Regulation of CX3CL1/Fractalkine Expression in Endothelial Cells

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CX3CL1/fractalkine is a chemokine with a unique CX3C motif. Fractalkine is synthesized in endothelial cells as a membrane protein, and the N-terminal domain containing a CX3C motif is cleaved and secreted. CX3CR1, the specific receptor for fractalkine, is expressed in monocytes and lymphocytes. Membrane-bound fractalkine works as an adhesion molecule for these leukocytes and the secreted form as a chemotactic factor. Fractalkine is produced by endothelial cells stimulated with tumor necrosis factor- α , interleukin-1 (IL-1), lipopolysaccharide and interferon- γ . Expression of fractalkine in endothelial cells is inhibited by the soluble form of IL-6 receptor- α , 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂, and hypoxia. The expression of fractalkine is tightly regulated and fractalkine plays an important role in the interaction between leukocytes and endothelial cells. *J Atheroscler Thromb*, 2004; 11: 15-21.

Key words: Fractalkine, Endothelial cells, Chemokine

Endothelium-leukocyte Interactions in Atherosclerosis

The interactions of vascular endothelial cells with leukocytes are critical in recruiting blood leukocytes into the vascular wall. Chemokines and adhesion molecules produced by vascular endothelial cells are essential in endothelial cell-leukocyte interactions: chemokines induce chemotaxis and activation of leukocytes, and adhesion molecules mediate the adhesion of leukocytes. Recruitment of circulatory monocytes to the arterial intima is an important process in the formation of foam cells that are a central feature of atherosclerotic lesions (1). The activation of blood leukocytes at the vascular endothelium may facilitate the release of various factors by leukocytes and trigger cellular events that eventually may lead to atherosclerotic vascular changes. Thus, the regulation of chemokines and adhesion molecules in vascular endothelial cells is important in inflammatory responses (2), and inflammation in vascular wall tissue is implicated in atherogenesis (3).

Fractalkine: A Unique Chemokine with a CX3C Motif

Chemokines have conserved cysteine residues and are classified into C, CC, CXC and CX3C subfamilies. Lymphotactin is the only member of the C chemokine family. CC chemokine family includes monocyte-chemoattractant protein-1, macrophage inflammatory protein-1 α , eotaxin, etc. Interleukin-8 (IL-8), epithelial neutrophil activating peptide-78 (ENA-78), growth-related oncogene- α (GRO- α), and interferon- γ inducible protein-10 (IP-10) are the members of the CXC chemokine family. Fractalkine was identified as a chemokine with a unique CX3C motif (4), and was designated as CX3CL1. No other CX3C chemokines are identified.

Fractalkine is synthesized as a membrane protein and the cell surface-bound molecule serves as a potent adhesion molecule along with others such as vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin. The N-terminal of fractalkine, containing the CX3C motif, can be cleaved by a protease called tumor-necrosis factor- α (TNF- α)-converting enzyme (TACE; also known as ADAM17) (5) and released from the membrane-associated molecules. TACE is expressed in human endothelial cells (6). The secreted form of fractalkine has potent chemotactic activity, and this is a unique feature of fractalkine, com-

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pared to other adhesion molecules or chemokines. For example, VCAM-1 is also a potent adhesion molecule, and the N-terminal domain of VCAM-1 can be released from the membrane-bound molecules. The secreted form of VCAM-1 has no leukocyte chemotactic activity and is thought to function as an antagonist.

The cell surface receptor for fractalkine, CX3CR1, is expressed in T cells and monocytes (7), and fractalkine functions both as a chemokine and an adhesion molecule for these leukocytes. Knockout of the gene encoding CX3CR1 in apoE^{-/-} mice resulted in a significant reduction of atherosclerotic lesions induced by a high-fat diet (8). In humans, a polymorphism of the CX3CR1 gene, which reduces binding to fractalkine, is associated with reduced risk for atherosclerotic cardiovascular diseases (9) and increases the risk for HIV disease (10). These observations suggest that fractalkine and its receptor are important players in atherogenesis and other diseases, and elucidation of the molecular mechanisms of fractalkine expression in vascular endothelial cells may contribute to the understanding of part of atherogenesis.

Proinflammatory Cytokines Induce the Expression of Fractalkine in Endothelial Cells

Expressions of chemokines and adhesion molecules are usually upregulated by inflammatory stimuli, and fractalkine was first reported as a factor induced by TNF- α , IL-1 and bacterial lipopolysaccharide (LPS) (4). TNF- α , IL-1 and LPS stimulate a similar spectrum of signal transduction systems: both the nuclear factor- κ B (NF- κ B) system and mitogen-activated protein kinases (MAPKs) are activated by them. These factors thereby induce a variety of molecules including chemokines (11), adhesion molecules, growth factors (12), colony-stimulating factors (13) and enzymes (14, 15) in endothelial cells. Under resting conditions, NF- κ B binds to the inhibitory subunit of κ B (I κ B) and exists in cytoplasm. Upon specific stimulation, I κ B is phosphorylated, ubiquitinated and subsequently degraded by a proteasome-dependent pathway. Then, NF- κ B is translocated into the nucleus and activates the transcription of target genes. Treatment of endothelial cells with proteasome inhibitors, such as MG115 and MG132, suppresses the degradation of I κ B and activate the NF- κ B system. Proteasome inhibitors also activate MAPKs in inducing the expression of IL-6 and GRO- α (16, 17). In rat aortic endothelial cells, the induction of fractalkine by IL-1 β , TNF- α and LPS is reported to be dependent on NF- κ B (18).

The two subtypes of helper T cells, Th1 and Th2, produce distinct profiles of cytokines. Interferon- γ (IFN- γ), one of the most important proinflammatory and immunomodulatory cytokines, is produced by Th1 type cells. Th2 type cells are involved in allergic reactions and produce cytokines such as IL-4 and IL-5. Vascular en-

dothelial cells express the receptor for IFN- γ , and IFN- γ induces various factors such as IP-10 (19), cell death regulatory protein Bak (20), and galectin-9 (21). IFN- γ also induces fractalkine mRNA in rat aortic endothelial cells (18). We demonstrated the expression of fractalkine in human umbilical vein endothelial cells (HUVEC) stimulated with IFN- γ (22). IFN- γ controls the target cells by binding to its specific receptor and activating Janus kinase 1 (JAK1), the signal transducer and activator of transcription 1 (STAT1) and the STAT1-dependent transcription of genes. The human fractalkine gene is located on chromosome 16 (23), and there are binding sites for NF- κ B and STAT in the promoter region of the gene.

Expression of Fractalkine in Other Types of Cells

Fractalkine mRNA is expressed in most human organs, including the heart, brain, lung, kidney, intestines and skeletal muscles (4). This chemokine is constitutively expressed in dendritic cells and upregulated upon dendritic cell maturation (24), and this suggests the important role of fractalkine in immune reactions. Intestinal epithelial cells also produce fractalkine and its mRNA level is enhanced in active Crohn's disease (25). In the nervous system, fractalkine is constitutively expressed in the olfactory bulb, cerebral cortex, hippocampus, etc. (26), but its expression is not affected by experimentally-induced inflammation of central nervous tissue (27). TNF- α and IFN- γ synergistically induce fractalkine in human astrocytes (28). IFN- γ also enhances fractalkine expression in human bronchial epithelial cells and mediates adherence of macrophages (29). Fractalkine is induced by TNF- α and IFN- γ in dermal fibroblasts (30). Recently, accumulating evidence has shown that fractalkine is involved in the pathogenesis of various diseases, such as glomerulonephritis (31), cardiac allograft rejection (32), and rheumatoid arthritis (33).

Soluble Form of IL-6R α Inhibits the Expression of Fractalkine

IL-6 is a multifunctional cytokine and mediates a variety of inflammatory and cell growth responses. The cell surface receptor for IL-6 consists of a cytokine-binding α subunit (IL-6R α) and a transmembrane signaling gp130 subunit. Vascular endothelial cells have gp130 but lack IL-6R α , and IL-6 alone does not have an agonistic activity on endothelial cells. Leukocytes express IL-6R α , and the soluble form of IL-6R α (sIL-6R α) is released by the shedding of the membrane-bound forms from activated neutrophils. sIL-6R α binds to gp130 on the endothelial cell surface and thereby activates the signaling via JAK, STAT (34) and MAPK (35). This leads to the expression of chemokines and adhesion molecules, such as IL-8, E-

selectin, VCAM-1 (36) and GRO- α (35). We examined whether sIL-6R α also induces the expression of fractalkine in HUVEC and, unexpectedly, sIL-6R α was found to inhibit the expression of fractalkine induced by IL-1 or IFN- γ (37). Preincubation of HUVEC with an anti-gp130-neutralizing antibody reversed the inhibition of fractalkine expression by sIL-6R α , suggesting that the effect was dependent on specific signaling via gp130. sIL-6R α also inhibited the secretion of chemotactic activity for mononuclear cells by endothelial cells and the adhesion of mononuclear cells to the endothelial monolayer stimulated with IFN- γ .

Role of 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ in the Regulation of Fractalkine

When endothelial cells are activated, arachidonic acid is released from membrane phospholipids. The arachidonic acid is metabolized to prostaglandins via the arachidonic acid cascade. Cyclooxygenase-2 (COX-2) is a key enzyme in prostaglandin synthesis, and COX-2 in endothelial cells is upregulated in response to various stimuli (14). 15-Deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) is a metabolite of PGD₂, and detected, by immunohistochemistry, in the cytoplasm of foamy macrophages in human atherosclerotic plaques (38). 15d-PGJ₂ is an agonist for peroxisome proliferator-activated receptor- γ (PPAR- γ), a member of the nucleic hormone receptor superfamily. Three subtypes of PPARs (α , β , γ) have been described, and PPAR- γ was originally identified in adipose tissue as a factor that induces adipocyte differentiation and triglyceride synthesis. PPAR- γ is also expressed in vascular tissues and leukocytes, and it mediates the apoptotic death of vascular endothelial cells (39). 15d-PGJ₂ regulates the expression of various genes in an PPAR- γ -dependent or -independent manner. It induces the expression of IL-8 (40), but does not affect the expression of GRO- α (41). However, 15d-PGJ₂ inhibits the expression of IP-10, monokine-induced by IFN- γ , IFN-inducible T cell α chemoattractant (42), galectin-9 (43), granulocyte-colony stimulating factor (13) and ENA-78 (41). We found the inhibition of endothelial fractalkine expression by 15d-PGJ₂ (44); however, ciglitazone, another agonist for PPAR- γ , did not inhibit cytokine-induced fractalkine expression, and the effect of 15d-PGJ₂ may be independent of PPAR- γ . 15d-PGJ₂ is shown to inhibit the signaling through NF- κ B, activating protein-1 (AP-1) and STAT1 (43, 45), and the effects on these transcriptional factors may be responsible for the downregulation of fractalkine by 15d-PGJ₂. The expression of other adhesion molecules such as VCAM-1 and ICAM-1 is also regulated by agonists for PPARs (46).

Hypoxia Inhibits the Expression of Fractalkine

Hypoxia constitutes the pathophysiological state in many disorders including ischemic vascular diseases, inflammatory diseases and malignant diseases. Hypoxia modulates the expression of various genes, and this may represent part of the mechanisms for the adaptation of cells to hypoxic stress. Hypoxia-inducible factor-1 α (HIF-1 α) is a transcriptional factor and involved in the activation of several hypoxia-responsive genes. Under a normoxic condition, HIF-1 α is rapidly degraded by the ubiquitin-proteasome system, because normally it is ubiquitinated by a ubiquitin ligase called von Hippel-Lindou (VHL) (47). Mutations in the VHL are responsible for the VHL disease, which is characterized by hemangioma and cancer. When the cells are exposed to hypoxia, the ubiquitination and degradation of HIF-1 α is inhibited, which results in the expression of genes such as erythropoietin, vascular endothelial growth factor (48) and plasminogen-activator inhibitor-1 (49), regulated by HIF-1 α . Hypoxia also induces the expression of COX-2 in endothelial cells, and another transcriptional factor, high mobility group protein-I(Y), is involved in this reaction (50).

Hypoxia impairs the functions of many cell types by inhibiting the expressions of various genes induced by factors implicated in pathological conditions. Hypoxia inhibits the expression of argininosuccinate synthase in lung endothelial cells (51) and the PPAR- α /retinoid X receptor gene regulatory pathway in cardiac myocytes (52). Heme oxygenase 1 (HO-1) is the rate-limiting enzyme in heme catabolism, and the expression of HO-1 mRNA is repressed by hypoxia in human endothelial cells and astrocytes (53). We found the inhibition, by hypoxia, of the IFN- γ -induced expression of fractalkine in HUVEC (54). Desferrioxamine (DFX) is an iron chelator which mimics hypoxia *in vitro*, and DFX also inhibited the expression of fractalkine in HUVEC stimulated with IFN- γ (54). Tissue hypoxia and reoxygenation commonly occur during the course of circulatory shock or other pathological conditions. Inhibition of fractalkine expression by hypoxia was reversed on returning the HUVEC cultures to a reoxygenated condition (54). Thus, the inhibition of fractalkine expression by hypoxia is reversible, and there is a specific intracellular regulatory system for the hypoxia-mediated fractalkine inhibition.

Expressions of cytokines are also regulated by oxygen- or redox-sensitive mechanisms. Hypoxia is known to induce the generation of reactive oxygen species. N-acetyl-L-cysteine (NAC), a radical scavenger, did not affect the expression of fractalkine, and reactive oxygen species may not take part in the inhibition of fractalkine expression in a hypoxic condition.

Effect of Heparin on the Expression of Fractalkine

Heparin is an N-sulfated polysaccharide and commonly used as an anticoagulant. Heparin and heparin-related molecules are also known to inhibit leukocyte-endothelial interactions by binding chemokines (55) and adhesion molecules (56).

The luminal surface of vascular endothelium is covered by a dense layer of proteoglycans. The main functional components of this layer are polyanionic glycosaminoglycans (GAGs), and the most abundant endothelial GAG is heparan sulfate, which is structurally and biosynthetically related to heparin and chondroitin sulfate (57). Exogenous chondroitin-6-sulfate, but not dextran sulfate, inhibited the fractalkine mRNA expression in HUVEC stimulated with IFN- γ . Various cytokines including IFN- γ are known to bind to heparin or heparin-related molecules (58). Therefore, heparin may regulate the activities of IFN- γ by competing for cell surface GAGs or proteoglycans in IFN- γ binding (59). We demonstrated the inhibition, by heparin, of fractalkine expression in HUVEC induced by IFN- γ , but not by IL-1 β (60). Adhesion of blood mononuclear cells to IFN- γ -stimulated HUVEC monolayers was reduced by heparin, but heparin had no effect on the IL-1 β -induced adhesion of mononuclear cells. Heparin was demonstrated to directly bind IFN- γ , partially block the binding of IFN- γ to its receptor, and thereby inhibit the phosphorylation of STAT1 (60). Heparin may inhibit in-

flammatory and immune responses by regulating leukocyte trafficking across the vascular endothelium, and it may be particularly effective in conditions such as disseminated intravascular coagulation associated with infectious diseases.

Conclusion

Fractalkine is expressed in vascular endothelial cells in response to inflammatory stimuli and the expression is regulated by various factors. This cytokine constitutes part of the molecular mechanisms of the interactions between the vascular wall and leukocytes and plays an important role in the pathogenesis of inflammatory reactions in the vascular wall (Fig.1).

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Fig. 1. Regulation and functional significance of the fractalkine expression in endothelial cells. LPS from *E. coli* and cytokines such as TNF- α , IL-1 or IFN- γ induce the expression of fractalkine in endothelial cells. Membrane-bound fractalkine works as an adhesion molecule and the secreted form as a chemotactic factor for mononuclear cells expressing CX3CR1. sIL-6R α , 15d-PGJ $_2$, and hypoxia inhibit the induction of fractalkine. Heparin inhibits the IFN- γ -induced expression of fractalkine by interfering with the binding of IFN- γ to its receptor.

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