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The role of fractalkine in the recruitment of monocytes to the endothelium

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Abstract

Recombinant fractalkine possesses both chemoattractive and adhesive properties in vitro. Previous studies have demonstrated an upregulation of this molecule on the membranes of activated human endothelial cells and hypothesised that fractalkine plays a role in the recruitment and adherence of monocytes to the activated endothelium. Here we present data analysing both the adhesive and chemoattractive properties of this chemokine expressed by activated human umbilical vein endothelial cells. We demonstrate that both recombinant fractalkine and endogenously produced fractalkine function as adhesion molecules, tethering monocytes to the endothelium. However, our data demonstrate that although recombinant fractalkine has the potential to function as a potent monocyte chemoattractant, the endogenous fractalkine cleaved from activated human umbilical vein endothelial cells is not responsible for the observed chemotaxis in this model. Instead, we show that monocyte chemoattractant protein-1 (MCP-1), secreted from the activated human umbilical vein endothelial cells, is responsible for the chemotaxis of these monocytes.

Keywords: Fractalkine; Chemokine; Chemotaxis; Adhesion; Umbilical vein, human; Endothelial cell

1. Introduction

Fractalkine is a recently described novel chemokine with a CX_3C motif that differs from other chemokines in that it comprises a chemokine domain (1-76AA) which sits on a heavily glycosylated mucin-like stalk (Bazan et al., 1997; Pan et al., 1997; Boddeke et al., 1999; Maciejewski-Lenoir et al., 1999). Fractalkine exists in two forms, as a membrane-associated protein with a cytoplasmic tail that has cell adhesion properties and as a cleaved, soluble form that is chemotactic for monocytes and lymphocytes in vitro (Bazan et al., 1997) and for neutrophils in vivo (Pan et al., 1997). The cell adhesion and chemoattractive properties of fractalkine are mediated via the CX_3CR_1 receptor which is a G-protein-coupled receptor that is expressed on T-lymphocytes, monocytes (Imai et al., 1997), natural killer cells (Al-Aoukaty et al., 1998), microglia (Harrison et al., 1998; Nishiyori et al., 1998) and hippocampal neurons (Meucci et al., 1998).

Activation of human umbilical vein endothelial cells for 24 h with tumour necrosis factor-α (TNF-α) or interleukin-1β leads to an increase in the expression of fractalkine message and membrane-bound protein (Bazan et al., 1997). Since fractalkine has cell adhesion and chemotactic activities, and the fractalkine receptor is expressed on a number of leukocytes, it has been suggested that fractalkine may play a role in the migration and adhesion of leukocytes to the endothelium.

In order to understand more about the role of fractalkine in the inflammatory event, a time course of fractalkine expression on endothelial cells was analysed. The results were then related back to the potential role of fractalkine in the recruitment and adhesion of monocytes.

In this report, we have evaluated the expression of fractalkine message and membrane-bound protein in activated human umbilical vein endothelial cells over a 24-h period. The ability of this transmembrane protein to adhere monocytes to the endothelial cells has subsequently been analysed. In addition, we have followed the cleavage of

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the chemotactic fractalkine protein from the endothelial cell membrane and have analysed the role of this cleaved protein in the chemotaxis of monocytic cells. Our data suggest that fractalkine plays a key role in the adhesion of monocytes to endothelial cells under these conditions, but surprisingly, the cleaved protein is not responsible for the monocytic chemotaxis observed.

2. Materials and methods

2.1. RNA preparation and reverse transcription real time polymerase chain reaction analysis (RT-PCR) of human fractalkine mRNA from human umbilical vein endothelial cells

RNA was prepared from cells lysed in Tri-reagent (Sigma, Dorset, UK) using 1 ml of Tri-reagent per 10 cm^2 plate. Total RNA was extracted from the tissue according to the manufacturer's suggested protocol with the addition of an extra chloroform extraction step and phase separation and an extra wash of the isolated RNA in 70% ethanol. The RNA was resuspended in autoclaved, double-distilled water and the concentration calculated by A_{260} measurement. RNA quality was assessed by electrophoresis on a 1% agarose gel.

First strand cDNA was synthesised using oligo$(dT)_{15}$ and 1 μg of each RNA sample: 0.01 M dithiothreitol, 0.5 mM each dNTP, 0.5 μg oligo$(dT)_{15}$ primer, 40 U RNAseOUT ribonuclease inhibitor (Life Technologies, Paisley, UK), 200 U Superscript II reverse transcriptase (Life Technologies). Triplicate RT reactions were performed along with an additional reaction in which the reverse transcriptase enzyme was omitted to allow for assessment of genomic DNA contamination of the RNA. Taqman PCR was carried out using an ABI prism 7700 sequence detector (Perkin Elmer, Cheshire, UK) under the following conditions: 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. The reaction mixture contained cDNA samples (1 μl of 20 μl RT reaction): 2.5 mM $MgCl_2$, 0.2 mM dATP, dCTP, dGTP and dUTP, 0.1 μM each primer, 0.05 μM Taqman probe, 0.01 U AmpErase uracil-*N*-glycosylase (Perkin Elmer), 0.0125 U Amplitaq Gold DNA polymerase (Perkin Elmer). Taqman primer and probe sets for human fractalkine and the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were designed using Primer Express software (Perkin Elmer). Primer and probe sequences (5′–3′) (forward primer, reverse primer, Taqman probe); GAPDH: GAACATCATCCCTGCATCCA, CCAGTGAGCTTCCCGTTCA, CTTGCCCACAGCCTTGGCAGC; hFractalkine: CTTCCTTGGCCTCTTCTG, CATCTCTCCTGCCATCTTTCGA, TTCACCTACCAGAGCCTCCAG; additional reactions were performed on each 96-well plate using known dilutions of human genomic DNA as a PCR template to allow construction of a standard curve relating threshold cycle to template copy number.

2.2. Cytokine stimulation of human umbilical vein endothelial cells (time course)

Normal human umbilical vein endothelial cells (Bio-Whittaker, Wokingham, UK) at passage four were seeded at a density of 1.5×10^5 cells/30 mm dish. Prior to cytokine stimulation, the culture media were replaced with serum-free media (1 ml); cells were then stimulated with TNF-α (25 ng/ml) for various lengths of time (0, 0.5, 1, 2, 6, 12, 24 h), with each time point represented in triplicate. After 24 h, conditioned media were harvested and cell lysates were prepared for either protein analysis or RNA isolation. For analysis of protein, cells were harvested in lysis buffer (100 μl) [NaCl (50 mM), Tris–HCl (pH 7.5) (20 mM), EDTA (5 mM), 0.1% Triton X-100, complete protease inhibitors; Boehringer Mannheim, East Sussex, UK]. Cells were harvested in Tri-reagent (Sigma) following the manufacturer's instructions for subsequent isolation of nucleic acids. Media samples were concentrated (×10) using Centriprep-3 filtration units (Amicon, Herts, UK).

2.3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting

Proteins from whole cell lysates and concentrated media samples were separated by SDS-PAGE (4–20% Tris–glycine gels; Novex, Germany) and transferred onto Immobilon-P™ membranes (Millipore, Herts, UK). Membranes were incubated in blocking buffer [phosphate-buffered saline (PBS), 0.05% Tween-20, 5% milk powder] at room temperature for 1 h and probed with a polyclonal antibody serum (raised in rabbit against the human fractalkine chemokine domain) for 1 h at room temperature. Washing was followed by a second 1 h, room temperature incubation with a horseradish-peroxidase-linked anti-rabbit Ig (Amersham, Bucks, UK) (1/20,000). Detection of the anti-rabbit Ig (immunoglobin) was carried out using enhanced chemiluminescence (ECL; Amersham).

2.3.1. Polyclonal antibody production

Rabbits were immunised subcutaneously with 100 μg of recombinant human fractalkine chemokine domain (R&D Systems, Oxon, UK) and Freund's complete adjuvant. Four weeks later, the animals were boosted with 100 μg antigen plus Freund's incomplete adjuvant via the same route; this was repeated at 8 weeks. At 12 weeks post-initial immunisation, the terminal bleed was carried out and serum harvested. The resultant antibody was used at a dilution of 1:500 in PBS-Tween and 1% foetal calf serum. Pre-incubating the antibody for 1 h at room temperature

with 10 μM recombinant human fractalkine (R&D Systems) prevented the antibody from recognising fractalkine on a Western blot.

2.4. Chemotaxis assays

Human monocyte isolation and chemotaxis assays were performed as described by Berkhout et al., (1997) and Turner et al., (1998). The chemotactic activity of human umbilical vein endothelial cell conditioned medium on human monocytes was determined in the absence and presence of a 30-min pre-incubation of the monocytes with an antibody to the CCR2 receptor (15 μg/ml CCR2 Mab 150; R&D Systems); the concentration of antibody employed is sufficient to completely inhibit the binding of monocyte chemoattractant protein-1 (MCP-1) to membranes of Chinese hamster ovary cells transfected with the human CCR2B receptor (Berkhout et al., 1997).

2.5. Determination of MCP-1 and interleukin-8 concentrations in activated human umbilical vein endothelial cells medium

The concentrations of MCP-1 and interleukin-8 in activated human umbilical vein endothelial cells medium were determined by enzyme-linked immunosorbent assay (ELISA) using quantikine kits for MCP-1 and interleukin-8 (R&D Systems). Analysis was performed according to the manufacturer's instructions. For assaying cell culture supernatants, samples were diluted 10-fold and plates were read at 450 nM (with correction at 540 nM) using a Spectramax 250 plate reader (Molecular Devices, Wokingham, UK).

2.6. Cell adhesion assays

The adherence of THP-1 cells (2×10^6/ml) to activated and non-activated endothelial cells was determined by seeding 70,000 human umbilical vein endothelial cells per well into Nunc 24-well plates. For TNF-α time course study, 30,000 cells were seeded overnight into black-sided 96-well plates (Polyfiltronics, MA, USA). The following protocol was then adhered to during both experiments: 24 h after seeding, human umbilical vein endothelial cells were activated with TNF-α (25 ng/ml) for different periods of time (0–24 h) prior to the addition (2×10^6/ml) of fluorescently labelled THP-1 cells or human monocytes. The cells were labelled with 10 μM calcein AM™ dye (Molecular Probes, Cambridge Bioscience, Cambridge, UK) for 20 min at room temperature; excess dye was removed by centrifugation of the cells and replacement of the media with PBS. The human umbilical vein endothelial cell conditioned media were aspirated and the cells were washed with PBS. The THP-1 cells were added to the human umbilical vein endothelial cells at 100 μl/well (96-well plate) or 500 μl/well (24-well plate) and incubated at 37°C for 45 min. The plate was washed by inversion and by the addition of PBS [250 μl/well (96-well plate); 1 ml/well (24 well plate)] followed by inversion. Calcein AM™ fluorescence was measured in a Fluorskan plate reader (Labsystems, Yorkshire, UK) using Ascent 2.2 programme to determine adherence; filter pair: Ex 485 Em 538.

3. Results

3.1. Time course of fractalkine mRNA levels in activated human umbilical vein endothelial cells

A profile of fractalkine mRNA expression by human umbilical vein endothelial cells, following TNF-α activation, was determined using quantitative RT-PCR. Significant levels of human fractalkine message were first detected in the human umbilical vein endothelial cells at 2 h post-activation with TNF-α (25 ng/ml), with maximal expression occurring between 6 and 12 h.

The fractalkine mRNA levels expressed as a percentage of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase, are shown in Fig. 1a. Parallel profiles were obtained using cyclophilin and acidic ribosomal phosphoprotein P0 as control genes (data not shown).

3.2. Western blot analysis of human fractalkine from activated human umbilical vein endothelial cells cultures

A profile of fractalkine protein expression following activation of human umbilical vein endothelial cells was also studied over the time course (shown in Fig. 1b and c). The data indicate that membrane-bound fractalkine (with an apparent MW ~ 100 kDa) is first detected 6 h after TNF-α stimulation, and is maximal at 12 h. The appearance of the soluble form in the human umbilical vein endothelial cell media (apparent MW ~ 95 kDa) lags behind the expression of the membrane-bound form, with first detection of the soluble fractalkine observed at 12 h.

3.3. Chemotaxis assays

Validation of the chemotaxis assay was carried out using recombinant human fractalkine (chemokine domain) in which the chemotaxis of THP-1 cells and human monocytes to the recombinant protein was analysed (Fig. 2a and b). THP-1 cells and monocytes chemotax to recombinant fractalkine with an EC_{50} of 0.24 ± 0.08 nM and 0.25 ± 0.03 nM, respectively, with maximal effect at 1 nM for each cell type; as with other chemokines, fractalkine appears to generate a bell-shaped dose–response curve (Fig. 2a and b).

Conditioned human umbilical vein endothelial cell media were analysed for chemotactic activity to provide a measure of the levels of monocyte chemoattractant pro-

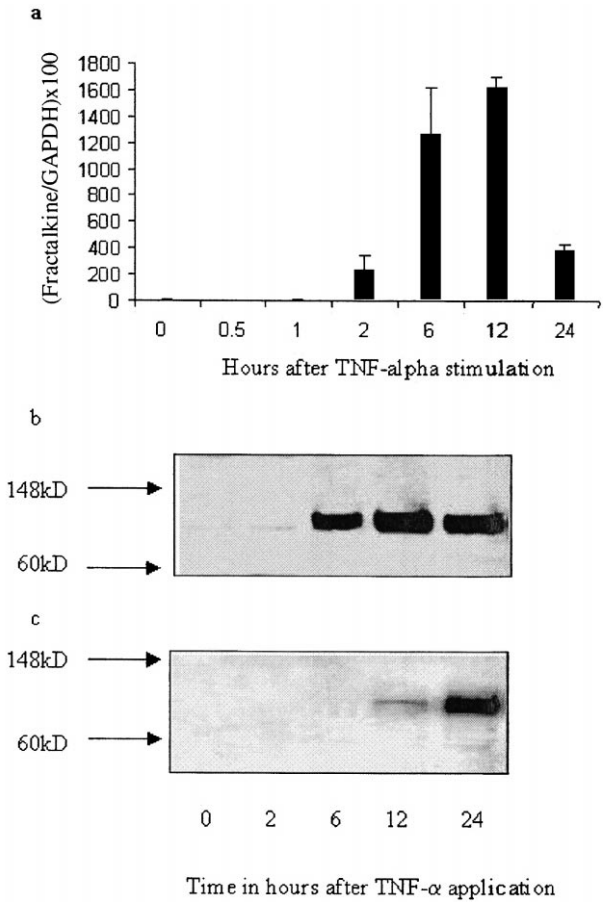

Fig. 1. Time course RT-PCR and protein analysis of human fractalkine from human umbilical vein endothelial cells stimulated with TNF-α (25 ng/ml). (a) Fractalkine message levels are expressed as a percentage of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase ($n = 3$ for all samples; error bars represent standard deviation). (b and c) Western blot analysis of human fractalkine from human umbilical vein endothelial cell cultures. The human umbilical vein endothelial cells were stimulated with TNF-α (25 ng/ml) for varying lengths of time before harvesting the conditioned media and whole cell lysates. Membrane-bound fractalkine was detected from human umbilical vein endothelial cells whole cell lysate (8 μg protein in each lane) (b). Soluble fractalkine was detected from human umbilical vein endothelial cell conditioned media (4 μg protein in each lane) (c).

teins secreted by the endothelial cells over the time course analysed. The ability of conditioned medium (collected at times 0.5, 1, 2, 6, 12 and 24 h after activation with TNF-α) to attract human monocytes is illustrated in Fig. 3a (filled bars). Increases in the chemotactic index for conditioned human umbilical vein endothelial cell media were observed for medium collected between 0.5 and 6 h with the maximum effect occurring at 2 h. The chemotactic effect of the human umbilical vein endothelial cell medium appears to decline at 6 h post-activation although it is clear from Figs. 1c and 3b that both fractalkine and MCP-1 continue to accumulate in the media until 24 h post-induction. This bell-shaped dose–response is typical of chemokine-induced chemotaxis and is also observed in Fig. 2a and b. It is thought that above an optimal concentration of chemokine, receptors become desensitised and a feedback inhibition of the biological mechanisms involved in locomotion results in inhibition of the response (Newton et al., 1997). It is of interest to note that this maximal chemotactic index of the medium occurs 10 h prior to the detection of soluble fractalkine protein by Western blot. Pre-incubating the monocyte cells with a blocking antibody against the MCP-1 receptor (CCR2) resulted in a complete inhibition of the chemotactic properties of the conditioned human umbilical vein endothelial cell media at all time points (Fig. 3a, open bars).

Analysis of the conditioned human umbilical vein endothelial cell media for other chemoattractive proteins revealed that both MCP-1 and interleukin-8 were present within the media and, not surprisingly, accumulated with time (Fig. 3b). MCP-1 concentrations reached 14 nM after 24 h; however, after 2 h, the maximum time for chemotactic activity, the human umbilical vein endothelial cell medium had concentrations of MCP-1 around 250 pM. The presence of MCP-1 at levels known to be active in vivo led us to believe that this chemokine, rather than fractalkine, was the predominant chemoattractant in the media (EC_{50} MCP-1 ~ 0.37 nM). This hypothesis was confirmed by antagonising the interaction of MCP-1 with its receptor (Fig. 3a). Although we have measured increasing levels of MCP-1 in the human umbilical vein endothelial cell medium with time, it must be noted that the CCR2 is also a functional receptor for MCP-2, 3 and 4; these chemokines may also potentially play a role in this system.

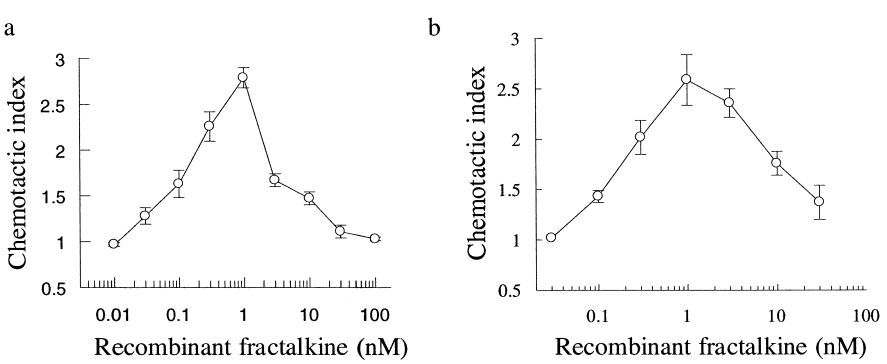

Fig. 2. Fractalkine-mediated chemotaxis of THP-1 and fresh human monocytes. Chemotaxis of THP-1 cells (a) and monocytes (b) induced by recombinant fractalkine–chemokine domain ($n = 3$; error bars represent standard deviations).

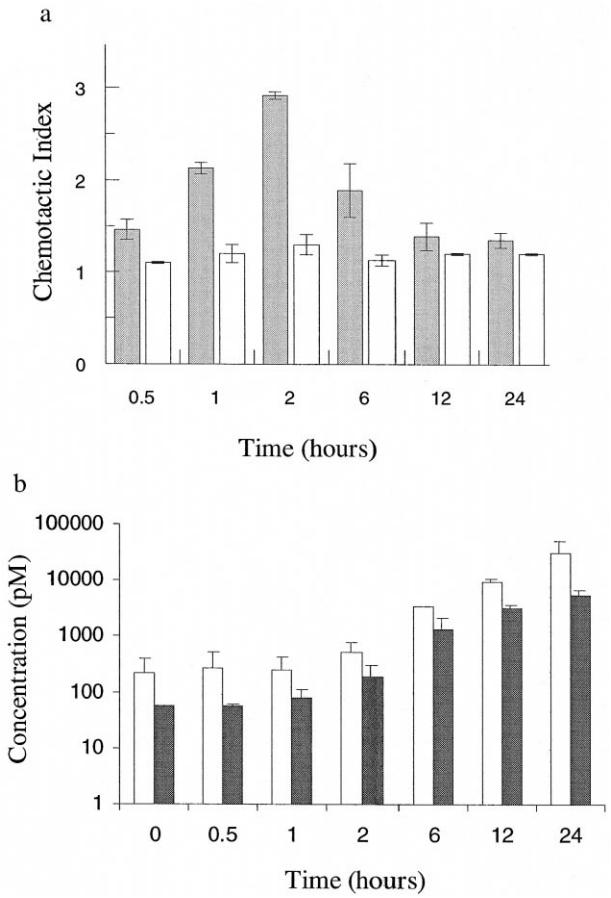

Fig. 3. Monocyte chemotactic activity and chemokine immunoreactivity in media from conditioned human umbilical vein endothelial cells. (a) Chemotaxis induced by medium collected from human umbilical vein endothelial cells at various times after activation with TNF-α (25 ng/ml) in the absence (filled bars) and presence (open bars) of monoclonal antibody (15 μg/ml) to the human CCR2 receptor ($n = 3$; standard deviation). The values of antibody-treated samples were significantly lower than those of untreated ones at all time points (t-test, $p < 0.05$). (b) Analysis of conditioned human umbilical vein endothelial cell medium for MCP-1 and interleukin-8. Determination by ELISA of interleukin-8 (dark bars) and MCP-1 concentrations (light bars) in human umbilical vein endothelial cell conditioned medium harvested at various times after activation with 25 ng/ml TNF-α ($n = 2$).

3.4. Cell adhesion assays

Fractalkine is a membrane-bound chemokine and has therefore been proposed to have cell adhesion properties. In order to analyse this possibility, THP-1 cells were incubated with human umbilical vein endothelial cells, which had been activated for 12 h with TNF-α (25 ng/ml), and adherence was then ascertained. Maximal adherence of the THP-1 cells to the monolayer of activated human umbilical vein endothelial cells was seen after a 45-min incubation; THP-1 cells did not adhere to non-activated human umbilical vein endothelial cells (Fig. 4a). Fig. 4b shows the adherence of THP-1 cells and human monocytes to human umbilical vein endothelial cells activated for increasing periods of time with TNF-α (25 ng/ml). The adherence of both cell types followed a very similar profile with significant adherence apparent at 4 h and maximal adherence occurring between 6 and 8 h post-activation of the human umbilical vein endothelial cells.

The ability of recombinant human fractalkine to block the interaction between membrane-bound fractalkine and the receptor on THP-1 cells was validated using an endothelial cell line stable expressing human fractalkine. Using these cells, it was not necessary to stimulate production of fractalkine with TNF-α. The non-transfected endothelial cell line (WT-ECV304) did not bind THP-1 cells (Fig. 5a). However, ECV304's stable expressing human fractalkine-bound THP-1 cells at significant levels and pre-incubation of the THP-1 cells with the recombinant protein (up to 3 nM) reduced adhesion by approximately 75% (Fig. 5a), $EC_{50} = 0.19$ nM.

The role of fractalkine in the adherence of human umbilical vein endothelial cells to monocytic cells was investigated by inhibiting the interaction between fractalkine and its receptor. This was attained by pre-in-

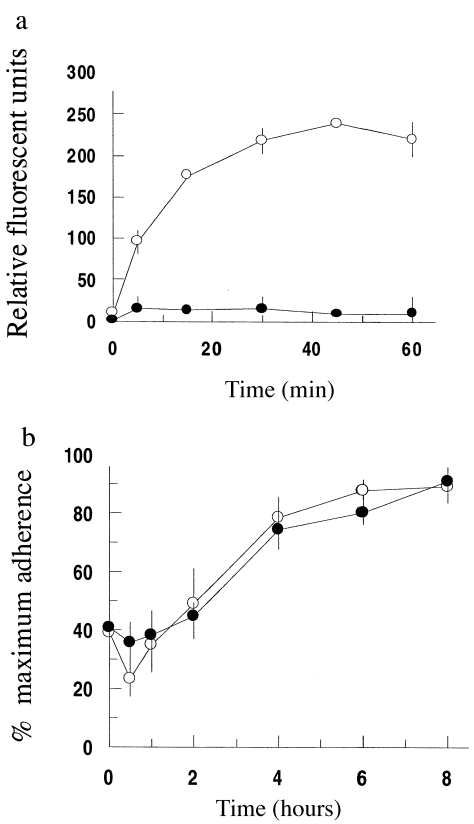

Fig. 4. Adhesion assays. (a) The adherence of THP-1 cells to activated (TNF-α, 25 ng/ml) (clear circles) and non-activated (filled circles) human umbilical vein endothelial cells was determined at various times after the addition of fluorescently labelled THP-1 cells to a monolayer of human umbilical vein endothelial cells. Experiments were carried out in a 24-well plate and represent the mean ± S.E.M. of four determinations. (b) The adherence of THP-1 cells (clear circles) and human monocytes (filled circles) to a monolayer of human umbilical vein endothelial cells activated for different periods of time with TNF-α (25 ng/ml). Data from one experiment (eight determinations) out of two are shown. Data are expressed as the percent maximum adherence (mean ± S.E.M.).

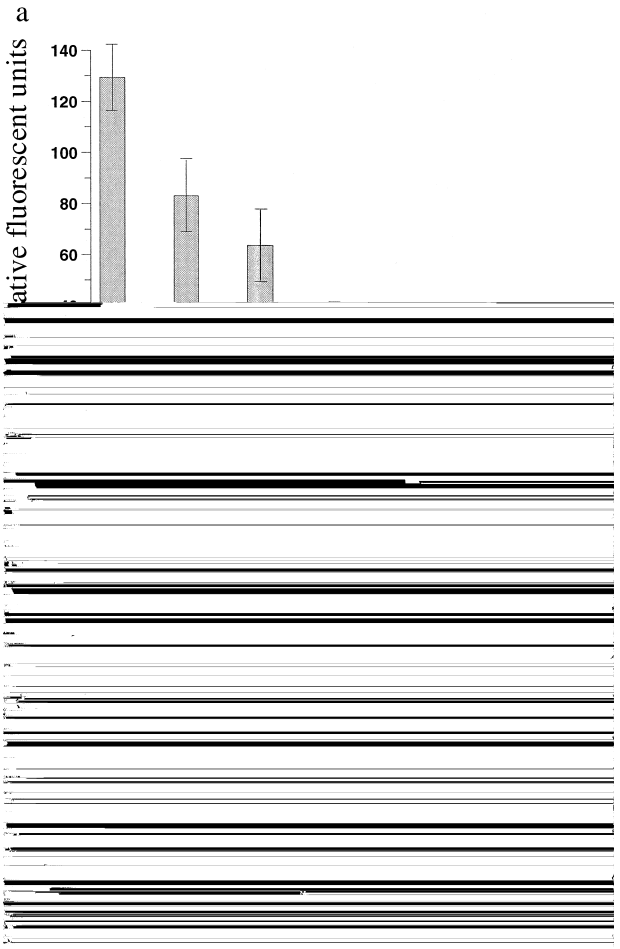

Fig. 5. Effect of pre-incubation of human monocytes with recombinant fractalkine on their adhesion to fractalkine-expressing cells. (a) Effect of increasing concentrations of recombinant human fractalkine (chemokine domain) on the adhesion of THP-1 cells to ECV304 cells stably expressing full-length human fractalkine. Filled bars represent stable clones; light bars represent WT ECV304 cells. (b) Effect of increasing concentrations of recombinant human fractalkine (chemokine domain) on the adhesion of THP-1 cells to TNF-α activated human umbilical vein endothelial cells. Filled bars represent human umbilical vein endothelial cells activated with TNF-α (25 ng/ml); light bars represent non-activated human umbilical vein endothelial cells.

cubating the THP-1 with an excess of recombinant human fractalkine protein (as determined above) to block the receptor sites on the THP-1 cells. Repeating the adhesion assay (human umbilical vein endothelial cells stimulated for 24 h) with the blocked receptor reduced the binding of the THP-1 to the fractalkine of the human umbilical vein endothelial cells significantly but not entirely (Fig. 5b). Post-activational binding was reduced by 30% when concentrations of recombinant fractalkine up to 100 nM were used. However, the level of binding was not reduced to the basal levels, which were seen when THP-1 cells were incubated with non-activated human umbilical vein endothelial cells. As expected, pre-incubating the THP-1 cells with recombinant fractalkine had no effect on the binding between THP-1 cells and non-activated human umbilical vein endothelial cells (m. 5b).

4. Discussion

The data presented in this communication demonstrate that fractalkine expression is upregulated by human umbilical vein endothelial cells after an inflammatory insult. Stimulation of these endothelial cells with the inflammatory cytokine, TNF-α, results in an increase in mRNA encoding fractalkine which is first detected 2 h post-insult. The RNA message levels encoding fractalkine peak at 12 h before decreasing dramatically by 24 h. The detection of full-length cell associated fractalkine parallels message levels; here, the protein is observed at 6 h post-insult, levels peak at 12 h before returning towards basal levels by 24 h. The soluble form of fractalkine present in the human umbilical vein endothelial cells conditioned media is first detected after 12 h in culture; as expected, this soluble protein accumulates in the media and is therefore maximal at 24 h post-stimulation. These data support previous hypotheses suggesting that full-length fractalkine is produced and directed to the membrane of endothelial cells following stimulation with TNF-α. The protein is then thought to be released from the cell membrane by a cleavage event. The time course of fractalkine mRNA expression and detection of both the full length and soluble forms of fractalkine support this.

There are two conflicting hypotheses as to why this cleavage occurs. The first suggests that the release of fractalkine from the membrane of the cell sets up a chemotactic gradient attracting peripheral blood leukocytes (such as monocytes and T-lymphocytes) to the endothelium where they are bound by the transmembrane form of fractalkine. A second possibility is that the cleavage of fractalkine from the membrane represents a ''terminating event'', removing fractalkine from the membrane and thus downregulating its adhesion properties; our data support this latter hypothesis since we show that fractalkine is cleaved from the cell but a role for this cleaved protein in subsequent chemotaxis events appears unlikely.

The increasing adherence of monocytes to activated human umbilical vein endothelial cells is mirrored by an increase in the levels of transmembrane fractalkine detected on the human umbilical vein endothelial cells. Blocking the fractalkine receptor on the monocytes by pre-incubation with recombinant human fractalkine (100 nM) reduced adhesion of these cells to human umbilical vein endothelial cells by ~30%. This work is supported by the findings of Imai et al. (1997) who showed that soluble recombinant fractalkine could reduce the adhesion of CX_3CR_1-transfected K562 cells to human umbilical vein endothelial cells (activated with TNF-α, 50 ng/ml, 10 h) cells by ~50%. Whereas soluble fractalkine could completely abolish the adherence of CX_3CR_1-transfected K562 cells to ECV-304 endothelial cells transfected with

fractalkine, our own unpublished observations would support these findings of Imai et al. (1997). Consideration of all these data strongly suggest that the fractalkine/CX_3CR_1 interaction is responsible for a significant fraction of the adhesion between human peripheral blood leukocytes and activated human endothelial cells.

The observed increases in the concentration of MCP-1 and interleukin-8 secreted into human umbilical vein endothelial cells medium shortly after activation with TNF-α may also be of importance in the light of work reported recently by Gerszten et al. (1999). This report described a role for MCP-1 and interleukin-8 in triggering the firm adhesion of monocytes to human umbilical vein endothelial cell monolayers expressing E-selectin. These proteins are therefore also likely to contribute to the adhesion in this system. Interestingly, a recent report by Feng et al. (1999) suggested a central role for the fractalkine/CX_3CR_1 interaction in the adherence of macrophages and $CD8^+$ T-lymphocytes to the endothelium of the renal glomeruli. It is currently accepted that chemokines present themselves on the vascular endothelium wall by binding to proteoglycans. This low-affinity interaction may not withstand the shear environment found in tissues with a high blood flow rate such as the renal glomerulus. Here, the contribution of a membrane-bound chemokine, such as fractalkine, in ''capturing'' and adhering leukocytes to the endothelium may be significantly elevated. Other chemokines, such as interleukin-8 and MCP-1, may be washed away under these high shear conditions.

Our findings indicate that MCP-1 is released from TNF-α-stimulated human umbilical vein endothelial cells prior to the release of soluble fractalkine. We also show that MCP-1 in the conditioned medium, rather than fractalkine, is responsible for monocyte chemotaxis. These data suggest that fractalkine does not play a critical role as a chemoattractant in the acute reaction to an inflammatory event at the endothelium.

However, we demonstrate that fractalkine does play a significant role as an adhesion molecule under the low shear conditions of this model. The unique structure of fractalkine, referred to in a recent review as a ''chemokine-on-a-stick'', may place fractalkine in a critical position among the chemokines in the recruitment of monocytes under conditions of high shear. Future studies will aim to dissect the relative contribution of fractalkine, and other adhesion molecules and chemokines, in leukocyte adhesion to the endothelium under conditions of increasing shear.

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