells and cytotoxic T lymphocytes, which contain high levels of intracellular perforin and granzyme B, and on macrophages. Soluble fractalkine causes migration of NK cells, cytotoxic T lymphocytes, and macrophages, whereas the membrane-bound form captures and enhances the subsequent migration of these cells in response to secondary stimulation with other chemokines. Furthermore, stimulation through membrane-bound fractalkine activates NK cells, leading to increased cytotoxicity and interferon-γ production. Recently, accumulating evidence has shown that fractalkine is involved in the pathogenesis of various clinical disease states or processes, such as atherosclerosis, glomerulonephritis, cardiac allograft rejection, and rheumatoid arthritis. In addition, polymorphisms in CX3CR1, which reduce its binding activity to fractalkine, have been reported to increase the risk of HIV disease and to reduce the risk of coronary artery disease. This review will examine new concepts underlying fractalkine-mediated leukocyte migration and tissue damage, focusing primarily on the pathophysiological roles of fractalkine in various clinical conditions, especially in atherosclerosis and vascular injury. (Arterioscler Thromb Vasc Biol. 2003;23:1235–1240.)

Key Words: fractalkine • endothelial cells • vascular biology • atherosclerosis • inflammation

From Basic Research

The migration of leukocytes into extravascular tissues involves a cascade of molecular events, including the elaboration of chemotactic factors and chemokines, the response to these factors, the interaction of leukocytes with endothelial cells (ECs), and leukocyte transmigration through the blood vessel wall.1–4 Chemokines can be divided broadly into 2 categories: (1) inflammatory chemokines, which recruit leukocytes in response to physiological stress, and (2) homeostatic chemokines, which are responsible for basal leukocyte trafficking and the forming of the architecture of secondary lymphoid organs.5,6 Expression of inflammatory chemokines can be elicited by almost any stimulus that alters cellular homeostasis, such as infections and immune disorders.7 However, inappropriately elevated expression of inflammatory chemokines may result in extensive tissue damage caused by activated leukocytes.6,8 Inasmuch as fractalkine is expressed on ECs activated by proinflammatory cytokines9 and has both chemotactic and adhesive functions,10,11 it is likely that fractalkine is involved in the extravasation of leukocytes into inflamed tissues.12

Structure of Fractalkine
Chemokines were first described as chemoattractant cytokines synthesized at sites of inflammation and are now known to be major regulatory proteins for leukocyte recruitment and trafficking. More than 40 chemokines have been identified to date, and they are subdivided into 4 subfamilies, C-, CC-, CXC-, and CX3C-chemokines, according to the number and spacing of the first 2 cysteines in a conserved cysteine structural motif.3 Different chemokine classes tend to exhibit different ranges of leukocyte specificity, and a particular set of chemokines produced during an inflammatory process determines the extent, quality, and duration of the cellular infiltrate.1,13–15 Fractalkine is the only CX3C-chemokine to have been described.9,16 Fractalkine and CXCL16 (Bonzo ligand) contain multiple domains and are structurally distinct from other chemokines (Figure 1). The first 76 amino acids of the extracellular domain of fractalkine constitute a chemokine domain with a novel arrangement of cysteines (CXXXC: 2 cysteines separated by 3 other amino acids). The extracellular domain connects to an extended mucin-like stalk, followed by
a transmembrane domain and an intracellular domain of 37 amino acids. The expression of membrane-bound fractalkine can be markedly induced on primary ECs by inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and interferon (IFN)-γ. Soluble fractalkine can also be released, presumably by proteolysis at a membrane-proximal region by TNF-α-converting enzyme (TACE [ADAM17]) and ADAM10. This soluble form exhibits efficient chemotactic activity for monocytes, natural killer (NK) cells, and T cells. Because the endothelium is the first obstacle to leukocyte transmigration, the properties and functions of fractalkine on ECs support its role as a gateway controlling leukocyte extravasation at sites of inflammation.

**Adhesive Function of Fractalkine**

Chemokines together with adhesion molecules regulate the appropriate "addressing and delivery" of each leukocyte subtype to healthy or diseased body compartments. The first step in the classical pathway of leukocyte migration involves transient, selectin-mediated interactions between rolling leukocytes and the endothelium. Next, integrins on leukocytes are activated by chemokines that have been produced locally; they are presented on glycosaminoglycans (triggering), resulting in firm adhesion between leukocytes and ECs (firm adhesion). Leukocytes then extravasate through the vascular wall and into the surrounding tissue (transmigration, Figure 2a). Before the identification and description of fractalkine, it had been assumed that all chemokines are secreted as soluble molecules that must associate with cell surface proteoglycans and tissue matrix components, such as glycosaminoglycan, to retain the local chemokine gradient. After this association, the interaction between chemokines and their specific receptors on leukocytes triggers the activation of members of the integrin family of adhesion molecules through a G-protein–dependent mechanism.

In the case of fractalkine, the chemokine domain is presented at the top of a cell-bound extended mucin-like stalk, and fractalkine itself functions as an adhesion molecule, thereby obviating the need for both the association with proteoglycans and other adhesion molecules. Indeed, CX3CR1-expressing cells bind rapidly and with high affinity to immobilized fractalkine or fractalkine-expressing cells in both static and physiological flow conditions. Using video microscopy, Haskell et al have found that CX3CR1-expressing cells adhere more rapidly to immobilized fractalkine than to vascular cell adhesion molecule (VCAM)-1 without cell tethering and dislodging in flow conditions. Thus, fractalkine may facilitate the extravasation of circulating leukocytes by mediating cell adhesion through the initial tethering and the final transmigration steps (Figure 2b).

In addition to the intrinsic adhesion function of fractalkine, CX3CR1 can also transduce signals through G proteins that enhance the avidity of integrin binding to its ligands. Therefore, the engagement of both CX3CR1 and integrins through the coexpression of fractalkine and integrin ligands, such as intercellular adhesion molecule (ICAM)-1 and VCAM-1, results in greatly enhanced cell adhesion compared with each system alone. This cooperative adhesive function of fractalkine and integrin has been confirmed to occur under conditions of physiological flow.

**CX3CR1 and Leukocyte Subsets**

CD4+ helper T cells (Th) as well as CD8+ cytotoxic T cells (Tc) are subdivided into 2 distinct populations based on the profile of cytokine production. Th1 and Tc1 cells secrete IFN-γ, TNF-β, and IL-2, mediate immune responses against intracellular pathogens, and are associated with pathological process, such as organ-specific autoimmune diseases. Conversely, Th2 and Tc2 cells produce IL-4, IL-5, IL-6, and IL-13, mediate immune responses against extracellular pathogens, and are associated with allergic immune responses.

Recent studies have shown that various lymphocyte subsets with differential tissue tropism, in accordance with their particular developmental stages and/or functional properties,
express specific chemokine receptors. Although this conclusion is still controversial, a number of groups have reported that Th1 cells preferentially express CCR5 and CXCR3 (Th1-associated chemokine receptors), whereas Th2 cells preferentially express CCR4 and possibly CCR3 and CCR8 (Th2-associated chemokine receptors). Kim et al have reported that overlapping patterns of expression of chemokine receptors effectively distinguish Th1 from Th2 cells, ie, CCR4 and CXCR3 and CCR4 and CXCR3 for Th1 and Th2 cells, respectively.

Helper T cells are further subdivided into 2 distinct subsets according to the expression of CCR7, the homing chemokine receptor to secondary lymphoid organs. A linear differentiation from CCR7 naïve cells to CCR7 lymph node–homing memory cells and, finally, to CCR7 tissue-homing effector memory cells has been reported. Thus, memory T cells lacking CCR7 produce the effector cytokine IFN-γ with rapid kinetics (effector memory T cells), whereas, T cells expressing CCR7 represent a pool of central memory T cells. On the other hand, it has been reported that the majority of polarized effector T cells are CCR7 and that CCR7 ligands are able to attract naïve as well as the vast majority of activated and effector/memory T cell stages.

CD8 cytotoxic T cells start to express lytic mediators, perforin and granzymes, during differentiation to memory/effector stages after antigenic stimulation. Terminally differentiated effector CD8 T cells do not express CD27, CD28 (costimulatory molecules), or CD62L (L-selectin), and they possess high cytolytic activity that produces IFN-γ and TNF-α. We have previously identified CX3CR1 and have demonstrated that it is expressed on most CD16 NK cells, the majority of CD14 monocytes, and a substantial fraction of CD33 T cells. Recently, Nishimura et al characterized the phenotypes of lymphoid cells expressing CX3CR1. The majority of CX3CR1-expressing CD4 and CD8 T cells coexpress CCR5, but not CXCR3, suggesting that CX3CR1-expressing T cells partly overlap Th1 and Th2 cells, respectively.

Fractalkine is also known to exert an effect on monocytes. Bazan et al have reported that fractalkine induces the migration of monocytes, and Imai et al have demonstrated that CD14 monocytes express CX3CR1. Furthermore, fractalkine induces migration and enhances integrin-dependent cell adhesion in the monocyctic cell line, THP-1 cells, as well as other cell types.

![Figure 2. Schematic model of classical and fractalkine-mediated pathways in the adhesion cascade.](http://atvb.ahajournals.org)
as in fresh monocytes. Very recently, it has been reported that CD14 low CD16+/H11001 monocytes preferentially express CX3CR1 and undergo efficient binding to fractalkine-expressing cells and transendothelial migration in response to fractalkine. Thus, the fractalkine/CX3CR1 system may contribute to the pathogenesis of vascular and tissue injury by enhancing cell adhesion and facilitating transmigration of CX3CR1-expressing monocytes as well as lymphocytes.

Fractalkine and Cytotoxicity

Almost all CD16+ NK cells express CX3CR1, suggesting that they are important targets of the biological effects of fractalkine (ie, chemotaxis, adhesion, and activation) while also having cytoplasmic granules containing perforin and granzyme B. Indeed, soluble fractalkine can induce the transmigration of NK cells and granule exocytosis by NK cells in a dose-dependent and pertussis toxin-sensitive manner, in association with enhanced cytolytic function against NK-resistant target cells.

Similar to NK cells, CX3CR1-expressing CD8+ T cells and CD4+ T cells, but not those without surface CX3CR1, showed terminally differentiated effector phenotypes with cytotoxic granules. CD8+ T cells sorted into the CX3CR1-positive population indeed possess much greater cytotoxic activity than do presorted or CX3CR1-negative CD8+ T cells by CD3 monoclonal antibody-mediated redirected cytotoxicity assay. Because excessive activation of cytotoxic lymphocytes causes incidental vascular and tissue damage, the expression of fractalkine on ECs may be involved in vascular injury. This hypothesis is supported by experiments using ECV304 cells or human umbilical vein ECs transfected with fractalkine cDNA, resulting in the de novo expression of fractalkine on the cell surface membrane while not changing the expression of ICAM-1 or VCAM-1. The transfected cells showed increased interaction with NK cells and enhanced susceptibility to NK cell–mediated cytolysis compared with mock-transfected control cells. These findings suggest that the expression of fractalkine at the site of inflammation can attract and activate NK cells through CX3CR1 and that NK cells, once activated under such conditions, can lyse neighboring ECs despite MHC class I expression, which inhibits NK cell activation signals through interaction with inhibitory receptors that recognize MHC class I, such as certain killer cell immunoglobulin-like receptors (Figure 3).

Fractalkine and Inflammation

Nishimura et al. observed that in addition to the original chemotactic function of soluble fractalkine, membrane-bound fractalkine enhanced the effect of other chemokines on the migration of CX3CR1-expressing lymphocytes in a transmigration assay using fractalkine-expressing ECV304 cells. The transmigration of CD8+ T cells (CX3CR1+/CCR5+) and NK cells (CX3CR1+/CXCR1+) to secondary chemokines (MIP-1α, a ligand for CCR5, and IL-8, a ligand for CXCR1, respectively) was significantly increased in the presence of membrane-bound fractalkine. Thus, fractalkine expressed on inflamed endothelium may play a role as a vascular gateway for cytotoxic effector cells (CX3CR1-expressing cells) by rapidly capturing them from the blood and by promoting their migration into tissue, where Th1 polarization may occur through IFN-γ production (Figure 3).

NK cells are important in innate immunity through the production of cytokines, including IFN-γ, TNF-α, granulocyte-macrophage colony-stimulating factor, IL-3, IL-5, IL-10, and IL-15. IFN-γ produced by NK cells and γδ T cells as well as Th1 cells, has also been shown to be related to Th1 cell polarization. Recently, Yoneda et al. have reported that stimulation of NK cells with immobilized...
fractalkine, but not with soluble fractalkine, or coculture of NK cells with fractalkine-expressing cells markedly induces IFN-γ production, suggesting a role for fractalkine expressed on ECs in developing Th1 responses. IFN-γ also enhances the expression of fractalkine on ECs, indicating the existence of a paracrine-feedback loop system in which ECs may be activated to produce more fractalkine (Figure 3).

To Clinical Disease

Cytotoxic lymphocytes, including NK cells, γδ T cells, and CD8+ T cells, function in the immune defense against infections and tumors. However, in a variety of pathological conditions, excessive activity by such cells may damage tissues, including the endothelium.1,6,12,44 Although the possible involvement of fractalkine in such damage has not been thoroughly examined, accumulating evidence regarding the physiological effects of fractalkine might provide an insight into the pathogenesis of various diseases.

Atherosclerosis and Cardiovascular Disease

Atherosclerotic lesions contain large numbers of immune cells, particularly macrophages and T cells, which orchestrate inflammatory responses.45 There is growing evidence to suggest that fractalkine may be involved in atherogenesis and cardiovascular pathophysiology. High levels of fractalkine mRNA expression, as well as mRNA encoding other 16q13-chromosome–linked chemokines, CCL17 (thymus-and activation-regulated chemokine [TARC]), and CCL22 (macrophage-derived chemokine [MDC]), have been observed in some, but not all, human arteries with advanced atherosclerotic lesions.46 Similar to atherosclerotic coronary artery disease, vessels from diabetic patients have been reported to express fractalkine in the deep intima.47 Circulating monocytes are the precursors of foam cells in the atherosclerotic lesion, and chemokines are important in directing monocyte migration from the blood to the vessel wall.48 Lesnik et al49 and Combadiere et al50 have reported that fractalkine expression is upregulated in atherosclerotic lesions of apolipoprotein E−/− mice and that crossing CX3CR1−/− into the apoE−/− background results in decreased atherosclerotic lesion formation with reduced macrophage accumulation.49,50 Gene polymorphisms at amino acids 249 and 280 of human CX3CR1 have been reported to be a genetic risk factor for coronary artery disease.51,52 CX3CR1-Y249F/T280M heterozygosity is associated with a markedly reduced risk of acute coronary events. This protective effect could be explained by the decreased ability of monocytes to adhere to vascular endothelium.

Although atherosclerosis is a multifactorial disease, often occurring as a complication of hypertension, obesity, and diabetes, it is also likely that infectious agents contribute to the development of atherosclerosis and to plaque instability and rupture.53,54 Two chronic human infections, the intracellular parasitic bacterium Chlamydia pneumoniae and human cytomegalovirus, are considered candidates. Interestingly, several studies have suggested that C pneumoniae infection may contribute to atherosclerotic plaque progression and rupture, at least in part, by accumulation of CD8+ T cells.55,56 In addition, the viral chemokine receptor US28, encoded by human cytomegalovirus, binds a broad spectrum of chemokines, including fractalkine, with high affinity and also recognizes membrane-associated fractalkine.57,58 A possible role for cytomegalovirus infection in exacerbating vascular pathology after angioplasty or organ transplantation has been also reported.59 Both of these infections induce the production of inflammatory chemokines and proinflammatory cytokines, such as TNF-α, IL-1, and IFN-γ, which probably activate ECs. Despite the apparent lack of impact of polymorphisms in CX3CR1 on peripheral arterial disease,60 these findings suggest that it is likely that the fractalkine/CX3CR1 system may nevertheless be important in the pathogenesis of atherosclerotic and coronary vascular diseases.52,61,62

Allograft Rejection

Acute allograft rejection is characterized by an intense cellular immune response marked by the influx of circulating leukocytes into the transplant.61 Robinson et al63 have reported that fractalkine expression is significantly enhanced in rejecting cardiac allografts and is particularly prominent on vascular tissues and endothelium. Moreover, the treatment of recipients with anti-CX3CR1–blocking antibodies significantly prolonged allograft survival.63 Haskell et al64 have demonstrated that the survival time of allogeneic cardiac transplants is significantly increased in the presence of subtherapeutic levels of cyclosporin A in CX3CR1-knockout mice and that this prolongation is associated with a reduction in the infiltration of macrophages, NK cells, and other leukocytes. These findings suggest that the NK cells act in concert with cyclosporin A–sensitive T cells to effect graft rejection. Together, these results indicate an important role of the fractalkine/CX3CR1 system in graft rejection.

Renal Disease

There are a number of reports suggesting a role for fractalkine in human renal diseases (glomerulonephritis, renal tumors, and renal transplants) and in kidney disease in animal models. For example, a viral chemokine similar to macrophage inflammatory protein II, with antagonistic activity for CC-, CXC-, and CX3C chemokine receptors, reduced the infiltration of leukocytes significantly and attenuated proteinuria in the rat crescent glomerulonephritis model.65 The expression of fractalkine and the presence of CX3CR1-expressing cells, such as CD16+ NK cells, have been demonstrated in patients with various types of nephropathies.66–70 Feng et al71 have reported that anti-CX3CR1 antibody treatment dramatically blocked leukocyte infiltration into the glomeruli, prevented crescent formation, and improved renal function, suggesting a role for fractalkine and CX3CR1-expressing cells in the pathogenesis of human glomerulonephritis.

HIV Infections

Increased expression of fractalkine has been detected in lymph nodes and brain tissue from patients with HIV. The increased expression of fractalkine protects neurons from 2 HIV-1 neurotoxins (Tat and platelet-activating factor, which play key roles in neural apoptosis in the brain)72 but induces the depletion of CX3CR1-positive Th cells by contact with dendritic cells.73 It has also been reported that CX3CR1 polymorphism may influence the pathogenesis of HIV infec-
tion. HIV-infected patients homozygous for CX3CR1-I249M progress to AIDS more rapidly than do those with other haplotypes. Functional CX3CR1 analysis showed that fractalkine binding is reduced among patients homozygous for this particular haplotype. Thus, it has been concluded that the specific polymorphism, CX3CR1-I249M, is a recessive genetic risk factor in HIV.74

Other Inflammatory Diseases

The involvement of fractalkine as an inflammatory mediator has also been reported in the immunopathogenesis of various Th1-dominated inflammatory diseases. Synovial tissue macrophages, fibroblasts, ECs, and dendritic cells express fractalkine and CX3CR1 in patients with rheumatoid arthritis as well as in an adjuvant-induced arthritis model in rats.75–77 Furthermore, fractalkine in synovial fluid from patients with rheumatoid arthritis promotes angiogenic activity in vitro.78 It has been reported that EC dermal dendrocytes and keratinocytes from patients with lichen planus or psoriasis express high levels of fractalkine.35,79,80 The involvement of fractalkine/CX3CR1 has been also reported in pulmonary arterial hypertension,81 lung cancer,82 and acute hepatitis83 in humans and in prediabetic NOD mice.84 Taken together, these reports indicate that fractalkine may be expressed in many tissues and may be involved in the accumulation of CX3CR1-positive T cells at inflammation sites in Th1-dominated diseases.

Conclusions

Fractalkine, a unique chemokine, can fulfill the dual functions of an adhesion molecule and a chemoattractant. Fractalkine is expressed on activated ECs and functions as a vascular gateway by attracting CX3CR1-expressing NK cells, CTLs, and macrophages with immediate cytolytic function. Inappropriate expression or function of fractalkine might well be involved in inflammatory conditions leading to vascular and tissue damage. We now appreciate that the fractalkine/CX3CR1 system is important in various clinical diseases, such as atherosclerosis, cardiovascular disease, graft rejection, HIV infection, and inflammatory diseases. Efforts to elucidate the precise physiological role of fractalkine will provide conceptual, diagnostic, and therapeutic intervention for fractalkine-mediated pathophysiological conditions.

Acknowledgments

This work was supported by grants 13877075, 13226124, and 13557160 from the Japanese Ministry of Education, Science, and Culture (to H.U.). We acknowledge the help of numerous colleagues who have provided us with insightful discussion on the fractalkine and CX3CR1 system, in particular, Drs O. Yoneda, S. Goda, H. Inoue, and M. Nishimura.

References


Fractalkine in Vascular Biology. From Basic Research to Clinical Disease
Hisanori Umehara, Eda T. Bloom, Toshiro Okazaki, Yutaka Nagano, Osamu Yoshie and Toshio Imai

_Arterioscler Thromb Vasc Biol_, published online September 11, 2003;
_Arteriosclerosis, Thrombosis, and Vascular Biology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2003/09/11/01.ATV.0000095360.62479.1F.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Arteriosclerosis, Thrombosis, and Vascular Biology_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Arteriosclerosis, Thrombosis, and Vascular Biology_ is online at:
http://atvb.ahajournals.org//subscriptions/