Molecular Uncoupling of Fractalkine-mediated Cell Adhesion and Signal Transduction

RAPID FLOW ARREST OF CX₃CR1-EXPRESSING CELLS IS INDEPENDENT OF G-PROTEIN ACTIVATION*

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Christopher A. Haskell‡, Michael D. Cleary‡, and Israel F. Charo‡‡‡¶¶

From the ‡Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94141-9100, the ¶Cardiovascular Research Institute, and the §§Department of Medicine, University of California, San Francisco, California 94143

Fractalkine is a novel multidomain protein expressed on the surface of activated endothelial cells. Cells expressing the chemokine receptor CX₃CR1 adhere to fractalkine with high affinity, but it is not known if adherence requires G-protein activation and signal transduction. To investigate the cell adhesion properties of fractalkine, we created mutated forms of CX₃CR1 that have little or no ability to transduce intracellular signals. Cells expressing signaling-incompetent forms of CX₃CR1 bound rapidly and with high affinity to immobilized fractalkine in both static and flow assays. Video microscopy revealed that CX₃CR1-expressing cells bound more rapidly to fractalkine than to VCAM-1 (60 versus 190 ms). Unlike VCAM-1, fractalkine did not mediate cell rolling, and after capture on fractalkine, cells did not dislodge. Finally, soluble fractalkine induced intracellular calcium fluxes and chemotaxis, but it did not activate integrins. Taken together these data provide strong evidence that CX₃CR1, a seven-transmembrane domain receptor, mediates robust cell adhesion to fractalkine in the absence of G-protein activation and suggest a novel role for this receptor as an adhesion molecule.

The interaction of leukocytes with the vascular wall and their subsequent migration into surrounding tissues are central components of the inflammatory response. This leukocyte trafficking is directed by chemokines (chemotactic cytokines), a rapidly growing family of low molecular weight, soluble proteins. There are two major subfamilies of chemokines, classified by the sequence of a conserved di-cysteine motif near the amino-terminal end of the protein. The CC or α chemokines contain a sequential cysteine-cysteine motif and are predominately mononuclear cell agonists. The CXC or β chemokines contain a single amino acid between these two cysteines and are agonists for polymorphonuclear leukocytes. The mechanism of leukocyte migration to sites of inflammation involves the sequential actions of selectins, which mediate rolling along the vessel wall, and integrins, which mediate firm adhesion and diapedesis (1, 2). Chemokines appear to play a key role in this process by providing a chemotactic gradient to direct cell migration and by activating integrins (3).

Fractalkine (CX₃C) is a recently discovered chemokine that contains three amino acids between the first two cysteines and may thus be the first member of a new family of chemokines. Fractalkine possesses a number of structural characteristics and biological activities not previously found within the chemokine family (4, 5). In contrast to soluble chemokines, fractalkine is anchored to vascular wall cells by an extended mucin stalk linked to a transmembrane domain (5). This domain may serve to position fractalkine for efficient interactions with leukocytes expressing CX₃CR1, the receptor for fractalkine (6, 7). Unlike most chemokines, fractalkine is not synthesized by leukocytes. Instead, it is expressed on activated endothelial cells in the vasculature and on neurons in the central nervous system, where it appears to be involved in regulating the activity of microglia (8, 9). Soluble forms of fractalkine have been detected and may be the result of proteolytic cleavage at a di-basic consensus sequence located next to the transmembrane domain (5).

The location of fractalkine at the luminal surface of endothelial cells suggested a potential role in the arrest of cells from flowing blood. Consistent with this idea was the observation that CX₃CR1-expressing cells bound to fractalkine-coated glass and to cells expressing fractalkine on their surface (6, 10). A unique feature of this interaction was the failure of pertussis toxin (PTX) to block cell adhesion to fractalkine (6, 10). Other chemokines induce cell adhesion but do so indirectly by up-regulating integrins, a process that is dependent upon G-protein activation (3, 11, 12). The finding that fractalkine mediated cell adhesion in the presence of PTX raised the intriguing possibility that CX₃CR1 acts primarily as an adhesion molecule, rather than as a signaling molecule. To test this hypothesis, we used an in vitro system to study the binding of flowing cells to fractalkine. Here, we report that fractalkine promotes the rapid arrest of CX₃CR1-expressing cells under conditions of physiological shear stress. Unlike adhesion mediated by integrins, fractalkine-induced adhesion was independent of G-protein activation. Adhesion of CX₃CR1-expressing cells to fractalkine did not enhance integrin-dependent cell adhesion, suggesting that the presence of fractalkine may provide an alternative to integrin-mediated cell adhesion.

EXPERIMENTAL PROCEDURES

Materials—The recombinant human chemokines secondary lymphoid tissue chemokine (SLC) and fractalkine were obtained from R & D Systems (Minneapolis, MN). Two forms of fractalkine were used. The full-length extracellular domain, including a carboxyl-terminal polyhistidine epitope tag, was used for cell adhesion studies. A second form consisting of the chemokine domain portion only (designated soluble

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‡ To whom correspondence should be addressed; Gladstone Institute of Cardiovascular Disease, P. O. Box 419100, San Francisco, CA 94141-9100. Tel.: 415-826-7500; Fax: 415-285-5632; E-mail: icharo@gladstone.ucsf.edu.

1 The abbreviations used are: PTX, pertussis toxin; SLC, secondary lymphoid tissue chemokine; VCAM-1, vascular cell adhesion molecule 1; DR/NN, D127N/R128N double mutant.
fractalkine was used in signaling assays and in competitive cell binding studies. The anti-idiotype monoclonal antibody was from R & D Systems. Soluble VCAM-1 (13) was kindly provided by Dr. Ted A. Yednock (Athena Neurosciences, South San Francisco, CA). Minimal essential medium, Opti-MEM, and RPMI were from Life Technologies. Myo myosin was from Hybridoma Laboratory (Logan, UT). m30-2[H]Inositol was obtained from NEN Life Science Products. PTX was from List Biological Laboratories (Eugene, OR) or Sigma. All other chemicals were from Sigma.

DNA Constructs—A genomic clone of murine CX3CR1 was isolated by a PCR-based technique, in which genomic DNA from embryonic stem cells was used as a template. The following primers were used: 5′-GGTGTGCACTCAGCCTCTCCGGATCCG and 5′-ATGGGGCCTCTAGAGCAAGGAGAGCTATCTC (receptor-coding regions are underlined) and added 5′-SalI and 3′-NcoI sites to the amplified cDNA. The receptor-coding region was sequenced, and the nucleotide sequence matched that recently deposited in GenBank™ (accession number AF102289). The cDNA was ligated into an expression vector that added the prolactin signal sequence followed by a FLAG epitope to the amino terminus (14). Murine and human CX3CR1 showed equivalent binding affinity and signaling in response to human fractalkine (data not shown), and the murine form of the receptor was used in all experiments reported. Mutations were generated with the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) and overlapping primer PCR. The desired mutation PCR product was isolated from HUT-78 mRNA with a reverse transcription PCR-based technique and subcloned into the expression vector described above. All constructs were confirmed by sequencing of both strands. The G-protein construct (Go,αi) (15) was kindly provided by Dr. Bruce R. Conklin (Gladstone Institute of Cardiovascular Disease).

Cell Culture and Transfection—Human embryonic kidney cells (HEK-293) were purchased from the American Type Culture Collection (Manassas, VA), and the murine pre-B cell line (300-19) (16, 17) was a generous gift of Dr. G. L. Rossa (LeukoSite, Cambridge, MA). Cells were grown and transfected as described (17). To further select for cells expressing high numbers of receptors, cells were labeled with the anti-FLAG epitope antibody (M1), as described below, and sorted on a fluorescence-activated cell sorter (Vantage, Becton Dickinson, Franklin Lakes, NJ). Sixteen-well glass chamber slides (Nunc, Naperville, IL) were coated overnight with a monoclonal antihistidine antibody (Viggo, Lake Success, NY). After a 3-min perfusion, the anti-FLAG monoclonal antibody (H10) from Hybridoma Laboratory (Logan, UT) was added at a final concentration of 10 nM in adhesion buffer (150 mM NaCl, 10 mM HEPES, 1 mM MgCl2, 1 mM CaCl2, pH 7.4). The slides were incubated in a humidified box at 4 °C overnight. Before the next reagent was added, the slides were washed by applying 150 μl of adhesion buffer with a pipettor and then aspirating the buffer until the slide was almost dry. The second reagent (fractalkine-stalk-polyhistidine (100 μg/ml) or VCAM-1 (2 μg/ml) in flow adhesion buffer (150 mM NaCl, 10 mM HEPES, 1 mM MgCl2, 1 mM CaCl2, pH 7.4) was incubated for 1 h at 37 °C and washed. The entire mounted area was then coated with fetal bovine serum for 15 min at 37 °C. Before use in the assay, the entire slide was washed by dipping into adhesion buffer, and the flow chamber was aligned with the coated area.

Cell Preparation and Adhesion Assay—Cells were washed once, resuspended in flow adhesion buffer (2 × 106 cells/ml), and perfused through the chamber. The calculated wall shear stress was 1.5 dynes/cm2 (assuming viscosity equaled 0.01 poise), unless otherwise noted. Images were captured with a TMS microscope (Nikon, Garden City, NJ), a charged-coupling device video camera (Sony SSC-S20, Park Ridge, NJ), and a videorecorder (Panasonic, Secaucus, NJ).

Analysis of Numbers of Cells Adhering—After a 3-min perfusion, the entire chamber was resuspended (10,000 cells/well) and captured on video. The number of adherent cells was determined by counting the cells in these fields during video playback.

Analysis of Cell Behavior and Time to Capture—Time to capture was determined by replaying the video frame by frame (17 ms/frame = 60 frames/s). Cells that interacted with the substrate and subsequently stopped were counted. The “zero” time was defined as the time at which the cell first entered the focal plane of the substrate-coated glass, indicating an interaction with the substrate. Cells transiently stopping were not included in this analysis. These transient interactions, which we observed commonly in integrin-dependent adhesion to VCAM-1, were never seen in fractalkine-mediated adhesion. Therefore, to compare stable adhesion, we included in the data set only cells that remained attached for more than 30 s. Cell position as a function of time was determined by analysis of videocap with NIH Image 1.61 (National Institutes of Health). These data were analyzed at 20 frames/s.

Statistical Analysis—Statistics for all assays were done with Instat software (GraphPad Software, San Diego, CA) for the Macintosh. The Mann-Whitney test or Welch’s t test was used, where appropriate.

RESULTS

To determine if fractalkine-mediated adhesion is dependent upon signal transduction, we changed critical intracellular domains of CX3CR1 to uncouple it from G proteins. CX3CR1, like many seven-transmembrane domain receptors, has in its second intracellular loop a highly conserved aspartate-arginine-tyrosine (DRY) sequence that is required for G-protein activation (20–23). Receptors with both single (R128N) and double (D127N plus R128N, denoted DR/NN) point mutations were generated because the double mutant tended to be expressed at lower levels than wild-type CX3CR1. All receptors were constructed with the FLAG epitope at the amino terminus to facilitate quantitation of surface expression.

The three receptor isofoms (wild-type, R128N, and DR/NN) were transiently transfected into HEK-293 cells, and the transfected cells were assayed for their ability to signal in response to saturating levels of fractalkine. HEK-293 cells expressing wild-type CX3CR1 gave a robust signal, as measured by an increase in inositol phosphate release (Fig. 1). In contrast, cells expressing the R128N or DR/NN form of CX3CR1 failed to signal. Similar results were obtained when signaling was assayed by measuring agonist-dependent intracellular calcium fluxes in chemotaxis (data not shown). Inflow adhesion buffer (150 mM NaCl, 10 mM HEPES, 1 mM MgCl2, 1 mM CaCl2) was added to the cell suspension to a density of 3.2 × 106 cells/ml. Fifty microliters of cell suspension were added to a 35-μm cell strainer cap tube (Becton Dickinson, Franklin Lakes, NJ) to eliminate cell aggregates. Cells were washed with adhesion buffer and resuspended to a density of 3.2 × 106 cells/ml. Fifty microliters of the cell suspension were added to each well, and adhesion was allowed to progress for 30 min at room temperature. Nonadherent cells were washed from the slide by dipping the entire chamber in phosphate-buffered saline, removing the snap-off wells, and dipping the slide twice more. The cells were fixed in 1% glutaraldehyde for 10 min and rinsed in phosphate-buffered saline. Adherent cells were counted with an inverted microscope and ×40 objective.

Flow Chamber Adhesion Assay—Cells were perfused over a 20 × 3-mm area of a glass slide (Corning Glass Works, Corning, New York) in a laminar flow chamber (GlycoTech, Rockville, MD) as described previously (12).

Slide Preparation—A template and a solvent-resistant pen were used to mark a 20 × 3-mm region, which was subdivided into two sections. The slide was then inverted and the coagulation reagents were “drawn” onto the slide with an Eppendorf pipettor, with the markings on the opposite side serving as a guide. One of the two regions was coated with the “test” ligand, and the other was left uncoated as a control. The initial coating (9 μl) was either anti-polyhistidine antibody (10 μg/ml) or VCAM-1 (2 μg/ml) in flow adhesion buffer (150 mM NaCl, 10 mM HEPES, 1 mM MgCl2, 1 mM CaCl2, pH 7.4). The slides were incubated in a humidified box at 4 °C overnight. Before the next reagent was added, the slides were washed by applying 150 μl of adhesion buffer with a pipettor and then aspirating the buffer until the slide was almost dry. The second reagent (fractalkine-stalk-polyhistidine (100 μg/ml)) was added in the same manner. The slides were incubated for 1 h at 37 °C and washed. The entire mounted area was then coated with fetal bovine serum for 15 min at 37 °C. Before use in the assay, the entire slide was washed by dipping into adhesion buffer, and the flow chamber was aligned with the coated area.
conditions. Addition of a polyhistidine tail to the carboxyl terminus allowed us to “tether” fractalkine to an antipolyhistidine antibody previously coated onto glass slides. HEK-293 cells expressing wild-type CX3CR1 bound well to fractalkine; this binding was completely blocked by the addition of soluble fractalkine (chemokine domain only) (Fig. 2). Preincubating the cells with PTX (10 ng/ml, 16 h) did not block adhesion, although this concentration of PTX completely eliminated the fractalkine-induced increase in intercellular calcium (data not shown). Although unable to signal in response to soluble fractalkine, the DR/NN form of CX3CR1 bound as well as the wild-type receptor to tethered fractalkine (Fig. 2). Similar results were obtained when the wild-type or mutated forms of CX3CR1 were expressed in a 300–19 pre-B cells (Fig. 2). These data indicate that receptor-mediated signaling is not required for cells expressing CX3CR1 to bind to fractalkine.

We next asked whether the expression of CX3CR1 would cause rapidly flowing cells to adhere to a surface coated with fractalkine. Under flow conditions that produced a wall shear stress of 1.5 dyn/cm², cells expressing wild-type CX3CR1 adhered well to immobilized fractalkine; this adherence was completely blocked by soluble fractalkine (Fig. 3). Cells expressing the DR/NN mutation of CX3CR1 adhered as well as those expressing the wild-type receptor; this adherence was not reduced by pretreatment with PTX. These data suggest that fractalkine induces the capture of cells flowing at physiologically relevant shear rates and that cell capture is not dependent upon receptor activation.

Next, we used the flow assay to compare cell adhesion to fractalkine with adhesion to VCAM-1. Cell adhesion to VCAM-1 is mediated by the integrin αβ₁. To induce adhesion to VCAM-1, we used the chemokine SLC to activate αβ₁ on the cell surface. Upon coming into contact with VCAM-1, the cells slowed and rolled for varying periods of time before coming to a full stop (Fig. 4). These cells often adhered transiently and reentered the flow before stopping. In contrast, cells expressing CX3CR1 came to a rapid and complete stop virtually coincident with encountering the fractalkine-coated surface. Rolling was rare, if ever, observed on the fractalkine-coated slides (Fig. 4).

Next, we quantitated the time necessary for cells to stop after their initial contact with fractalkine or VCAM-1. Cells were captured after a mean of 60 ms (±42 ms) when the slide was coated with fractalkine; in contrast, cells were captured after a mean of 190 ms (±192 ms) when the slide was coated with VCAM-1 (Fig. 5). These data indicate that the cell adhesion to fractalkine is qualitatively and quantitatively different from adhesion mediated by integrins.

To determine whether adhesion to fractalkine up-regulated the cell’s integrins, we examined the adherence of CX3CR1-expressing cells to slides coated with VCAM-1, fractalkine, or VCAM-1 and fractalkine in the flow assay. The results are shown in Fig. 6. On VCAM-1-coated slides, there was a low level of binding. On fractalkine-coated slides, increased cell binding was observed. The cell binding to fractalkine was specific binding because it was virtually eliminated by the addition of soluble fractalkine (data not shown; see Fig. 2). On slides coated with VCAM-1 and fractalkine, the number of cells bound was equal to the sum of the cells binding to each substrate individually. Thus, adhesion to fractalkine did not induce integrin-dependent adhesion. Rather, cells appeared to bind either to fractalkine or to VCAM-1. We next asked whether the soluble form of fractalkine, which induces robust CX3CR1-dependent signaling (Fig. 1), also induces integrin-dependent effects.
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adhesion. Soluble fractalkine did not increase integrin-dependent adhesion to VCAM-1. In control studies, we showed that integrin activation by the chemokine SLC did induce binding of these cells to VCAM-1; as expected, this binding was blocked by EDTA.

**DISCUSSION**

This study demonstrates that immobilized fractalkine mediates the rapid arrest of cells flowing at physiological shear rates. In mediating cell adhesion, the fractalkine receptor, CX₃CR1, acts as a primary adhesion molecule rather than as a signaling molecule. Mutation of intracellular residues critically required for G-protein coupling demonstrated that fractalkine-dependent signaling by CX₃CR1 was not required for cell arrest. Furthermore, activation of CX₃CR1 by soluble fractalkine did not up-regulate integrin-dependent binding to VCAM-1. The dissociation of cell adhesion from signal transduction suggests a novel role for CX₃CR1, a G-protein coupled receptor, as an adhesion molecule.

Evidence from many groups supports a multistep model for leukocyte emigration from the bloodstream (1, 2). The initial step in this model is the rolling of unactivated cells along the vessel wall, which is thought to be mediated by the interaction of leukocyte selectins with glycosylated proteins or lipids on the surface of endothelial cells. In the next steps, firm adhesion to the endothelium and migration into the tissues are dependent upon activation of leukocyte integrins. Recent data suggest that integrin activation is an important physiological end point of chemokine receptor activation and that specific chemokines play an important role in recruiting lymphocytes to secondary lymphoid organs (3, 11, 12). Because PTX blocks chemokine-induced chemotaxis (24) and cell adherence (3), the observation that it failed to block fractalkine-dependent cell adhesion (6) was surprising and suggested a novel role for fractalkine as an adhesion molecule.

PTX does not uncouple chemokine receptors from all classes of G proteins, however, and so a role for signaling in mediating adhesion was not ruled out. We (18) and others (25) have shown that CCR2 and CXCR1 couple to multiple G proteins, including Go₁₆ and Go₁₅, which are not sensitive to PTX. Evidence that intracellular signal transduction might be important in fractalkine-mediated adhesion came from the observation that cells did not attach to fractalkine-coated surfaces at 4 °C (6). Since it is not known which G proteins bind to CX₃CR1, we chose to uncouple it from second messengers by mutating highly conserved intracellular amino acids known to be crucially involved in G-protein interactions (20–23).

Several lines of evidence indicated that the mutated CX₃CR1 failed to activate signaling pathways. First, we were unable to detect an increase in fractalkine-dependent hydrolysis of phosphoinositol. Second, fractalkine did not mobilize intracellular calcium in these cells. Third, cells transfected with the mutated receptor failed to undergo chemotaxis in response to fractalkine. The failure to support chemotaxis was especially significant because it provided a functional end point, independent of specific second messengers. In both static and flow assays, the signaling-incompetent form of CX₃CR1 mediated cell adhesion as well as wild-type fractalkine. These results indicate that the interaction between full-length fractalkine and CX₃CR1 leads to rapid cell arrest independent of receptor activation and G-protein coupling. These properties make fractalkine unique among the chemokines.

Fractalkine captured flowing cells extremely rapidly. In the

**FIG. 3.** Flow adhesion of cells expressing wild-type and mutant CX₃CR1. A, 300-19 cells stably expressing wild-type CX₃CR1 or the DR/NN mutant were perfused over antibody-tethered fractalkine at a wall shear stress of 1.5 dyn/cm². Cells adhering after 3 min were counted and averaged. There was no significant difference between the wild-type and mutated forms of the receptor (CX₃CR1 versus CX₃CR1 DR/NN: p > 0.05). Where indicated, soluble fractalkine (100 nm) was added to the cells before transfer into the flow chamber. Error bars represent S.D. values, and each data point was determined by counting cells in 10 or more fields (0.5 mm²/field) on each of two slides. Data shown are representative of two similar experiments. B, 300-19 cells expressing CX₃CR1 were untreated (Control) or treated with PTX (+PTX) (100 ng/ml) for 16 h and assayed as described above. There was no significant difference in adhesion (p > 0.05). Data shown are representative of four similar experiments.

**FIG. 4.** Behavior of 300-19 cells attaching under shear. 300-19 cells expressing transfected CX₃CR1 were perfused over slides coated with fractalkine or VCAM-1. Video recordings of flowing cells were analyzed for cell position at 20 frames/s with NIH Image software. Representative traces are shown. “No interaction” indicates free flow without substrate contact (600–800 μm/s) and is equal to the bulk flow rate through the chamber. “Deceleration” is the time during which a cell in the plane of the substrate-coated glass comes to a full stop. “Rolling,” which was seen only on the VCAM-1/SLC-coated slides, denotes cells that are transiently attaching to the substrate but are not fully stopped. Tracings are offset to allow tracking of individual cells.
parallel-plate assay, cells expressing CX3CR1 adhered to fractalkine in less than 60 ms. In contrast, cells required approximately 190 ms to adhere to VCAM-1. Videomicroscopy clearly showed that cells exhibited rolling and transient binding to VCAM-1, whereas they stopped almost instantaneously after encountering a fractalkine-coated surface. This rapid and firm adhesion of cells is consistent with the hypothesis that fractalkine, which is expressed on activated endothelial cells (5), may serve to capture cells from flowing blood.

Although signaling by CX3CR1 was not required for cell adhesion to fractalkine, it was unclear whether activation of this receptor would up-regulate integrins, as is the case for the chemokines SLC and SDF-1 (11), and thus further increase cell attachment to VCAM-1. Cells expressing CX3CR1 exhibited robust, fractalkine-dependent signaling as measured by phosphoinositol turnover, intracellular calcium mobilization, and chemotaxis, indicating that the wild-type receptor coupled well to G proteins. Up-regulation of integrins did not, however, appear to be an end point of fractalkine-mediated signal transduction, since soluble fractalkine did not increase the binding of cells to VCAM-1. Thus, unlike SLC, fractalkine does not activate the integrins. This result is consistent with the data of Campbell et al. (11), who reported that soluble fractalkine failed to increase the attachment of lymphocytes to ICAM-1, an adhesive interaction that is dependent upon the integrin αMβ2.

It was still possible, however, that when presented on the end of the mucin stalk, fractalkine could activate integrins. Our data suggest that this is not the case, however, because full-length fractalkine also failed to induce any additional binding of α5β1-expressing cells to VCAM-1. Taken together, these data suggest a model in which high-affinity binding of cells to fractalkine obviates the need for these cells to adhere through integrin-dependent mechanisms.

The molecular basis for the unique cell-adhesion properties of fractalkine is not yet clear. Other chemokines, such as MCP-1 and MIP-1α (11) and TARC and ELC (10), do not support the rapid capture of cells expressing the appropriate cognate receptors. However, it is possible that the these chemokines would have to be expressed at the end of a stalk, in a manner similar to the chemokine domain of fractalkine, in order for this to be a fair comparison. While no other known chemokines contain a mucin stalk or similar structure, the binding of chemokines to proteoglycans could be functionally equivalent to the presentation of fractalkine at the end of a stalk. Whether the remarkable ability of fractalkine to arrest cells flowing at high-shear rates is due to a unique property of the chemokine itself or to the manner in which it is presented is currently under investigation.

While this work was in progress, Fong et al. (10) showed that fractalkine mediated the capture of leukocytes under physiologic flow. Our results are consistent with those of Fong et al. (10) and further show that after mutation to remove all G-protein coupling, CX3CR1 functioned as well as the wild-type receptor in mediating cell adhesion. In addition, we show that activation of the wild-type receptor by fractalkine does not lead to up-regulation of integrins. Finally, we show that the adhesion of cells expressing CX3CR1 to fractalkine is much more rapid, and qualitatively different, from that mediated by integrins.

In summary, we have shown that fractalkine, a structurally unique chemokine, mediates the rapid capture of cells under physiological flow. The adhesion is not dependent upon cellular activation or signal transduction. Fractalkine is up-regulated on activated endothelial cells and is ideally placed to capture cells from rapidly flowing blood, such as is found in the cardio-

![Fig. 5. Time to capture of flowing 300-19 cells.](image)

**Fig. 5. Time to capture of flowing 300-19 cells.** 300-19 cells expressing CX3CR1 were perfused over antibody-tethered fractalkine (coating concentration, 100 nM) or over VCAM-1 (2 μg/ml) plus SLC (2 μM) at 1.5 dyn/cm² in a parallel-plate flow chamber. The mean time to capture was significantly less on fractalkine than on VCAM-1 plus SLC (60 ± 42 versus 190 ± 192 ms, p < 0.0001), as were the median (33 versus 150 ms) and range (17–217 versus 33–900 ms). Data shown are representative of four independent experiments that were analyzed.

![Fig. 6. Fractalkine does not activate integrin-mediated adhesion to VCAM-1.](image)

**Fig. 6. Fractalkine does not activate integrin-mediated adhesion to VCAM-1.** 300-19 cell lines stable expressing CX3CR1 were assayed for adhesion under flow (1.5 dyn/cm²). A, slides were coated with VCAM-1 (2 μg/ml) or antibody-tethered fractalkine (100 nM) or both. In addition, CX3CR1 cells were assayed for adhesion to VCAM-1 in the presence of soluble fractalkine (100 nM). The number of cells attaching to slides coated with fractalkine and VCAM-1 did not differ significantly from the sum of the number attaching to each individually. B, 300-19 cells stably expressing CCR7 were perfused over slides coated with VCAM-1 (2 μg/ml) alone or VCAM-1 and SLC (2 μM). EDTA (5 mM) was added to the cells before they were placed in the flow chamber. The asterisk indicates p < 0.05 versus SLC. Representative results from one of three assays are shown.
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nary arteries or the renal glomerulus. Whether fractalkine plays a role in the pathophysiology of diseases affecting these organs remains to be determined.

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