Unique Role of the Chemokine Domain of Fractalkine in Cell Capture

KINETICS OF RECEPTOR DISSOCIATION CORRELATE WITH CELL ADHESION*

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

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

The chemokine fractalkine (FK) has two structural features that make it unique in the chemokine family: a CX₃C motif and an extended carboxyl terminus that anchors it to the cell surface. This mucin-like stalk or an equivalent spacer is required for FK to mediate the adhesion of cells expressing its receptor, CX₃CR1. To determine whether the ability of FK to act as a cell adhesion molecule is due to the unique presentation of a chemokine domain on a stalk or to properties of the chemokine domain itself, we created a series of chimeras in which other soluble chemokines (RANTES (regulated on activation normal T cell expressed), monocyte chemoattractant protein 1, macrophage inflammatory protein 1β, secondary lymphoid tissue chemokine, and interleukin 8) were fused to the mucin stalk. When tested in a static-cell adhesion assay, many of these chemokine chimeras demonstrated activity equivalent to that of FK. In flow assays, however, none of the chimeras captured cells as efficiently as FK. Interestingly, FK captured cells expressing either CX₃CR1 or the viral receptor US28. Cells bound to FK without rolling or detaching, whereas the interleukin 8 and monocyte chemoattractant protein 1 chimeras induced primarily cell rolling and detaching, respectively. In binding studies, FK has a significantly slower off-rate from its receptors than any of the other chemokine chimeras had for their cognate receptors. We conclude that presentation of a chemokine atop a mucin-like stalk is not, in and of itself, sufficient to capture cells. The unique ability of FK to mediate adhesion under flow may be a function of its slow receptor off-rate.

In the development of the inflammatory response, leukocytes interact with vascular wall endothelial cells in a multistep, sequential process that includes rolling, firm adhesion, and diapedesis (1, 2). Firm adhesion is thought to be dependent on integrin binding, a process that can be regulated by the activity of the chemokine family of soluble proteins (3–6). However, recent studies have demonstrated that fractalkine (FK),¹ a novel member of the chemokine family, can capture leukocytes in an integrin-independent manner (7–9).

FK is a structurally unique molecule in which a chemokine domain is located atop a mucin stalk connected to a transmembrane domain (10). It is expressed on the surface of endothelial cells (10) and neurons (11) and is up-regulated by pro-inflammatory cytokines, such as lipopolysaccharide, interleukin-1, and tumor necrosis factor-α (9, 10, 12–14). We and others have recently shown that under physiological flow conditions FK efficiently captures leukocytes and cell lines transfectected with CX₃CR1, the FK receptor (7, 9). The cell capture function of FK is not dependent on integrin activation or on activation of G proteins by CX₃CR1 (7), but it is dependent on the presentation of the FK chemokine domain on a stalk (8, 9, 15). These studies revealed an unusual role for FK in directly mediating cell adhesion and raised the question of whether other chemokines presented in a similar manner would also directly support cell capture and adhesion.

To determine whether the ability of FK to act as a cell adhesion molecule was due to its unique presentation atop a rigid stalk or rather to properties of the FK chemokine domain itself, we created a series of chimeras in which other soluble chemokines were fused to the FK mucin stalk. In this study, we have examined the ability of these chemokine/stalk chimeras to capture cells expressing the appropriate cognate receptors in both static and flow adhesion assays.

EXPERIMENTAL PROCEDURES

Materials—The recombinant human chemokines monocyte chemoattractant protein 1 (MCP-1), interleukin 8 (IL-8), macrophage inflammatory protein 1β (MIP-1β), RANTES, secondary lymphoid tissue chemokine (SLC), full-length murine FK, and both soluble and full-length human FK were obtained from R & D Systems (Minneapolis, MN). ¹²⁵I-FK and ¹²⁵I-IL-8 were from PerkinElmer Life Sciences (Boston, MA). The antibodies for detection of chemokines were all from R&D Systems (Minneapolis, MN).

Experimental Design—The shuttle vector was generated containing the cDNA encoding the extracellular domain of FK with an additional carboxyl-terminal 6-His epitope. The cDNA for human FK was obtained from the American Type Culture Collection (ATCC, Manassas, VA; IMAGE clone 44145G). The primers 5′-ttgagggcggcactagctgctctttggtgtgg and 5′-tttggggctgttaagtatagtaatgtagatgggtcatggctgttggtg were used to add 5′-NotI and 3′-AscI.

Chemokine Chimera Constructs—A shuttle vector was generated containing the cDNA encoding the extracellular domain of FK with an additional carboxyl-terminal 6-His epitope. The cDNA for human FK was obtained from the American Type Culture Collection (ATCC, Manassas, VA; IMAGE clone 44145G). The primers 5′-ttgagggcggcactagctgctctttggtgtgg and 5′-tttggggctgttaagtatagtaatgtagatgggtcatggctgttggtg were used to add 5′-NotI and 3′-AscI.

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restriction endonuclease sites to the FK cDNA in a polymerase chain reaction (PCR). In addition, the 3′-primer introduced the amino acids KQN(His), and a stop codon, following glutamine 341, near the predicted transmembrane domain of FK. This product was inserted into the pcDNA.1 vector with the TA cloning kit (Invitrogen, Carlsbad, CA). The expression of the FK cDNA in each cell line with the chemokines, a unique BamHI restriction site was introduced at the sequence coding for the junction of the chemokine domain and the mucin stalk (after glycine 100). The primers were 5′-gcatctcgtaagttcgctgtgccggc-3′ and 5′-ctgtttctgaaggccgctttgaggcagggc-3′. The QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) was used to introduce conservative changes in the amino acid sequence. After replacing threonine 102 with serine, the modified FK was subcloned into JH1104R (a gift from R & D Systems). The DNAs for the other chemokines were PCR-amplified in the process adding flanking 5′-NotI and 3′-BamHI sequences and removing the stop codon. The cDNAs were obtained from the following sources: RANTES (ATCC number 105475), MIP-1β (ATCC number 185267), and MCP-1 (GenBank accession number X14768) were from ATCC, and SLC was a generous gift from Dr. Jason Cyster (University of California, San Francisco). cDNA for IL-8 was amplified from human tumors and used in reverse transcription-PCR with the Pro-Star first-strand reverse transcription-PCR kit (Stratagene). The primers used were 5′- and 3′- RANTES, 5′-aggaaaaaagaaggctgttctcggctgtgcgctgcggcggccgctaatatgatggc and 5′-cggcggatccagtcttcggagtttgggtttgc; MIP-1β, 5′-aggaaaaaagaaggctgttctcggctgtgcgctgcggcggccgctaatatgatggc and 5′-cggcggatccagtcttcggagtttgggtttgc; SLC, 5′-aggaaaaaagaaggctgttctcggctgtgcgctgcggcggccgctaatatgatggc and 5′-cggcggatccagtcttcggagtttgggtttgc; and IL-8, 5′-attgagaagctctgcgtgactgtccgctgcggcggccgctaatatgatggc and 5′-attgagaagctctgcgtgactgtccgctgcggcggccgctaatatgatggc.

These chemokines were inserted into the JH1104R shuttle vector in frame with the mucin stalk of FK. At R & D Systems, these constructs were subcloned into an insect cell expression vector, and protein was generated and purified using the polyclonistep epitope tag. All chimeras were tested for chemokine activity as described under “Results.”

Cell Culture and Transfection—The murine pre-B cell line (300-19) (16, 17) was a generous gift from Dr. G. La Rosa (Millennium Pharmaceuticals, Cambridge, MA). Cells were grown and transfected as described (17). The human CX3CRI receptor cell line was derived from the cDNA containing the Ade-745, Cyt-839 haplotype. This corresponds to the receptor isoform Ile-249, Thr-280 that has a reported affinity for FK (20 h, 10 ng/ml) of six histidines following FK Arg-340. The sizes of the different regions of six histidines following FK Arg-340. The sizes of the different regions include the signal peptide.

Calculation of Chemokine/Stalk Coating Density on Flow Adhesion Dishes—Flow adhesion dishes were coated as described above. The laminar flow chamber was placed on the dish, and the adhesion buffer (200 μl) was added. Cells were perfused over the coated dishes for 20 to 30 min to mimic the conditions used in the cell adhesion assay. The dish was then removed from the flow chamber, excess liquid was removed by aspiration, and vacuum grease (Dow Corning Corporation, Midland, MI) was “painted” around the substrate coated area with a cotton swab. α-Indole running buffer (bromphenol blue 0.004%), Tris base (120 mM, pH 6.8), glycerol (20%), SDS (6%), and 2-mercaptoethanol (10%) (30 μl) was added to solublize the chemokine/mucin stalk chimera coating the plate (15 min, room temperature). Samples were run on an 8% SDS-polyacrylamide gel and analyzed by Western blotting with the ECL system (Amersham Pharmacia Biotech, Piscataway, NJ). Primary antibodies were specific for the chemokine domain of each stalk chimera. All primary antibodies were used at 0.1 μg/ml except anti-SLC, which was used at 1.0 μg/ml. Secondary antibodies were selected to be specific to the primary antibody. Films of each gel were scanned, and the bands were analyzed by densitometry. The amount of chemokine protein was calculated by comparing the bands from solublized chemokine/stalk against standard concentrations of chemokine/stalk run on each gel.

Receptor Labeling with Chemokine/Stalk Constructs and Analysis by FACS—Cells were washed and resuspended at 10^6/ml in static adhesion buffer and then added to an equal volume of buffer containing the ligand (2× concentration of chemokine/stalk ± soluble chemokine) in a 96-well V-bottom microtiter plate (final volume, 100 μl) (Costar, Corning Inc., Corning, NY). Plates were incubated for 60 min at room temperature for ligand binding. When the assay was called for delayed addition of soluble chemokines, this was done at 30 min. All subsequent steps were performed on ice in FACS buffer (phosphate-buffered saline, bovine serum albumin (1%), sodium azide (0.1%) Cells were washed by spinning the plates (500 × g) for 3 min and then inverting and tapping the plate to remove the liquid from the cell pellet. Cells were labeled first with an antihistidinemonoclonal antibody (4 μg/ml, 60 min) and then with an anti-mouse fluorescein isothiocyanate-conjugated secondary antibody (1:100, 30 min) (Zymed Laboratories Inc.).

Chemokine Binding—Radioligand binding assays were performed as described (23) except that 1.25 × 10^5 cells were used for each data point, and binding was done at 4 °C for 24 h.

Static Analysis—Statistics for all assays were done with the Mann-Whitney test and Instat software (GraphPad Software, San Diego, CA) for Macintosh.

RESULTS

To determine whether or not the unique architecture of FK could completely explain its unusual ability to capture cells, we constructed a series of chimeras in which different chemokines were fused to the extracellular domain of the mucin-like stalk of FK (Fig. 1). Each chimera had a series of six histidine residues added to the extreme carboxyl terminus to immobilize the protein on glass slides coated with antihistidine antibodies.

**Fig. 1. Schematic of chemokine chimeras.** Chemokines were subcloned onto the extracellular domain of the FK mucin-like stalk. To generate a BamHI site, a threonine 102 to serine mutation was made in the FK sequence. A new carboxyl terminus was created by the addition of six histidines following FK Arg-340. The sizes of the different regions are indicated in amino acids (AA). The size of the chemokine domain includes the signal peptide.
A panel of 300-19 pre-B cells stably expressing epitope-tagged cognate receptors for FK and each of the chimeric chemokines was developed. Chemokine receptor expression was comparable in each of these cell lines, with the exception of US28, which was expressed at approximately half the level of the other receptors (Fig. 2). A binding isotherm using $^{125}$I-FK and the CX3CR1-expressing cell line revealed 1.4 $\pm$ 0.2 $\times$ 10$^5$ receptors/cell (not shown).

The ability of each chimera to interact with its receptor was first assessed by measuring agonist-dependent changes in intracellular calcium levels. Full-length human and murine FK (FK/stalk) and the smaller, soluble chemokine domain-only portion of FK were essentially equipotent in their ability to mobilize intracellular calcium in CX3CR1-transfected cell lines (Fig. 3A). Similar results were obtained for the MCP-1/stalk versus MCP-1 on CCR2-transfected cells (Fig. 3B), for the MIP-1$b$/stalk versus MIP-1$b$ on CCR5-transfected cells (Fig. 3C), and for SLC/stalk versus SLC on CCR7-transfected cells (Fig. 3D). IL-8 and IL-8/stalk gave similar results on CXCR1-transfected cells (not shown).

We began the adhesion studies by examining each of the chemokine/stalk chimeras in static assays (Fig. 4). FK captured cells expressing CX3CR1 and US28, but not cells expressing other chemokine receptors (e.g., CCR5), consistent with previous reports (8). The other chemokine/stalk chimeras varied in their ability to capture receptor-expressing cells. Some of these receptor-ligand pairs (MCP-1/stalk:CCR2; IL-8/stalk:CXCR1) were as potent as FK at mediating static adhesion. All pairs except MIP-1$b$/stalk:CCR5 mediated adhesion of cells above background levels. The ability to capture cells depended on the specific ligand-receptor pair. Thus, the MCP-1/stalk captured cells expressing CCR2 better than those expressing US28. Similarly, the RANTES/stalk captured cells expressing US28 better than cells expressing CCR1, even though CCR1 was expressed at a higher level than US28. In all cases, the addition of excess soluble chemokine (100 nM) reduced or eliminated adhesion of cells to the tethered chemokine (not shown). These data demonstrate that, under static conditions, at least a portion of the cell capture function of FK can be mimicked by chimeras that express other chemokine receptors. Some of these data demonstrate that, under static conditions, at least a portion of the cell capture function of FK can be mimicked by chimeras that express other chemokine receptors.

To determine whether the chemokine/stalk chimeras could capture cells under physiological flow conditions, we used a parallel-plate adhesion assay. At a shear rate of 0.8 dyn/cm$^2$, FK captured cells expressing CX3CR1 more efficiently than those expressing US28, and the RANTES/stalk chimera failed to capture cells expressing CCR1, CCR5, or US28 (Fig. 5A). Similar results were obtained for MCP-1/stalk and US28 cells, although some adhesion of CCR2 cells by MCP-1/stalk remained. In addition, the capture of CXCR1 cells by the IL-8/stalk remained robust. At a shear rate of 1.2 dyn/cm$^2$, however, only FK/stalk captured cells well (Fig. 5B). As previously demonstrated for FK-mediated adhesion of cells expressing CX3CR1, the IL-8/CXCR1 adhesion was not reduced by pertussis toxin treatment or by EDTA (not shown) (7, 8).

Next we attempted to determine the coating density of the chemokine/stalk chimeras presented on the antihistidine antibody. As assessed from Western blots, the calculated density of FK was approximately 400 molecules/μm$^2$ (60 pg/mm$^2$). The "active" concentration was probably lower, because it is unlikely that all the protein was presented in a functional form by the antibody. The other chimeras coated at similar densities. Assuming that the surface area of one face of an endothelial cell has an area of 10$^{-3}$ μm$^2$ (24), the density of FK in our assays would be equivalent to 4 $\times$ 10$^5$ molecules/cell. Although the actual density of FK on endothelial cells has not been reported, other adhesion proteins are expressed at comparable levels. For example, thrombin-activated endothelial cells express P-selectin at 50 molecules/μm$^2$ or a total number of 5 $\times$ 10$^4$ molecules/cell (25). It is also interesting to note that we can see adhesion, albeit with reduced numbers of cells, to FK coated at 10% of this concentration (not shown).

We observed during these flow adhesion assays that the behavior of captured cells depended on the specific chemokine/stalk substrate. We therefore further analyzed the interaction between the receptor-transfected cells and the chemokine/stalk chimeras. A single field of view in the flow chamber was selected, and cells that became tethered during an interval of 60 s were observed for an additional 30 s and classified as to whether they were arrested (remained stationary), rolled, or detached and re-entered the flow. Of the chemokine/stalk chi-
mers examined, only FK was able to capture and retain cells (Fig. 6). In the case of the IL-8/stalk chimera, the great majority of the cells that were “captured” subsequently rolled on the substrate. In the case of MCP-1/stalk and RANTES/stalk, cells adhered only briefly, and the majority detached during the observation period. Thus, only in the case of FK did cells attach and remain firmly adherent for the entire 30 s. In addition, both US28- and C3CR1-expressing cells showed a similar phenotype when interacting with FK (i.e., they arrested without rolling).

These data indicated that cell adhesion properties of FK were not simply due to the presentation of a chemokine-like domain at the top of a rigid stalk and thus suggested unique features of the interaction between FK and its receptor. To test this notion, we first asked whether cells expressing chemokine receptors could be “labeled” with the appropriate chemokine/stalk chimera. A variant of this approach, using epitope-tagged FK, has been used to evaluate receptor expression on cells (8, 9). FK/stalk bound to cells expressing both C3CR1 and US28 but not to wild-type, untransfected cells adhering to FK. FK-CCR5, mFK-US28, MIP-1α-CCR5, MCP-1-CXCR1, and IL-8-CXCR1. The others were significantly different (p < 0.05), indicating specific adhesion. Adhesion of US28-expressing cells to FK/stalk or RANTES/stalk was significantly greater than to MCP-1/stalk or mFK/stalk (p < 0.05).

Fig. 4. Static adhesion of chemokine receptor cells. 300-19 cells stably expressing chemokine receptors were assayed for adhesion to antibody-tethered chemokine/stalk chimeras. Cells were loaded with the fluorescent dye BCECF-AM and allowed to adhere for 30 min. Nonadherent cells were washed off, and the number of adherent cells was determined from the mean fluorescence in two wells. Each point represents an independent assay, and the bars represent the means. The following ligand receptor pairs showed no significant difference (p > 0.05) from wild-type, untransfected cells adhering to FK: FK-CCR5, mFK-US28, MIP-1α-CCR5, MCP-1-CXCR1, and IL-8-CXCR1. The others were significantly different (p < 0.05), indicating specific adhesion. Adhesion of US28-expressing cells to FK/stalk or RANTES/stalk was significantly greater than to MCP-1/stalk or mFK/stalk (p < 0.05).

Fig. 5. Flow adhesion by chemokine/stalk chimeras. 300-19 cells stably expressing chemokine receptors were perfused over antibody-tethered chemokine/stalk at a wall shear stress of 0.8 or 1.2 dyn/cm². Cells adhering after 3 min were counted in 10 or more fields, and the mean value was determined. Each point represents an independent assay, and the bars represent the means. A, 0.8 dyn/cm², there was no significant difference between FK and mFK in capturing CX3CR1-expressing cells. All others were significantly lower than FK-CX3CR1 (p < 0.005). B, 1.2 dyn/cm², all were lower than FK-CX3CR1 (p < 0.01), except US28, which could not be analyzed statistically, because it contains only one point.

We next asked whether the ability of FK to capture and retain cells under flow was due to a slow off-rate of the chemokine from its receptor. To address this question, we allowed the chemokine/stalk chimeras to bind to cells expressing the appropriate receptors and then added excess soluble chemokine to cells expressing both CX3CR1 and US28 but not to wild-type, untransfected cells (Fig. 7). The RANTES/stalk labeled cells expressing US28 but not label cells expressing either CCR1 or CCR5. MCP-1/stalk and SLC/stalk also failed to label cells expressing their cognate receptors, but IL-8/stalk did bind to cells expressing CX3CR1. These data indicated that the chemokine/stalk chimeras that supported cell adhesion under flow also bound to cells expressing the appropriate receptors with sufficient affinity to resist dissociation during the time and under conditions necessary to perform this flow cytometry assay.

In contrast, RANTES/stalk binding to US28 was almost completely eliminated by the addition of soluble RANTES, even after 30 min. Similar experiments could not be performed for the other chemokine/stalk-receptor pairs, because the initial binding affinity was too low (see Fig. 7).
DISCUSSION

In this study, we have created a series of chimeras in which soluble chemokines were fused to the mucin-like stalk of FK to test the hypothesis that the ability of FK to capture cells is due to the unique presentation of its chemokine-like domain. Under static conditions, a number of the chemokine/stalk chimeras bound cells expressing the cognate receptors. Under flow conditions, however, only FK/stalk and IL-8/stalk successfully captured cells. The majority of the CX3CR1-expressing cells rolled on the IL-8/stalk chimera and were not firmly adherent. Finally, the ability of FK to capture cells expressing CX3CR1 and US28 correlated well with the slow off-rate of the chemokine from these two receptors. We conclude that presentation of chemokines atop a rigid mucin-like stalk is not sufficient to confer the ability to capture cells under flow conditions.

Evidence from many groups supports a multistep model for leukocyte emigration from the bloodstream. The initial step is selectin-mediated rolling of unactivated cells along the vessel wall, followed by capture and diapedesis through the endothelium. One conclusion from the current study is that static adhesion assays are less discriminatory than simple flow assays in identifying molecules capable of capturing cells. Thus, under static conditions, human and murine FK/stalk, RANTES/stalk, and MCP-1/stalk had comparable abilities to bind cells, a result similar to that observed with various chemokine/stalk:receptor pairs (8, 26). However, under conditions of low shear, only the FK/stalk interactions remained robust, and all the other chemokine chimeras had significantly reduced adhesion. We were further able to distinguish between human and murine FK/stalk in their ability to capture cells at a slightly higher flow. Interestingly, the IL-8/stalk interaction represented cell rolling rather than firm adherence. Although rolling is typically attributed to selectin-mediated interactions, there...
is evidence that the integrin $\alpha_\beta_1$ (VLA-4) also mediates rolling (27). Similar to what was seen with $\alpha_\beta_2$, increased concentrations of IL-8/stalk induced firm adhesion, rather than rolling, in a subpopulation of the cells (not shown). Because IL-8 and other chemokines bind well to heparin sulfate proteoglycans, it is possible that they too contribute to rolling along the endothelium (28–30).

US28, a seven-transmembrane protein encoded by cytomegalovirus, is also a high-affinity receptor for FK (31). Although it is a promiscuous receptor that binds many chemokines, Kedal et al. (31) found that US28 binds FK preferentially with sub-nanomolar affinity and speculated that it has been “optimized” through evolution to promote viral entry through binding to FK on the host cell surface. Consistent with these data, we found that, although cells transfected with US28 adhered to many chemokines (FK/stalk, RANTES/stalk, MCP-1/stalk) under static conditions, only FK/stalk captured US28-expressing cells under flow conditions. Of the two receptors for FK, CX$_2$CR1 appeared to capture cells more efficiently than US28. A caveat to this conclusion, however, is that US28 was expressed at a somewhat lower levels than CX$_2$CR1 on the 300-19 cells. Whether or not the binding of US28 to FK actually contributes to the entry of cytomegalovirus into mammalian cells remains to be determined.

The three-dimensional structure of the chemokine-like domain of FK was recently solved by Mizoue et al. (32), and several features were found that distinguish it from the other chemokines whose structures are known. For example, FK exists as a monomer at high concentrations, and the CX$_2$C motif forms a “bulge” in the structure. It is not known if these structural features are important for FK’s unique ability to act as a cell adhesion receptor; however, the other chemokine/stalk chimera to support adhesion was IL-8, a CX$_4$ chemokine. It is also interesting to note that a potential N-glycosylation site is present within the CX$_2$C motif (AsNX(STP)) in human FK, but not in murine FK. In our studies, murine FK failed to support adhesion of US28-expressing cell lines. Mutation of this asparagine or treatment with N-glycosidases may reveal whether these functional differences are due to glycosylation of human FK in this critical region.

The ability of FK to capture and retain cells under flow conditions suggested that the off-rate from its receptor might be considerably slower than typical chemokine-receptor interactions. Indeed, preliminary experiments revealed that we could actually label cells expressing CX$_2$CR1 or US28 with epitope-tagged FK and that the binding was sufficiently robust to be easily detected by flow cytometry. Competition binding experiments revealed that, after 30 min at 22 °C, a substantial portion of the binding of FK/stalk to its receptor could no longer be displaced by soluble FK, whereas they competed equally for binding to the entry of cytomegalovirus into mammalian cells remains to be determined.

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It is important to note that cell adhesion represents the overall affinity of the cell for the immobilized ligand and is thus a much more complex parameter than the equilibrium dissociation constant. In general, however, the range of reported dissociation constants for FK/CX$_2$CR1 (30–740 pM) suggests that it has a higher affinity than the other chemokine/receptor pairs in this study (8, 15, 36). In our hands, chemokine/receptor pairs had dissociation constants as follows: FK/CX$_2$CR1 (100 ± 40 pM), IL-8/CX$_2$CR1 (1100 ± 400 pM), and MCP-1/CCR2 (260–650 pM) (17, 21). Others have reported a range of 1100–3600 pM for IL-8/CX$_2$CR1 and 35–680 pM for MCP-1/CCR2 (37–40). The wide range of values in the literature probably reflects differences in experimental systems and assay methods. It is possible, therefore, that FK’s unique ability to capture cells is due in part to a higher affinity for its receptor than those seen in other chemokine/receptor pairs.

In summary, we have shown that the ability of FK to act as a robust cell adhesion receptor is not simply due to its unique architecture, with prominent display of the chemokine-like domain. These results may be relevant to the question of whether the binding of other chemokines to heparin sulfate proteoglycans on the surface of endothelial cells functionally mimics FK. Although the basis for cell adhesion by FK is not revealed in these studies, it is clear that the chemokine-like domain is crucially important for this unique function. Whether or not this is due to its CX$_2$C motif remains to be determined.

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