Interaction of Very Late Antigen-4 with VCAM-1 Supports Transendothelial Chemotaxis of Monocytes by Facilitating Lateral Migration[1]

Christian Weber[2]*† and Timothy A. Springer*

The transient regulation of very late antigen (VLA)-4 avidity by CC chemokines may promote chemotaxis of monocytes across VCAM-1-bearing barriers, whereas late and prolonged activation of VLA-5 may mediate subsequent localization in the extracellular matrix. We demonstrate that interactions of VLA-4 with VCAM-1, fibronectin, or a 40-kDa fragment but not a 120-kDa fragment of fibronectin supported the lateral random migration of isolated blood monocytes induced by CC chemokines, termed chemokinesis. This effect was optimal at intermediate substrate concentrations. Moreover, coimmobilization of VCAM-1 with ICAM-1 allowed better migration than ICAM-1 alone. Chemokinesis on VCAM-1 appeared to be associated with transient regulation of VLA-4 avidity by CC chemokines, given that locking VLA-4 in a high avidity state markedly inhibited migration and the locomotion rate was inversely correlated with the adhesive strength of VLA-4 to VCAM-1 following stimulation with monocyte chemoattractant protein-1. Induction of VCAM-1 expression by endothelial activation with IL-4 improved chemokinesis and lateral migration toward a monocyte chemoattractant protein-1 or a monocyte inflammatory protein-1α gradient on endothelium and increased transendothelial chemotaxis of monocytes by a VLA-4-dependent mechanism. In contrast, endothelial activation with IL-4 did not affect the time required for diapedesis of monocytes itself. Hence, VCAM-1 may facilitate transendothelial chemotaxis by supporting lateral migration of attached monocytes along endothelium. *The Journal of Immunology,* **1998, 161: 6825–6834.**

The multistep model postulates how transendothelial migration of leukocytes into sites of inflammation is regulated by sequential action of traffic signal molecules, displayed on activated endothelium (1, 2). Binding of vascular selectins to carbohydrate ligands initiates tethering and rolling of leukocytes (3). This can result in exposure to chemoattractants and chemokines that are released by blood and tissue cells or bound to proteoglycans in the endothelial vicinity (4, 5). Soluble or immobilized chemokines induce adhesion of leukocytes to endothelium or purified ligands, implicating their role in integrin activation (6–10). Integrins bind to Ig superfamily members on endothelium and can support rolling of lymphocytes and mediate firm arrest on activation (3, 11, 12). Chemokine gradients promote transendothelial diapedesis of arrested leukocytes into the tissue (7), stimulating directional motility that is thought to require reversible integrin-ligand interactions, since migration involves attachment and extension of the leading edge, lamellipodia formation, and detachment of the trailing end to allow cells to advance over a substrate (13).

Monocytes, eosinophils, and lymphocytes express the β_1 integrin very late antigen (VLA)-4[3] ($\alpha_4\beta_1$, CD49d/CD29) (14), which binds to domains 1 and 4 of VCAM-1, an Ig superfamily member induced by cytokines on endothelium (15–18). VLA-4 also binds to an alternatively spliced domain of the extracellular matrix (ECM) protein fibronectin, while VLA-5 ($\alpha_5\beta_1$) binds to a distinct domain containing the sequence RGD (19, 20). In vitro studies suggest that β_2 integrins and ICAM-1 are important in transendothelial migration of leukocytes; however, interactions of VLA-4 and VCAM-1 additionally contribute to migration across cytokine-stimulated endothelium (21–25). This is consistent with in vivo studies showing a role of VLA-4 in migration of monocytes and lymphocytes into inflammatory sites or cytokine-induced lesions, following up-regulation of endothelial adhesion molecules (26–28).

The cytoplasmic domain of α_4 has been described to exhibit specialized functional properties essential for extravasation; as compared with the cytoplasmic tail of α_5, it supported better migration and less adhesion strengthening and spreading in transfectants expressing chimeric α subunits (29). We have recently shown that CC chemokines sequentially regulate adhesiveness of VLA-4 and VLA-5 in monocytes (9). Transient activation and deactivation of the adhesive strength of VLA-4 to VCAM-1 precedes activation of VLA-5 avidity for fibronectin. Transient activation of VLA-4 may follow initial tethering and rolling of leukocytes through selectins and α_4 integrins and appears to facilitate transendothelial chemotaxis of monocytes (9), whereas the late and prolonged activation of VLA-5 may support interactions with the underlying basement membrane and with the ECM. However, it remains to be elucidated whether the mechanism by which VLA-4 facilitates transendothelial chemotaxis involves increases in migration of attached leukocytes along endothelium or in diapedesis through interendothelial cell junctions.

*Center For Blood Research and Department of Pathology, Harvard Medical School, Boston, MA 02115; and †Institut für Prophylaxe der Kreislaufkrankheiten, Klinikum Innenstadt, Ludwig-Maximilians-Universität, Munich, Germany

Received for publication April 21, 1998. Accepted for publication August 18, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by Deutsche Forschungsgemeinschaft Grant We-1913/2 to C.W. and by National Institutes of Health Grant CA31798 to T.A.S.

[2] Address correspondence and reprint requests to Dr. C. Weber, Institut für Prophylaxe der Kreislaufkrankheiten, Pettenkoferstrasse 9, D-80336 München, Germany.

[3] Abbreviations used in this paper: ECM, extracellular matrix; MIP, monocyte inflammatory protein; MCP, monocyte chemoattractant protein; VLA, very late antigen; FN40, 40-kDa fragment of fibronectin; FN120, 120-kDa fragment of fibronectin; HSA, human serum albumin.

0022-1767/98/$02.00

Here we demonstrate that the interaction of VLA-4 with VCAM-1, fibronectin, or a 40-kDa fragment of fibronectin (FN40) containing the CS-1 binding site for VLA-4 but not the binding of VLA-5 to a 120-kDa fragment of fibronectin (FN120) containing the RGD binding site support CC chemokine-induced lateral random migration (chemokinesis) of isolated blood monocytes. This appeared to be associated with the transient regulation of VLA-4 avidity by chemokines. The induction of VCAM-1 on endothelium facilitated lateral chemokinesis and transendothelial chemotaxis of monocytes by a VLA-4-dependent mechanism. The transient nature of VLA-4 avidity regulation by chemokines may promote transendothelial chemotaxis by facilitating lateral random migration along endothelium rather than diapedesis itself.

Materials and Methods

Reagents and mAbs

The human recombinant chemokines and cytokines monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), and IL-4 were from Genzyme (Cambridge, MA). Accudenz was from Accurate Chemicals (Westbury, NY). Other reagents were from Sigma (St. Louis, MO). The murine IgG myeloma X63 (isotype control), TS1/18 (CD18) (30), R6.5 (ICAM-1) (31), and CBR-IC2/2 (32) were purified with protein A. Purified TS2/16 (β_1 activating) (33) was a kind gift from Dr. M. E. Hemler (Dana-Farber Cancer Institute, Boston, MA). Purified HP1/2 (CD49d) (34) was a kind gift from Dr. R. Lobb (Biogen, Cambridge, MA), purified 1G11 (VCAM-1) was from Camon (Wiesbaden, Germany), My4 (CD14) (35) was from Coulter (Hialeah, FL), and CD32 mAb was from PharMingen (San Diego, CA).

Blood and endothelial cell isolation

Blood was collected from healthy donors and citrate anticoagulated. Leukocyte-rich plasma was prepared by 0.6% dextran T500 sedimentation of erythrocytes for 45 min at room temperature. Monocytes were separated from lymphocytes by Accudenz 1.068 hyperosmotic gradient centrifugation of leukocyte-rich plasma (9, 24). Platelets were removed from monocytes by four washes at 300 g for 7 min. This protocol yielded a population of 85% monocytes as analyzed by CD14 staining and light scatter. Monocytes were not activated by this isolation procedure, inasmuch as expression of L-selectin, which is rapidly shed during monocyte activation, was identical with that on the monocyte fraction in minimally processed whole blood and L-selectin-mediated functions under flow were maintained (9). Isolation and culture of HUVEC was performed as described (9).

Time lapse videomicroscopy

VCAM-1 (a kind gift from Dr. R. Lobb), FN40, FN120, or fibronectin (Life Technologies, Gaithersburg, MD) were coated onto 96-well plates at 4°C overnight at 1, 3, or 10 μg/ml, respectively, and unspecific binding sites were blocked with 1% heat-treated human serum albumin (HSA) for 2 h at 37°C. ICAM-1 (10) was coimmobilized with VCAM-1 or coated alone at 10 μg/ml. HUVEC were seeded into 96-well plates and were stimulated with or without IL-4 (500 U/ml) for 24 h. Isolated monocytes were kept on ice for no longer than 3 h, and 10^4 cells were added to wells in 40 μl of assay medium (HBSS, 10 mM HEPES, 1 mM Ca^{2+}, 1 mM Mg^{2+}, 0.5% HSA) with or without TS2/16 (10 μg/ml) or Mn^{2+} (1 mM). HUVEC were also grown on collagen-coated transparent 2.5-cm-diameter Transwell culture inserts (Costar, 8 μm pore size) and treated with or without IL-4 (500 U/ml) for 24 h, and MCP-1 was added to 2 ml of assay medium in six-well plates containing the inserts. Monocytes were resuspended at 2×10^5 cells/ml in assay medium (1:1 RPMI 1640-medium 199, 0.5% HSA), and 500 μl were added to inserts. Cells were allowed to settle for 10 min on the stage of a Nikon Diaphot (Nikon, Instrument Group, Melville, NY) microscope in a Plexiglas incubator box at 37°C and densities of $\approx$10 cells/40$\times$ high power field were obtained to avoid interactions between cells. After addition of MCP-1 or MIP-1α, cells were observed with phase contrast optics, and images were recorded for 30 min at a speed 12-fold slower than normal using a Panasonic AG-6730 time lapse video recorder (Panasonic, Secaucus, NY. Sufficient experiments were performed to obtain 30 cells for analysis. To trace individual monocyte paths, images were captured as digitized movies, and cell edges were enhanced, outlined, chronologically colorized, and projected over each other, using National Institutes of Health image software (Bethesda, MD). The image of a hemocytometer was used for size calibration. The coordinates of the cell centroids were determined, and the lengths of individual cell paths were quantitated as the sum of the linear distances between the centroids after every 1-min interval, representing a scalar parameter of cell migration. The locomotion rate of every cell analyzed was determined by dividing the length of its cell path by the time elapsed and was expressed as micrometers per minute. The mean $\pm$ SD of all individual cells and the medians of the sample distributions are given. As indicated, the data were statistically analyzed by nonparametric signed rank test according to Wilcoxon, or by analysis of variance by rank according to Kruskal-Wallis, where appropriate. Locomotion rates were also presented as the percentage of cells in velocity categories to reflect the distribution of cells over the range occurring under different conditions, as previously described for similar cell numbers (36).

Transendothelial chemotaxis assay

HUVEC were grown on collagen-coated 6.5-mm-diameter Transwell culture inserts (Costar, 8 μm pore size) and treated with or without IL-4 (500 U/ml) for 24 h. Transendothelial chemotaxis assays were as reported (9). Monocytes were incubated with 5% human serum or CD32 mAb (10 μg/ml) for 30 min to block Fc receptors and to prevent binding of monocytes via Fc receptors to mAbs added in inhibition experiments. Cells were washed and resuspended at 5×10^6 cells/ml in assay medium (1:1 RPMI 1640-medium 199, 0.5% HSA). MCP-1 or MIP-1α was added to 24-well tissue culture plates in 600 μl of assay medium. Transwells were inserted into wells, cells were added to the top chamber in a final volume of 100 μl, and monocytes were allowed to transmigrate for 1 h. Transmigrated and input monocytes were detached with 5 mM EDTA and counted with a FACScan (Becton Dickinson, Mountain View, CA) using forward and side scatter gates for monocytes. In each experiment, the ability of HUVEC to prevent upward diffusion of low m.w. FITC-dextran was assessed. For mAb inhibition studies, cells were preincubated with mAb to β_2 (TS1/18, 20 μg/ml), VLA-4 (HP2/1, 10 μg/ml), or isotype control (X63, 20 μg/ml) for 20 min on ice. Data were statistically analyzed using analysis of variance, where indicated.

Flow cytometry

Monocytes were washed and reacted for 30 min with mAb on ice, stained with a 1:20 dilution of goat anti-mouse IgG FITC (Zymed, San Francisco, CA), and subjected to scatter-gated flow cytometry in a FACScan. Concentrations of mAb used for inhibition were saturating (data not shown). Surface expression of ICAM-1, ICAM-2, and VCAM-1 was analyzed by staining of HUVEC with mAb as described (37). After subtraction of unspecific staining, specific fluorescence intensity was expressed in channels.

Quantification of MCP-1 protein

The MCP-1 concentration in HUVEC supernatants was quantitated using a sandwich ELISA (R&D Systems, Minneapolis, MN). Supernatants were collected from resting and IL-4 (500 U/ml, 24 h)-stimulated HUVEC and sterile filtered. An ELISA was performed according to the manufacturer's protocols using protein standards provided.

Results

VLA-4 but not VLA-5 ligands support lateral random migration of monocytes

Transient regulation of VLA-4 avidity by CC chemokines has been suggested to promote chemotaxis of monocytes across VCAM-1-bearing barriers, whereas prolonged activation of VLA-5 may mediate subsequent localization within the extracellular matrix (9). To elucidate the mechanism by which interactions of VLA-4 and not VLA-5 with their ligands may support transendothelial chemotaxis, we studied lateral random migration of monocytes on VCAM-1 and the fibronectin fragments FN40 or FN120. On VCAM-1 (3 μg/ml), unstimulated monocytes showed spreading and spontaneous random migration (Fig. 1*A* and data not shown), and the percentage of cells moving $>$30 μm within 30 min was 30% (Fig. 2*A*). CC chemokines induced a marked cellular shape change with formation of leading filopodia and lamellipodia (not shown). MCP-1 and MIP-1α induced lateral migration in random directions (chemokinesis) (Fig. 1*B* and data not shown), with 86% and 80% of cells crawling $>$30 μm within 30 min, respectively

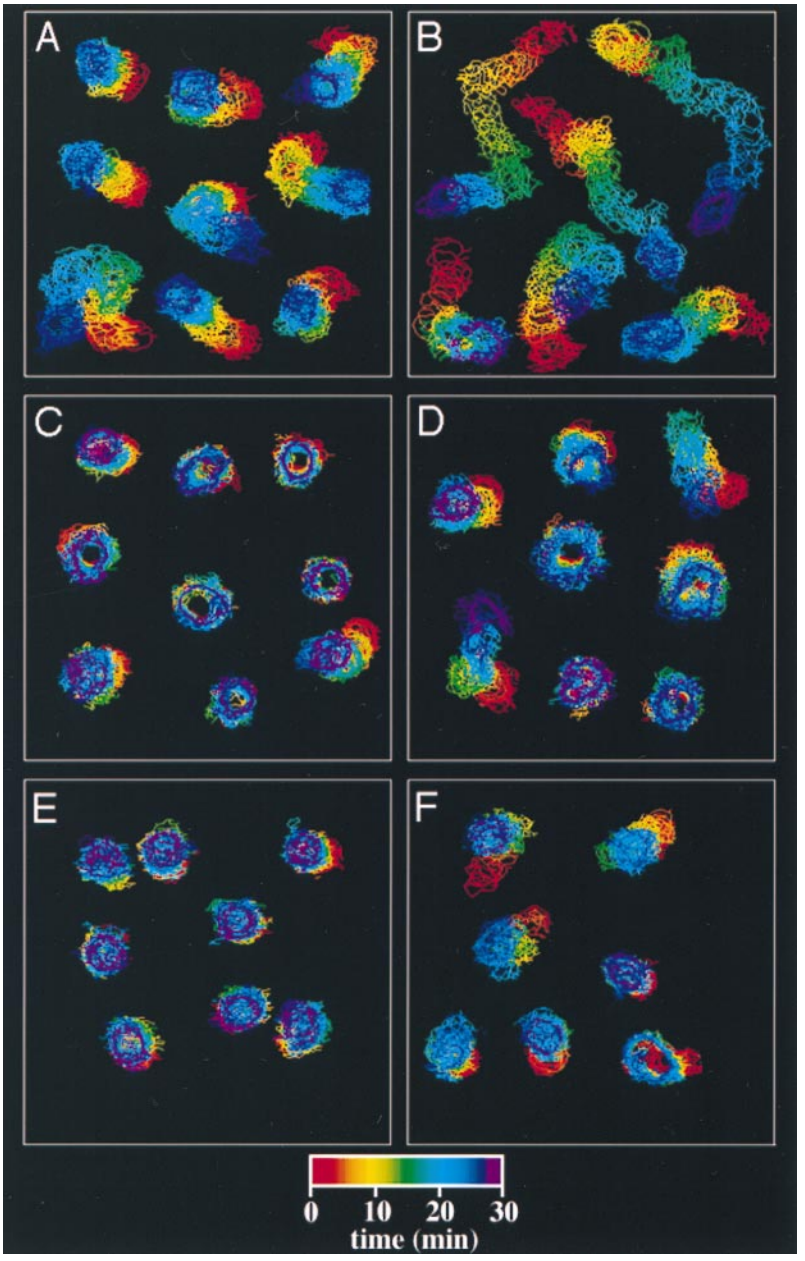

FIGURE 1. Random migration of monocytes on VCAM-1 or a 120-kDa fibronectin fragment (FN120). The random movement of monocytes in the absence (*A, C, E*) or presence (*B, D, F*) of MCP-1 (1 ng/ml) and after treatment without (*A, B, E, F*) or with (*C, D*) TS2/16 mAb (10 μg/ml) was recorded on VCAM-1 adsorbed at 2.5 μg/ml (*A–D*) or FN120 (*E, F*) adsorbed at 10 μg/ml over 30 min using time lapse video microscopy. Cell movement is indicated by traces of cell outlines in different colors corresponding to the elapsed time. The patterns shown are representative of three independent experiments.

(Fig. 2, *B* and *C*). The activating β_1 mAb TS2/16 or Mn^{2+} strongly inhibited spontaneous and MCP-1-induced random migration, restricting it to the area of cell attachment (Fig. 1, *C* and *D*; Fig. 2, *D–F*; and data not shown). This indicates that locking VLA-4 in a high avidity state impaired chemokinesis.

Similar results were seen on FN40 (10 μg/ml) which contains the binding site for VLA-4, with 35% of unstimulated cells and 70 or 65% of cells stimulated with MCP-1 or MIP-1α, respectively, moving for >30 μm within 30 min (Fig. 2, *J–L*). This indicates that migration was not restricted to one VLA-4 ligand and suggests that it is a function of the integrin. By comparison, attachment to FN120 (10 μg/ml) which contains the binding site for VLA-5, resulted in diffuse spreading but extension of few filopodia or lamellipodia in unstimulated or MCP-1-induced cells (not shown). Furthermore, unstimulated monocytes showed little spontaneous random migration on FN120 (Figs. 1*E* and 2*G*), and chemokinesis was hardly stimulated by MCP-1 or MIP-1α (Figs. 1*F* and 2, *H* and *I*).

MCP-1-induced chemokinesis on VCAM-1 and fibronectin is mediated by VLA-4 and optimal at intermediate substrate densities

To further define differential contributions of VLA-4 and VLA-5, we studied lateral random migration on intact fibronectin and VCAM-1 coimmobilized with ICAM-1. On fibronectin (3 μg/ml), unstimulated monocytes showed little random migration, with 14% of cells moving >30 μm within 30 min. MCP-1 induced chemokinesis, with 47% of cells moving >30 μm within 30 min ($p < 0.05$ vs unstimulated monocytes, Fig. 3, *A* and *B*). This increase in migration induced by MCP-1 was completely inhibited by mAb HP1/2 to α_4 ($p < 0.05$ vs isotype control) so that motility resembled that on FN120 or that without MCP-1, with 13% of cells crawling >30 μm within 30 min (Fig. 3*C*). By contrast, a mAb to α_5 increased locomotion rates ($p < 0.05$ vs isotype control, Fig. 3*C*). On ICAM-1 (10 μg/ml), migration of monocytes was induced by MCP-1 to an extent comparable to that on fibronectin and was

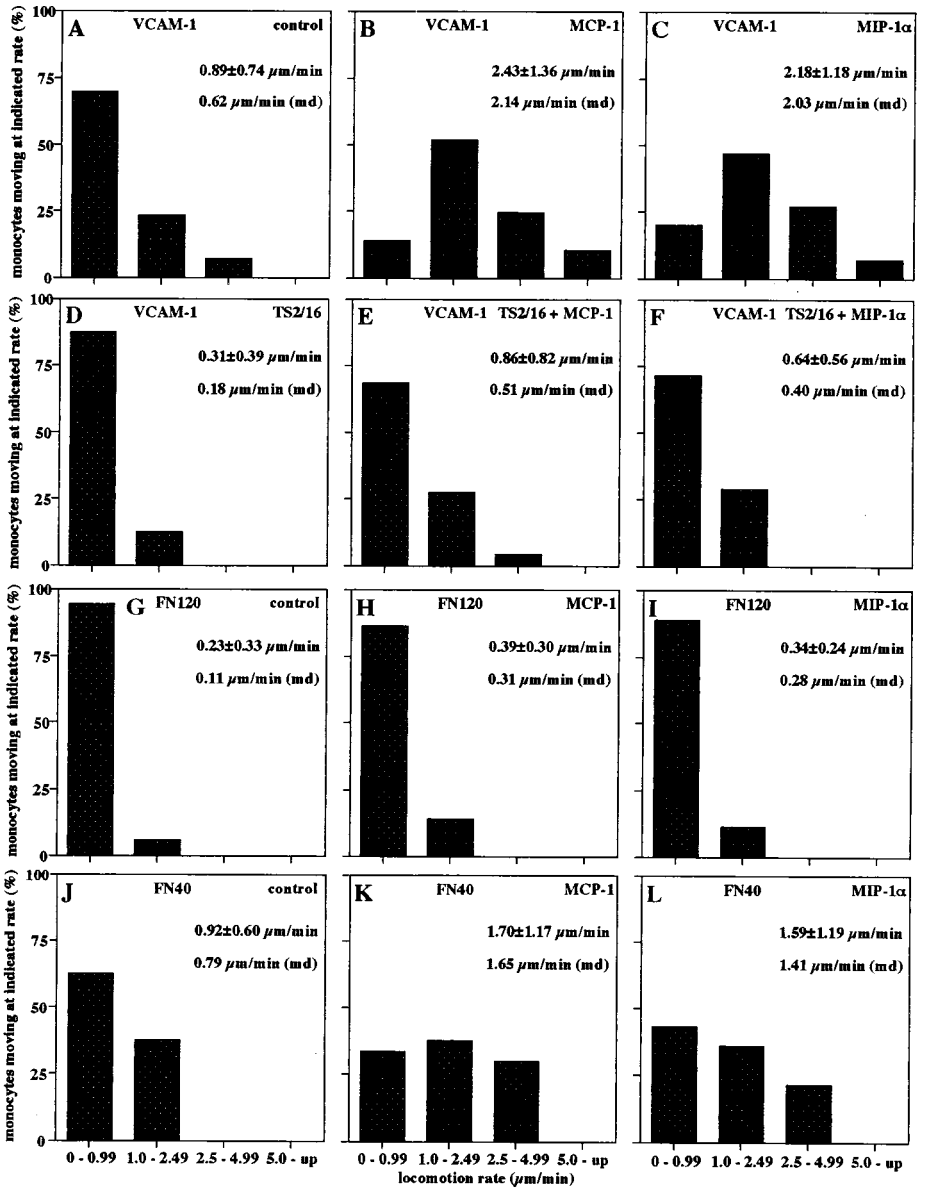

FIGURE 2. Random migration of monocytes on VCAM-1, a 40-kDa (FN40) or 120-kD fragment (FN120) of fibronectin. Random movement of monocytes was recorded on VCAM-1 (*A–F*) adsorbed at 2.5 μg/ml, FN40 (*G–I*) or FN120 (*J–L*) adsorbed at 10 μg/ml in the absence (*A, D, G, J*) or presence of MCP-1 (1 ng/ml, *B, E, H, K*) or MIP-1α (10 ng/ml, *C, F, I, L*) over 30 min using time lapse video microscopy. Some cells (*D–F*) were treated with TS2/16 mAb (10 μg/ml). The lengths of individual cell paths were determined by adding up the distances between the centroids of cell areas after after every 1-min interval, and locomotion rates within 30 min were calculated. Data are expressed as the percentage of cells moving at an indicated locomotion rate or as average locomotion rate (mean ± SD). Medians (md) are also given. For every condition, analysis included 30 cells recorded in at least three independent experiments.

not impaired by α_4 mAb (Fig. 3, *D–F*). Since adhesion is a prerequisite for migration, the ICAM-1 substrate represents an appropriate control in which neither fibronectin nor VCAM-1 was included. Coimmobilizing VCAM-1 with ICAM-1 did not affect migration of unstimulated monocytes but markedly improved chemokinesis of MCP-1-stimulated monocytes as compared with ICAM-1 alone (Fig. 3, *G* and *H*). Similar to results seen on fibronectin, addition of mAb HP1/2 to α_4 decreased chemokinesis on VCAM-1 coimmobilized with ICAM-1 to levels found on ICAM-1 alone (Fig. 3*I*). Thus, migration of monocytes on fibronectin or VCAM-1 was clearly mediated by interactions with VLA-4 and not VLA-5. We next tested whether the rate of MCP-1-induced chemokinesis was dependent on the concentration at which the substrate was adsorbed (Table I). Our results indicate that the locomotion rates were optimal at intermediate substrate concentrations of VCAM-1 and impaired at lower and higher substrate concentrations. Similarly, however, less marked effects of the substrate concentration were seen on fibronectin. In contrast, the chemokinetic locomotion rate on FN120 was low and was little altered over a 10 -fold range of FN120. Thus, our data suggest that intermediate site densities of VCAM-1 or fibronectin appeared to optimally support VLA-4-dependent chemokinesis of monocytes.

The locomotion rate of MCP-1-stimulated monocytes on VCAM-1 is time-dependent and may be inversely correlated with the adhesive strength of VLA-4

The kinetics of VLA-4 regulation by MCP-1 showed transient activation of adhesive strength to VCAM-1 with a peak at 10 min (9). Hence, we analyzed the locomotion rate of MCP-1-stimulated monocytes at different time points to correlate migration with the kinetics of avidity regulation. Interestingly, the mean locomotion rate on VCAM-1 was markedly higher in the first 6 min (Fig. 4*A*) and between 13 and 25 min (Fig. 4*C*) than between 6 and 13 min of stimulation (Fig. 4*B*). The percentage of cells crawling at >1 μm/min was 93% in the first 6 min, 64% between 7 and 12 min, and 93% between 13 and 25 min. Thus, the locomotion rates appeared to be inversely correlated to the adhesive strength for VCAM-1. In particular, deactivation of VLA-4 at later time points appeared to favor the motility of monocytes on VCAM-1. These

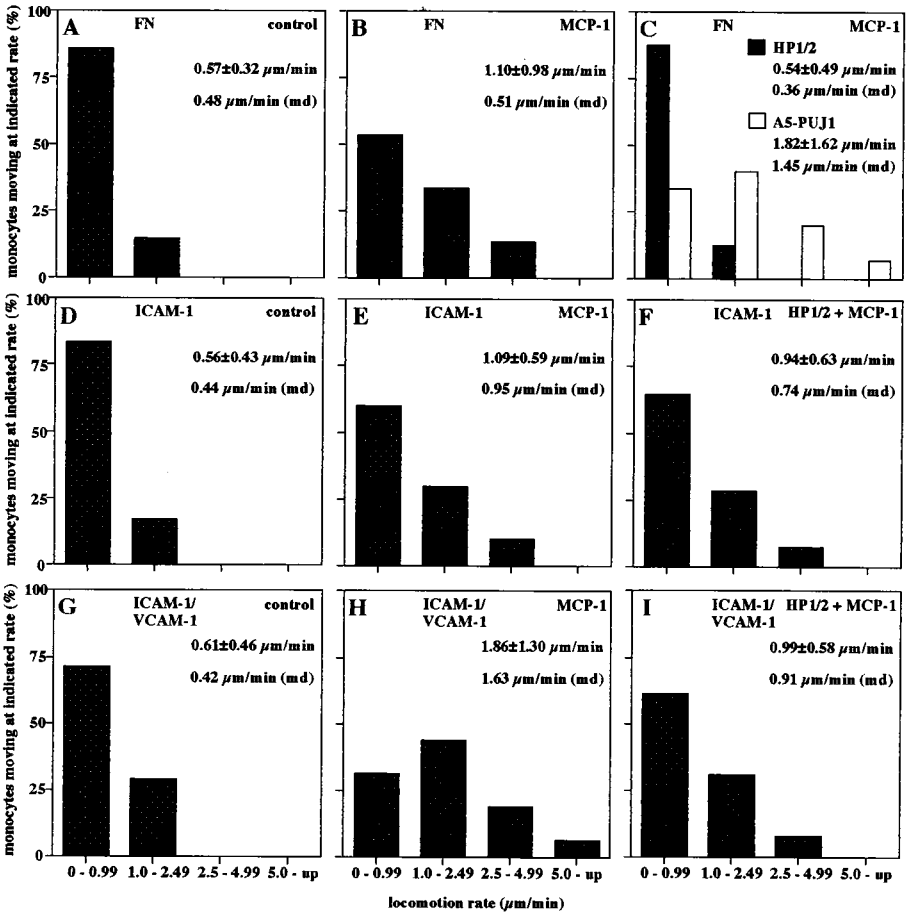

FIGURE 3. Random migration of monocytes on fibronectin or VCAM-1 is VLA-4 mediated. Random movement of monocytes pretreated with isotype control (*A, B, D, E, G, H*), mAb to α_4 (*C, F, I*), and α_5 (*C*) was recorded on fibronectin adsorbed at 2.5 μg/ml (*A–C*), ICAM-1 at 10 μg/ml (*D–F*), or ICAM-1 and VCAM-1 coadsorbed at 10 and 2.5 μg/ml (*G–I*), respectively, in the absence (*A, D, G*) or presence of MCP-1 (1 ng/ml, *B, C, E, F, H, I*) over 30 min using time lapse video microscopy. The lengths of individual cell paths were determined by the sum of the distances between cell centroids, and locomotion rates within 30 min were calculated. These data are expressed as the percentage of cells moving at indicated locomotion rates or as mean ± SD. Medians (md) are also given. Analysis included 30 cells recorded in at least three independent experiments.

data implicate the transient regulation of VLA-4 avidity in the induction of lateral migration of monocytes by MCP-1.

Induction of endothelial VCAM-1 increases VLA-4-dependent chemokinesis on endothelium and transendothelial chemotaxis of MCP-1-stimulated monocytes

To study how interactions of VLA-4 with VCAM-1 may facilitate migration across endothelium, we analyzed lateral random migration on resting and IL-4-activated HUVEC monolayers. Resting HUVEC expressed ICAM-1 and ICAM-2 but not VCAM-1 surface protein (Table II). Treatment of HUVEC with IL-4 for 24 h induced expression of VCAM-1 but did not affect that of ICAM-1 or ICAM-2 (Table II) (38, 39). Only few unstimulated monocytes crawled >30 μm in 30 min on resting or IL-4-activated HUVEC, indicating that random migration in the absence of chemokine was not altered by IL-4 activation (Fig. 5, *A* and *B*). MCP-1 stimulated the migration of monocytes on resting HUVEC ($p < 0.001$ vs unstimulated monocytes) with 44% of cells moving >30 μm in 30 min (Fig. 5*C*). On IL-4-activated HUVEC, MCP-1 more markedly increased migration of monocytes ($p < 0.0001$ vs unstimulated monocytes) with 63% of cells crawling >30 μm in 30 min (Fig. 5*D*). VLA-4 mAb only slightly reduced the locomotion rate of monocytes on resting HUVEC but markedly inhibited chemokinesis of monocytes ($p < 0.05$ vs isotype control) on IL-4-treated HUVEC (Fig. 5, *E* and *F*) to levels comparable with those seen on resting HUVEC (compare Fig. 5, *C* and *F*). The chemokinesis of monocytes that could still be observed on resting and IL-4-activated HUVEC after treatment with α_4 mAb was further blocked by a mAb to β_2 (Fig. 5, *G* and *H*). Induction of VCAM-1 expression by IL-4 may thus facilitate migration along activated endothelium by a VLA-4-dependent mechanism.

To show that VCAM-1 expression also supports chemotactic motility of monocytes, we studied transendothelial chemotaxis. Chemotaxis of monocytes to MCP-1 across resting HUVEC was mainly mediated by β_2 integrins with only a slight contribution of VLA-4, as demonstrated by mAb inhibition (Fig. 6*A*) (9). Treatment of HUVEC with IL-4 for 24 h did not induce a significant up-regulation of MCP-1 secretion (Table II) (40); hence, it did not appear likely to interfere with the MCP-1 gradient. Activation of HUVEC with IL-4 for 24 h, which induced VCAM-1, increased transendothelial migration of monocytes by up to 70% ($p < 0.05$, Fig. 6*B*). Inhibition with VLA-4 mAb revealed that the increase in chemotaxis was mediated by VLA-4 (Fig. 6*B*). Pretreatment with

Table I. *Effect of substrate density on locomotion rate of MCP-1 stimulated monocytes*[a]

	Locomotion Rate (μm/min) at Substrate Density		
	1 μg/ml	3 μg/ml	10 μg/ml
VCAM-1	1.38 ± 1.03	2.43 ± 1.36*	1.13 ± 0.52
Fibronectin	1.02 ± 1.01	1.10 ± 0.98	0.63 ± 0.44
FN120	0.49 ± 0.37	0.51 ± 0.30	0.39 ± 0.30

[a] The random movement of monocytes stimulated with MCP-1 (1 ng/ml) was recorded on VCAM-1, fibronectin, or FN120 adsorbed at indicated site densities over 30 min using time lapse video microscopy. The lengths of individual cell paths were determined by adding up the distances between centroids of cell areas after every 1-min interval, and locomotion rates were calculated within 30 min. Data are expressed as average locomotion rate (mean ± SD). For all conditions, 30 cells were recorded in at least three independent experiments. * $p < 0.01$.

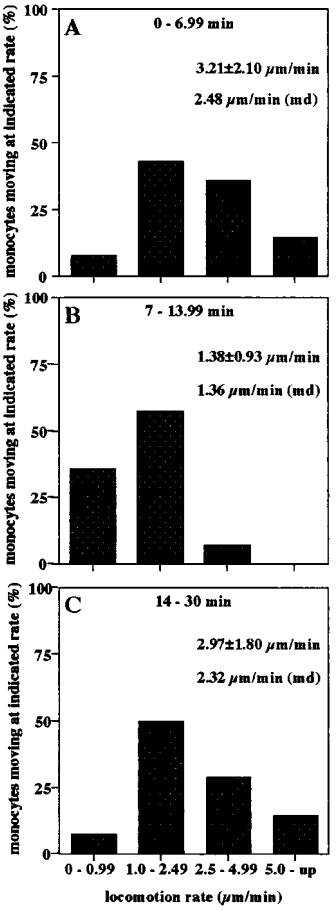

FIGURE 4. Time course of random migration of monocytes on VCAM-1. Random movement of monocytes was recorded on VCAM-1 adsorbed at 2.5 μg/ml in the presence of MCP-1 (1 ng/ml) over 30 min using time lapse video microscopy. The lengths of individual cell paths were determined by the sum of the distances between cell centroids after every 1-min interval, and locomotion rates for the intervals 0–6.99 min (*A*), 7–13.99 min (*B*), or 14–30 min (*C*) were calculated. These data are expressed as the percentage of cells moving at indicated locomotion rates or as mean ± SD. Medians (md) are also given. Analysis included 30 cells recorded in at least three independent experiments.

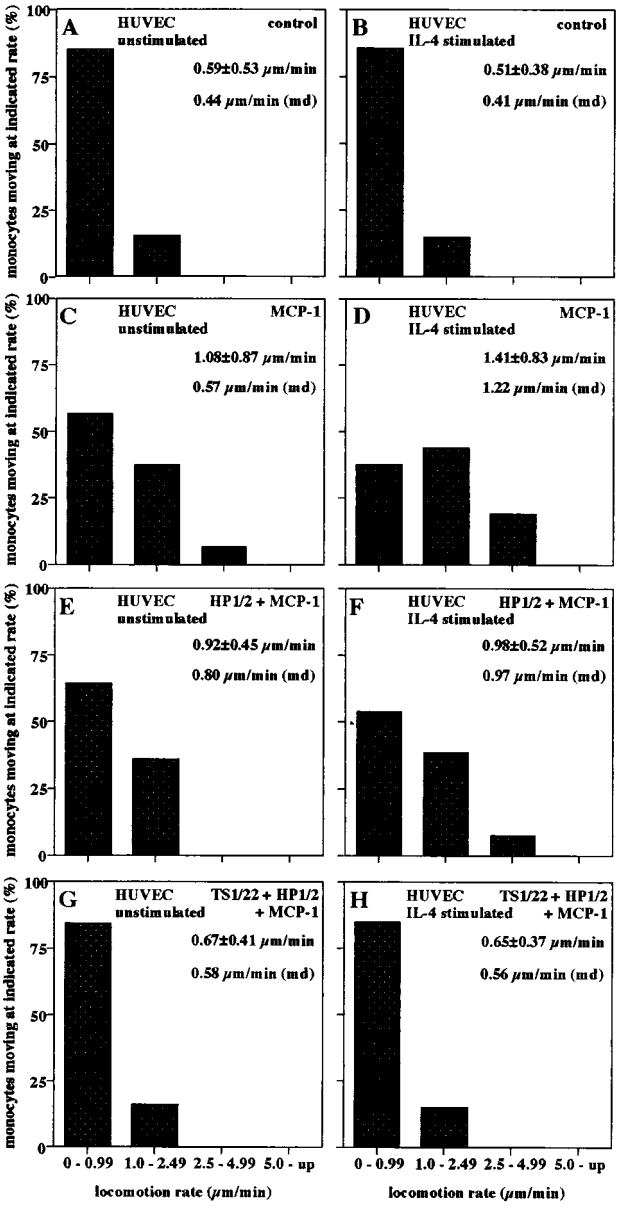

FIGURE 5. IL-4 activation of endothelium increases VLA-4-mediated chemokinesis of monocytes. Random movement of monocytes pretreated with isotype control (*A–D*), mAb to α_4 (*E, F*), or mAb to β_2 (*G, H*) was recorded on resting (*A, C, E, G*) or IL-4-activated (*B, D,F, H*) HUVEC in the absence (*A, B*) or presence of MCP-1 (1 ng/ml, *C–H*) over 30 min using time lapse video microscopy. The lengths of individual cell paths were determined by the sum of the distances between cell centroids after every 1-min interval, and locomotion rates were calculated within 30 min. These data are expressed as the percentage of cells moving at indicated locomotion rates or as mean ± SD. Medians (md) are also given. For every condition, analysis included 30 cells recorded in at least three independent experiments.

a combination of β_2 and α_4 mAb almost completely blocked chemotaxis across resting and activated HUVEC (data not shown). Visualizing the transendothelial chemotaxis of monocytes in the Transwell system revealed that MCP-1 in the lower chamber induced cell movement toward the filter pores (Fig. 6, *C* and *D*). The color code of the monocytes as they enter the filter pore corresponds to the elapsed time. Monocytes migrating across IL-4-treated HUVEC expressing VCAM-1 (Fig. 6*D*) reached the pores more rapidly than those migrating across resting HUVEC (Fig. 6*C*). This was due to higher locomotion rates on IL-4-activated HUVEC ($p < 0.001$ vs resting HUVEC, Fig. 6, *E* and *F*). The locomotion rate for lateral migration toward a gradient of MCP-1 (Fig. 6*F*) was higher than for MCP-1-induced chemokinesis (Fig.

Table II. *Effect of IL-4 activation on endothelial adhesion molecule and MCP-1 expression*[a]

	Control	IL-4
sMFI (channels)		
VCAM-1	4 ± 3	59 ± 18*
ICAM-1	139 ± 24	152 ± 22
ICAM-2	262 ± 41	265 ± 38
Protein (ng/ml)		
MCP-1	0.52 ± 0.15	0.73 ± 0.08

[a] HUVEC were activated with IL-4 (500 U/ml) or left untreated for 24 h, and supernatants were collected. Cells were reacted with VCAM-1, ICAM-1, or ICAM-2 mAb. The surface protein expression was analyzed by flow cytometry and given as specific mean fluorescence intensity (sMFI) corrected for nonspecific binding in channels. MCP-1 concentrations in supernatants were quantitated by ELISA. Data are mean ± SD of three independent experiments. * $p < 0.05$.

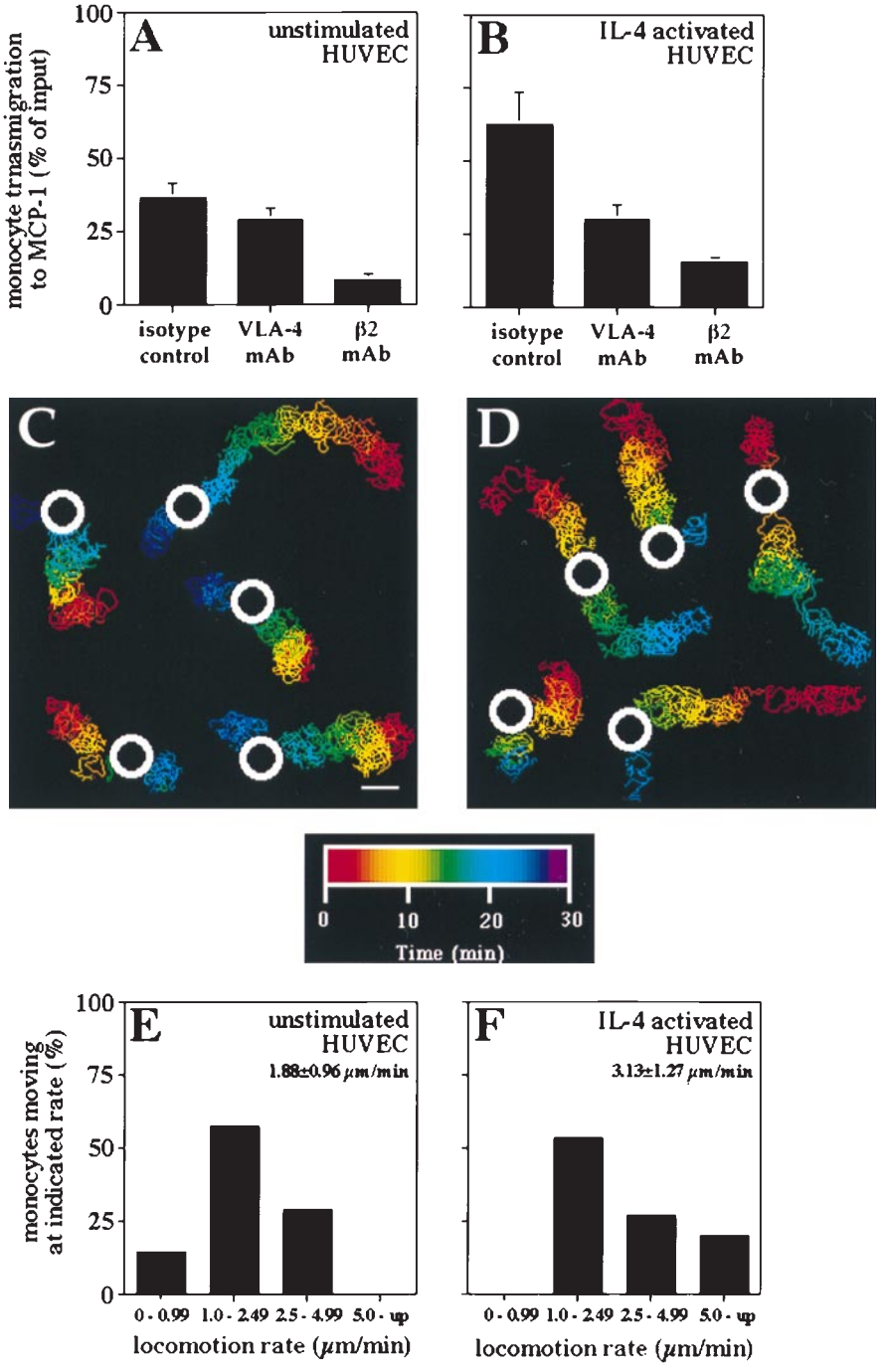

FIGURE 6. IL-4 activation of endothelial cells facilitates VLA-4-mediated transendothelial chemotaxis of monocytes by increasing lateral migration in response to a MCP-1 gradient. (*A*, *B*) Transendothelial chemotaxis of monocytes to MCP-1 (1 ng/ml) across HUVEC left unstimulated (*A*) or activated with IL-4 (*B*). MAb to α_4 (HP1/2) and β_2 (TS1/18) were at 10 μg/ml. Little spontaneous transmigration was seen both across untreated (1.8 $\pm$ 0.6%) or IL-4-stimulated HUVEC (2.5 $\pm$ 0.7%) in the absence of MCP-1. *C–F*, Lateral migration of monocytes on Transwell filters coated with HUVEC. Movement of monocytes was recorded on resting (*C*, *E*) or IL-4-activated (*D*, *F*) HUVEC with MCP-1 (1 ng/ml) in the lower chamber over 30 min using time lapse video microscopy. Cell movement is indicated by traces of cell outlines in different colors corresponding to the elapsed time (*C*, *D*). The bars indicate 10 μm. The lengths of individual cell paths were determined by the sum of the distances between cell centroids after every 1-min interval, and locomotion rates before entering pores were calculated (*E*, *F*). These data are expressed as the percentage of cells moving at indicated locomotion rates or as mean $\pm$ SD. The medians were 1.63 μm/min (*E*) and 2.52 μm/min (*F*), respectively. Analysis included 30 cells recorded in at least three independent experiments.

5*D*), probably due to the more directed migration toward a chemotactic gradient. The fraction of cells crawling at >2.5 μm/min before entering pores was 29% on resting vs 47% on IL-4-activated HUVEC. This complies with the findings that interactions of VLA-4 and VCAM-1 are favorable for monocyte chemokinesis. Monocytes that had transmigrated across IL-4-activated HUVEC appeared to pull free of the pores more readily, and particularly after early diapedesis continued to migrate on the lower surface of the Transwell filters before they detached (Fig. 6, *C* and *D*). Interestingly, the time monocytes spent between entering and exiting the pores was little changed by activation of HUVEC with IL-4 (4.6 $\pm$ 1.9 min vs 5.2 $\pm$ 1.5 min in resting HUVEC, mean $\pm$ SD), suggesting that diapedesis itself was not affected. Similarly, MIP-1α, a chemokine not produced by HUVEC, induced increased monocyte transmigration across IL-4-activated HUVEC that was VLA-4 dependent (Fig. 7, *A* and *B*) and increased the rate of lateral locomotion on IL-4-activated HUVEC (Fig. 7, *C* and *D*). These data suggest that VCAM-1 facilitates transendothelial chemotaxis by supporting lateral migration of attached monocytes along the endothelium before diapedesis.

Discussion

We have found that the VLA-4 ligands VCAM-1, FN40, and to a lesser extent fibronectin supported spontaneous lateral random migration and CC chemokine-induced chemokinesis of isolated blood monocytes. This migration was associated with a marked shape change and the induction of lamellipodia and uropods in

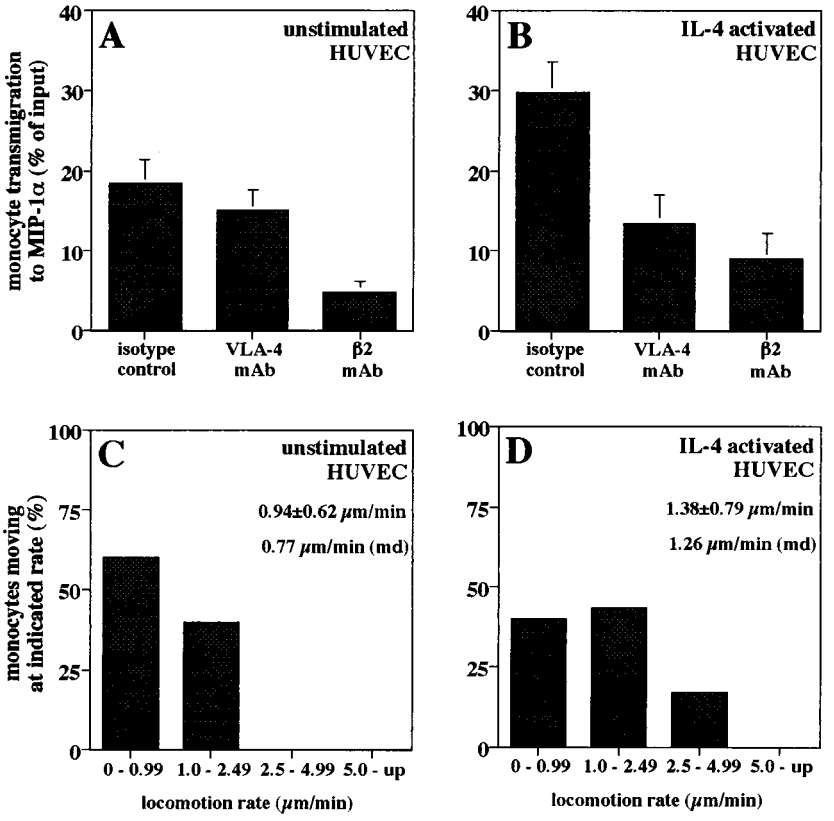

FIGURE 7. IL-4 activation of endothelial cells increases VLA-4-mediated transendothelial chemotaxis and lateral random migration of monocytes in reponse to a MIP-1α gradient. *A, B,* Transendothelial chemotaxis of monocytes to MIP-1α (10 ng/ml) across HUVEC left unstimulated (*A*) or activated with IL-4 (*B*). MAb to α_4 (HP1/2) and β_2 (TS1/18) were at 10 μg/ml. *C, D,* Lateral migration of monocytes on HUVEC. Movement of monocytes was recorded on resting (*C*) or IL-4-activated (*D*) HUVEC in the presence of MIP-1α (10 ng/ml) over 30 min using time lapse video microscopy. The lengths of individual cell paths were determined by the sum of the distances between cell centroids after every 1-min interval, and locomotion rates were calculated within 30 min. These data are expressed as the percentage of cells moving at indicated locomotion rates or as mean ± SD. Medians (md) are also given. Analysis included 30 cells recorded in at least 3 independent experiments.

migrating monocytes and was mediated by VLA-4, as demonstrated by mAb inhibition. In contrast, FN120, which contains the RGD binding site for VLA-5, allowed only marginal spontaneous or CC chemokine-induced lateral migration but promoted marked spreading of monocytes. We have recently shown that CC chemokines sequentially regulate the avidity of VLA-4 and VLA-5 in monocytes (9). In contrast to the transient activation and subsequent deactivation of VLA-4, we found prolonged activation of VLA-5 avidity for fibronectin and FN120. Accordingly, interaction of VLA-4 with its ligands allowed better monocyte chemotaxis to MCP-1 across filters coated with VCAM-1 or fibronectin at low but not high site densities, whereas interaction of VLA-5 with FN120 rather impaired transmigration (9). Taken together, our data suggest that integrin α subunits confer not only differential activation of adhesiveness but also differences in the potential to support lateral migration of monocytes.

Our data suggest that the induction of chemokinesis of monocytes on VCAM-1 may be promoted by the transient regulation of VLA-4 by MCP-1 in a subset of cells with relatively low or intermediate adhesive strength. CC chemokines transiently increase but then reduce the adhesive strength of VLA-4 in monocytes, resulting in attachment and subsequent detachment on its ligands (9). Locking VLA-4 in a high avidity state with stimulating mAbs, such as TS2/16, or Mn^{2+} interferes with the avidity regulation by CC chemokines and transendothelial chemotaxis (9, 41), and as demonstrated here markedly impaired lateral migration. The locomotion rate of monocytes appeared to be inversely correlated with the adhesive strength of VLA-4; i.e., it was the lowest when adhesive strength was maximally activated between 6 and 12 min. These data may further support an association of lateral migration with the transient regulation of VLA-4 avidity. Controlled detachment assays on VCAM-1 reveal that of the CC chemokines, MCP-1 most rapidly increased and deactivated the adhesive strength of VLA-4 (9). As observed for this regulation, our data now show that chemokinesis induced by MCP-1 was also optimally regulated at intermediate substrate concentrations of VLA-4 ligands and impaired at lower and higher substrate concentrations. Previous results (42) have established that immobilization of VCAM-1/Fc or fibronectin at various concentrations results in different site densities. In accordance with our data, this report (42) has indicated that the efficiency of spontaneous lymphocyte migration was optimal at intermediate site densities of VLA-4 ligands and higher across membranes coated with VCAM-1/Fc than with fibronectin. Hence, it is reasonable to assume that the changes in the locomotion rates we observed for monocyte chemokinesis were due to differences in site density under otherwise identical conditions. These data are also consistent with previous predictions that intermediate adhesive strength leads to maximal motility (43) and with recent studies implicating adhesive strength, which can be influenced by receptor expression and affinity, substrate density, and organization of adhesive complexes, as an important regulator of cell migration (44).

What determines different functions of VLA-4 and VLA-5? Directional cell migration involves formation of adhesive complexes and interaction with force generating components of the cytoskeleton to provide traction and detachment at the rear, allowing the cell to advance over its substrate (13, 45). Intermediate adhesive strength is a major determinant of cell speed (44) and may be different for VLA-4 and VLA-5. Extracellular ligand binding can induce an attachment of β_1 integrins to the retrograde-moving cytoskeleton critical for organized receptor movement and migration of adherent cells (46). In MCP-1-stimulated monocytes on VCAM-1, immunostaining revealed actin polymerization and asymmetrical VLA-4 distribution into uropod-like structures (C. Weber, unpublished data). Hence, migration may be associated with spatial redistribution of VLA-4. By contrast, VLA-5 has been implicated in the strengthening of integrin-cytoskeleton linkages stimulated by the ECM rigidity (47). Studies in Chinese hamster

ovary transfectants indicated that integrin mutations increasing cytoskeletal organization reduce locomotion rates, whereas mutations impairing cytoskeletal association and focal adhesion complex formation decrease haptotaxis but increase random migration (48). Whereas VLA-5 may thus favor a more rigid reinforcement of cytoskeleton linkages with high adhesive strength, spreading, and lower locomotion rates, the cytoskeletal associations of VLA-4 and its transient avidity regulation by chemokines may allow faster retrograde diffusion and better cell migration. Studies in K562 and Chinese hamster ovary transfectants showed that the α_4 cytoplasmic tail compared with that of α_5 promotes migration but reduces localization into focal adhesions and spreading and that α_4 cytoplasmic tail deletion decreases clustering and lateral diffusion of VLA-4 (29, 49). Thus, α subunit cytoplasmic domains may confer differences crucial for the ability of integrins to support locomotion of monocytes, a cell type without focal adhesions.

We assessed the contribution of VCAM-1 to the lateral random migration of MCP-1-stimulated monocytes on endothelium. Induction of VCAM-1 expression by activation of HUVEC with IL-4 resulted in a VLA-4-dependent increase in chemokinesis. Further analysis revealed that the chemokinesis on resting and IL-4-activated HUVEC after blocking VLA-4 was mediated by interactions of β_2 integrins with their ligands, as seen on ICAM-1 substrates. This is consistent with previous findings on the involvement of β_2 integrins in lateral and transendothelial migration of leukocytes and suggests that VLA-4-VCAM-1 interactions represent a major but not an exclusive pathway for lateral migration before transmigration (31, 50). To study the relevance of our findings in a chemotactic context, we analyzed chemotaxis across IL-4-activated HUVEC. Monocytes use either β_2 integrins or VLA-4 during spontaneous transendothelial migration or chemotaxis (23, 24). Activation of endothelium has been shown to support VLA-4-mediated monocyte transmigration (23), consistent with an essential role of VLA-4 in monocyte emigration to acute inflammatory sites in vivo (27, 28). Similar to findings with TNF-stimulated HUVEC (9), we found that mAb to β_2 or α_4 independently inhibited transendothelial chemotaxis. However, while the relative importance of β_2 integrins was reduced, that of VLA-4 was increased across IL-4-activated HUVEC expressing VCAM-1. This suggests that VLA-4 and β_2 integrins may synergize at distinct steps to achieve optimal transmigration across activated endothelium.

Induction of VCAM-1 by IL-4 increased both monocyte chemokinesis on HUVEC and transendothelial chemotaxis by a VLA-4-dependent mechanism. Notably, the time monocytes spent within Transwell pores was unchanged by activation of HUVEC with IL-4, suggesting that the process of diapedesis was not affected. Hence, VCAM-1 expression may facilitate the transendothelial chemotaxis by supporting lateral migration along endothelium. Diapedesis of monocytes across endothelium is promoted by a soluble gradient of MCP-1 rather than by haptotactic stimulation through immobilized MCP-1 (51). This may be due to a higher efficiency of migration with a directed gradient of soluble MCP-1. Indeed, locomotion rates for lateral migration toward a directed gradient were higher than for chemokinesis on IL-4-activated HUVEC. The extent of lateral migration on VCAM-1 may support transendothelial chemotaxis by improving the probability of encountering interendothelial cell junctions.

Alternatively, activation of endothelium with IL-4 may facilitate monocyte migration via distinct mechanisms, i.e., by inducing antiadhesive molecules or structures on endothelium. The VCAM-1-dependent adhesion of T lymphocytes to endothelium has been described to induce a 72-kDa gelatinase (52). Similarly, induction or activation of proteolytic enzymes by interaction of VLA-4 with VCAM-1 or by IL-4 activation of endothelial cells may contribute to the detachment of monocytes required for completing transmigration. This would comply with our findings that monocytes that had transmigrated across IL-4-activated HUVEC appeared to pull free of the pores more readily after diapedesis. Hence, it may be possible that migration on integrin ligands on the luminal side of IL-4-activated endothelium may transduce positive signals not only for completing monocyte diapedesis but also for the subsequent β_1 integrin-mediated migration on ECM proteins, likely to be present on the lower face of the Transwell filters, possibly generating a cross-talk to regulate sequential integrin functions (53). On the other hand, the crawling of monocytes on the lower surface of the Transwell filters after transgression of the filter pores may involve VLA-4-dependent lateral migration on components secreted into the subendothelial matrix, as shown on fibronectin substrates.

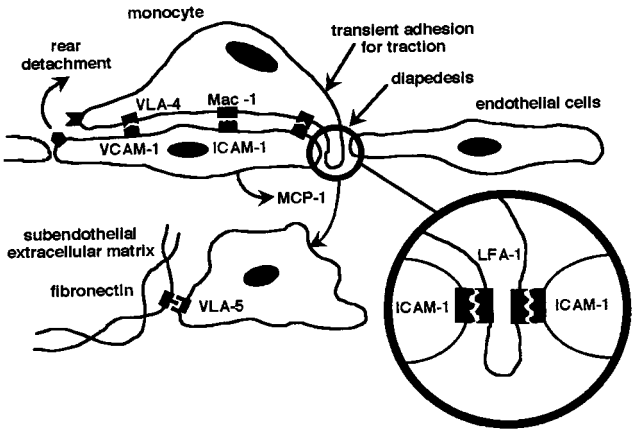

FIGURE 8. Schematic diagram depicting the putative role of VLA-4-mediated lateral migration in monocyte extravasation following arrest on endothelial cells and preceding diapedesis.

A version of the multistep model defined a role for α_4 integrins in bridging between selectins and β_2 integrin-mediated events (54). Distinct functions have been found for integrins in the interaction of monocytes with activated endothelium in flow, where VLA-4 participates in attachment, while β_2 integrins mediate transmigration (55). The VLA-4-dependent lateral migration induced by CC chemokines may follow initial rolling and attachment of leukocytes supported by selectins and α_4 integrins (11, 12). This may mediate the migration of arrested leukocytes along endothelium to interendothelial cell junctions where diapedesis can occur. In contrast, the β_2 integrin LFA-1 may be more important in transendothelial diapedesis itself, as also suggested by the crucial role of changes in its avidity for transendothelial chemotaxis (56). Migration and spreading on fibronectin associated with prolonged VLA-5 activation (9) may support interactions of leukocytes with the underlying basement membrane or ECM. Fig. 8 illustrates the putative sequence of monocyte extravasation with a potential role of VLA-4-mediated lateral migration following monocyte arrest on endothelial cells and preceding diapedesis and subendothelial localization. Thus, the functional specialization among integrins that we have demonstrated may be important to accomplish the complex process of leukocyte extravasation.

Acknowledgments

We thank Dr. R. Lobb for providing VCAM-1, Dr. E. Finger for help with image analysis, Professor Dr. R. Lorenz for help with statistical analysis, and Dr. K. S. C. Weber for assistance with ELISA.

References

1. Butcher, E. C. 1991. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell 67:1033.*
2. Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multi-step paradigm. *Cell 76:301.*
3. Lawrence, M. B., and T. A. Springer. 1991. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell 65:859.*
4. Rot, A. 1992. Endothelial cell binding of NAP-1/IL-8: role in neutrophil emigration. *Immunol. Today 13:291.*
5. Baggiolini, M., B. Dewald, and B. Moser. 1997. Human chemokines: an update. *Annu. Rev. Immunol. 15:675.*
6. Detmers, P. A., S. K. Lo, E. Olsen-Egbert, A. Walz, M. Baggiolini, and Z. A. Cohn. 1990. Neutrophil-activating protein 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. *J. Exp. Med. 171:1155.*
7. Huber, A. R., S. L. Kunkel, R. F. Todd, and S. J. Weiss. 1991. Regulation of transendothelial neutrophil migration by endogenous interleukin-8. *Science 254: 99.*
8. Tanaka, Y., D. H. Adams, S. Hubscher, H. Hirano, U. Siebenlist, and S. Shaw. 1993. T-cell adhesion induced by proteoglycan-immobilized cytokine MIP-1β. *Nature 361:79.*
9. Weber, C., R. Alon, B. Moser, and T. A. Springer. 1996. Sequential regulation of $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrin avidity by CC chemokines in monocytes: implications for transendothelial chemotaxis. *J. Cell Biol. 134:1063.*
10. Weber, C., J. Kitayama, and T. A. Springer. 1996. Differential regulation of β_1- and β_2 integrin avidity by chemoattractants in eosinophils. *Proc. Natl. Acad. Sci. USA 93:10939.*
11. Alon, R., P. D. Kassner, M. W. Carr, E. B. Finger, M. E. Hemler, and T. A. Springer. 1995. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J. Cell Biol. 128:1243.*
12. Berlin, C., R. F. Bargatze, U. H. von Andrian, M. C. Szabo, S. R. Hasslen, R. D. Nelson, E. L. Berg, S. L. Erlandsen, and E. C. Butcher. 1995.. α_4 integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell* 80:413.
13. Lauffenburger, D. A., and A. F. Horwitz. 1996. Cell migration: A physically integrated molecular process. *Cell 84:359.*
14. Hemler, M. E. 1990. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. *Annu. Rev. Immunol. 8:365.*
15. Elices, M. J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M. E. Hemler, and R. Lobb. 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell 60:577.*
16. Vonderheide, R. H., and T. A. Springer. 1992. Lymphocyte adhesion through VLA-4: evidence for a novel binding site in the alternatively spliced domain of VCAM-1 and an additional α_4 integrin counter-receptor on stimulated endothelium. *J. Exp. Med. 175:1433.*
17. Vonderheide, R. H., T. F. Tedder, T. A. Springer, and D. E. Staunton. 1994. Residues within a conserved amino acid motif of domains 1 and 4 of VCAM-1 are required for binding to VLA-4. *J. Cell Biol. 125:215.*
18. Osborn, L., C. Vassallo, B. G. Browning, R. Tizard, D. O. Haskard, C. D. Benjamin, I. Douglas, and T. Kirchhausen. 1994. Arrangement of domains, and amino acid residues required for binding of vascular cell adhesion molecule-1 to its counter-receptor VLA-4 ($\alpha_4\beta_1$). *J. Cell Biol. 124:601.*
19. Wayner, E.A., A. Garcia-Pardo, M. J. Humphries, J. A. MacDonald, and W. G. Carter. 1989. Identification and characterization of the T lymphocyte adhesion receptor for an alternative cell attachment domain (CS-1) in plasma fibronectin. *J. Cell Biol. 109:1321.*
20. Hynes, R. O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell. 69:11.*
21. Oppenheimer-Marks, N., L. S. Davis, D. T. Bogue, J. Ramberg, and P. E. Lipsky. 1991. Differential utilization of ICAM-1 and VCAM-1 during the adhesion and transendothelial migration of human T lymphocytes. *J. Immunol. 147:2913.*
22. Ebisawa, M., B. S. Bochner, S. N. Georas, and R. P. Schleimer. 1992. Eosinophil transendothelial migration induced by cytokines. I. Role of endothelial and eosinophil adhesion molecules in IL-1β-induced transendothelial migration. *J. Immunol. 149:4021.*
23. Chuluyan, E. H., and A. C. Issekutz. 1993. VLA-4 integrin can mediate CD11/CD18 independent transendothelial migration of human monocytes. *J. Clin. Invest. 92:2768.*
24. Meerschaert, J., and M. B. Furie. 1994. Monocytes use either CD11/CD18 or VLA-4 to migrate across human endothelium in vitro. *J. Immunol. 152:1915.*
25. Roth, S. J., M. W. Carr, S. S. Rose, and T. A. Springer. 1995. Characterization of transendothelial chemotaxis of T lymphocytes. *J. Immunol. Methods 100:97.*
26. Issekutz, T. B. 1991. Inhibition of in vivo lymphocyte migration to inflammation and homing to lymphoid tissues by the TA-2 monoclonal antibody: a likely role for VLA-4 in vivo. *J. Immunol. 147:4178.*
27. Winn, R. K., and J. M. Harlan. 1993. CD18-independent neutrophil and mononuclear leukocyte emigration into the peritoneum of rabbits. *J. Clin. Invest. 92: 1168.*
28. Issekutz, T. B. 1995. In vivo blood monocyte migration to acute inflammatory reactions, IL-1α, TNF-α, IFN-γ, and C5a utilizes LFA-1, Mac-1, and VLA-4: The relative importance of each integrin. *J. Immunol. 154:6533.*
29. Kassner, P. D., R. Alon, T. A. Springer, and M. E. Hemler. 1995. Specialized functional properties of the integrin α_4 cytoplasmic domain. *Mol. Biol. Cell. 6:661.*
30. Sanchez-Madrid, F., A. M. Krensky, C. F. Ware, E. Robbins, J. L. Strominger, S. J. Burakoff, and T. A. Springer. 1982. Three distinct antigens associated with human T lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. *Proc. Natl. Acad. Sci. USA 79:7489.*
31. Smith, C. W., S. D. Marlin, R. Rothlein, C. Toman, and D. C. Anderson. 1989. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J. Clin. Invest. 83:2008.*
32. de Fougerolles, A. R., S. A. Stacker, R. Schwarting, and T. A. Springer. 1991. Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. *J. Exp. Med. 174:253.*
33. Hemler, M. E., F. Sanchez-Madrid, T. J. Flotte, A. M. Krensky, S. J. Burakoff, A. K. Bhan, T. A. Springer, and J. L. Strominger. 1984. Glycoproteins of 210,000 and 130,000 M. W. on activated T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. *J. Immunol. 132:3011.*
34. Sanchez-Madrid, F., M. O. De Landazuri, G. Morago, M. Cebrian, A. Acevedo, and C. Bernabeu. 1986. VLA-3: a novel polypeptide association within the VLA molecular complex: cell distribution and biochemical characterization. *Eur. J. Immunol. 16:1343.*
35. Griffin, J. D., J. Ritz, L. M. Nadler, and S. F. Schlossman. 1981. Expression of myeloid differentiation antigens on normal and malignant myeloid cells. *J. Clin. Invest. 69:932.*
36. Dustin, M. L., O. Carpen, and T. A. Springer. 1992. Regulation of locomotion and cell-cell contact area by the LFA-1 and ICAM-1 adhesion receptors. *J. Immunol. 148:2654.*
37. Weber, C., E. Negrescu, W. Erl, A. Pietsch, M. Frankenberger, H. W. L. Ziegler-Heitbrock, W. Siess, and P. C. Weber. 1995. Inhibitors of protein tyrosine kinase suppress TNF-stimulated induction of endothelial cell adhesion molecules. *J. Immunol. 155:445.*
38. Thornhill, M. H., and D. O. Haskard. 1990. IL-4 regulates endothelial cell activation by IL-1, tumor necrosis factor, or IFN-γ. *J. Immunol. 145:865.*
39. Schleimer, R. P., S. A. Sterbinsky, J. Kaiser, C. A. Bickel, D. A. Klunk, K. Tomioka, W. Newman, F. W. Luszinskas, M. A. Gimbrone Jr., B. W. McIntyre, and B. S. Bochner. 1992. IL-4 induced adherence of human eosinophils and neutrophils but not neutrophils to endothelium. *J. Immunol. 148: 1086.*
40. Rollins, B. J., and J. S. Pober. 1991. Interleukin-4 induces the synthesis and secretion of MCP-1/JE by human endothelial cells. *Am. J. Pathol. 138:1315.*
41. Kuijpers, T. W., E. P. J. Mul, M. Blom, N. L. Kovach, C. A. Gatea, V. Tollefson, M. J. Elices, and J. M. Harlan. 1993. Freezing adhesion molecules in a state of high-avidity binding blocks eosinophil migration. *J. Exp. Med. 178:279.*
42. Chan, P.-Y., and A. Aruffo. 1993. VLA-4 mediates lymphocyte migration on the inducible endothelial cell ligand VCAM-1 and the extracellular matrix protein ligand fibronectin. *J. Biol. Chem. 268:24655.*
43. DiMilla, P. A., J. A. Stone, J. A. Quinn, S. M. Albelda, and D. A. Lauffenburger. 1993. Maximal migration of human smooth muscle cells on fibronectin and type IV collagen occurs at an intermediate attachment strength. *J. Cell Biol. 122:729.*
44. Palecek, S. P., J. C. Loftus, M. H. Ginsberg, D. A. Lauffenburger, and A. F. Horwitz. 1997. Integrin/ligand binding properties govern cell migration speed through cell/substratum adhesiveness. *Nature 385:537.*
45. Stossel, T. P. 1993. On the crawling of animal cells. *Science 260:1086.*
46. Felsenfeld, D. P., D. Choquet, and M. P. Sheetz. 1996. Ligand binding regulates the directed movement of β_1 integrins on fibroblasts. *Nature. 383:438.*
47. Choquet, D., D. P. Felsenfeld, and M. P. Sheetz. 1997. Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. *Cell. 88:39.*
48. Huttenlocher, A., M. H. Ginsberg, and A. F. Horwitz. 1996. Modulation of cell-migration by integrin-mediated cytoskeletal linkages and ligand-binding affinity. *J. Cell Biol. 134:1551.*
49. Yauch, R. L., D. P. Felsenfeld, S.-K. Kraeft, L. B. Chen, M. P. Sheetz, and M. E. Hemler. 1997. Mutational evidence for control of cell adhesion through integrin diffusion/clustering, independent of ligand binding. *J. Exp. Med. 186: 1347.*
50. Burns, A. R., S. I. Simon, G. L. Kukielka, J. L. Rowen, H. Lu, L. H. Mendoza, E. S. Brown, M. L. Entman, and C. W. Smith. 1996. Chemotactic factors stimulate CD18-dependent canine neutrophil adherence and motility on lung fibroblasts. *J. Immunol. 156:3389.*
51. Randolph, G. J., and M. B. Furie. 1995. A soluble gradient of endogenous monocyte chemoattractant protein-1 promotes the transendothelial migration of monocytes in vitro. *J. Immunol. 155:3610.*
52. Romanic, A. M., and J. A. Madri. 1994. The induction of 72-kD gelatinase in T cells upon adhesion to endothelial cells is VCAM-1 dependent. *J. Cell Biol. 125:1165.*
53. Porter, J. C., and N. Hogg. 1997. Integrin cross talk: activation of LFA-1 on human T cells alters $\alpha_4\beta_1$ and $\alpha_5\beta_1$-mediated function. *J. Cell Biol. 138:1437.*
54. Bargatze, R. F., M. A. Jutila, and E. C. Butcher. 1995. Distinct roles of L-selectin and integrins $\alpha_4\beta_7$ and LFA-1 in lymphocyte homing to Peyer's patch-HEV in situ: the multistep model confirmed and refined. *Immunity 3:99.*
55. Luscinskas, F. W., G. S. Kansas, H. Ding, P. Pizcueta, B. E. Schleiffenbaum, T. F. Tedder, and M. A. Gimbrone Jr. 1994. Monocyte rolling, arrest and spreading on IL-4-activated vascular endothelium under flow is mediated via sequential action of L-selectin, β_1 integrins, and β_2 integrins. *J. Cell Biol. 125:1417.*
56. Weber, C., C.-F. Lu, J. Casasnovas, and T. A. Springer. 1997. Role of $\alpha_L\beta_2$ integrin avidity in transendothelial chemotaxis of mononuclear cells, *J. Immunol. 159:268.*