Adhesion of Monocytes to Vascular Cell Adhesion Molecule-1–Transduced Human Endothelial Cells
Implications for Atherogenesis

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Abstract—To study the role of vascular cell adhesion molecule-1 (VCAM-1) in monocyte recruitment and atherogenesis, we constructed a recombinant adenovirus, AdRSVrVCAM-1, carrying the rabbit VCAM-1 cDNA. We have previously shown that AdRSVrVCAM-1–transduced human umbilical vein endothelial cells (HUVECs) support the adhesion of CD4+CD45RO+ memory T lymphocytes under laminar flow conditions. We now demonstrate that AdRSVrVCAM-1–transduced HUVECs support the adhesion of peripheral blood monocytes at a shear stress of \( \geq 1.5 \) dyne/cm\(^2\).

Although VCAM-1 supported only firm adhesion of lymphocytes, it was able to mediate monocyte rolling, firm adhesion, and transmigration when expressed in the context of otherwise unactivated vascular endothelium. VCAM-1–transduced HUVECs supported the adhesion of as many as 4-fold more monocytes than T cells under laminar flow. The greater monocyte adhesion was explained at least in part by leukocyte-leukocyte interactions (secondary adhesions), which were not seen with T cells. These secondary monocyte interactions were specifically blocked by monoclonal antibodies to L-selectin and P-selectin glycoprotein ligand-1. These data demonstrate that VCAM-1 expressed in the context of unactivated vascular endothelium supports the adhesion of the leukocyte populations present in atherosclerotic plaque and may contribute to the predominance of monocytes over lymphocytes. (Circ Res. 1998;82:871-878.)

Key Words: adenovirus ■ adhesion ■ atherosclerosis ■ mononuclear leukocyte

The association between monocytes and atherosclerotic lesions, both in animal models and in humans, has long been recognized. Monocytes constitute \( \approx >80\% \) of the leukocytes in atherosclerotic plaque. Lymphocytes constitute 5% to 20% of this cell population and are predominantly CD4+CD45RO+ (memory) T cells. Monocytes may contribute both to the development of atherosclerotic lesions and to events, such as acute plaque rupture, underlying acute coronary syndromes.

VCAM-1 supports the adhesion of mononuclear leukocytes, including monocytes and lymphocytes, in simple in vitro assays. It is expressed on the surface of endothelial cells during atherogenesis in animal models and is also detectable in human atherosclerotic lesions. The expression of VCAM-1 in human atherosclerotic plaques appears to be correlated with increased accumulation of mononuclear cells.

To address the role of VCAM-1 in mononuclear cell adhesion, we generated a recombinant adenovirus containing the seven-domain isoform of rVCAM-1 (AdRSVrVCAM-1).

Unlike more traditional methods of gene transfer, recombinant adenoviruses confer high-efficiency and high-level gene transduction in HUVECs, a well-characterized in vitro model of human vascular endothelium. We have also shown that adenoviral gene transfer does not globally activate HUVECs. Previous studies performed with immobilized VCAM-1 protein on plastic suggested that VCAM-1 alone, in the absence of an initial interaction with selectins, could support the adhesion and subsequent rolling of lymphocytes (unfractionated T cells) under flow. Using adenoviral gene transfer, we have shown that VCAM-1 alone, expressed on the surface of vascular endothelium, can support the adhesion of memory T cells, the predominant lymphocyte in the atherosclerotic plaque. In contrast to the experiments using purified proteins immobilized on plastic, memory T cells did not roll on the AdRSVrVCAM-1–transduced endothelium but remained firmly adherent at the site of attachment. This raises the possibility that immobilized protein may not fully reflect the endothelial milieu. Together, these studies constitute an exception to the previously accepted paradigm that initial
leukocyte adhesion events are selectin-mediated and that adhesion molecules of the immunoglobulin family participate only later in the multistep cascade. These data support the hypothesis that the increased VCAM-1 expression observed in the atherosclerotic plaque may be an important contributor to lymphocyte recruitment. However, these studies do not establish a role for VCAM-1 in monocyte recruitment under flow conditions. Moreover, they do not address whether VCAM-1 contributes to the impressive numerical predominance of monocytes over lymphocytes in atherosclerotic plaques.

In the present study, we extend our observations using AdRSVrVCAM-1–transduced endothelium to study the adhesion of monocytes under physiological flow conditions. Recently published data from antibody-blocking experiments under flow conditions have shown roles only for L- and P-selectin in the initial attachment of monocytes to TNF-activated endothelial cells. Antibody-blocking experiments on globally activated endothelium, however, cannot address the important question of whether VCAM-1 expressed in isolation is sufficient to support the adhesion of monocytes. In the present study, we also assess quantitative differences between the adhesion of monocytes and Jurkat T cells (T-cell line with adhesion properties similar to CD4+ memory T lymphocytes) to VCAM-1, the role of monocyte-monoocyte interactions in adhesion, and how the observed differences in adhesion could contribute to the predominance of monocytes in the atherosclerotic plaque.

Materials and Methods

Materials
RPMI 1640, DMEM, and Dulbecco’s PBS with or without Ca++ and Mg++ were purchased from BioWhittaker, Inc. Human serum albumin was obtained from Baxter Healthcare Corp. FBS was obtained from Hyclone Inc. Recombinant human TNF-α was obtained from Biogen. The 293 cell line was obtained from the American Type Culture Collection. Recombinant human TNF was purchased from Genzyme, Cambridge, MA and from Hyclone Inc. Recombinant human TNF was also obtained from Biogen. The E1/E3 deleted adenovirus was kindly provided by Dr. Lloyd Stoolman (University of Michigan, Ann Arbor). HUVECs were isolated from several normal-term umbilical veins, pooled, and cultured on 0.1% gelatin–coated tissue culture dishes as described in medium 199 with 20% FBS, endothelial cell growth factor (25 μg/mL, Biomedical Technologies), porcine intestinal heparin (50 μg/mL, Sigma Chemical Co.), and antibiotics. After infection with adenoviral vectors, HUVECs were cultured as above, but the serum concentration was reduced to 10%. For stimulation of HUVECs, TNF-α (200 U/mL) was added as indicated. For experimental use in the flow plate apparatus, HUVECs (passages 1 and 2) were plated at confluence on 25-mm fibronectin–coated glass coverslips, as previously described.

Recombinant Adenoviruses
Two recombinant type 5 adenoviruses were used in these studies: AdRSV-β-gal and AdRSVrVCAM-1. Both viruses use the dl 327 backbone, contain E1/E3 deletions, and were generated as previously described. Large-scale production of adenovirus and determination of viral titer was accomplished as described previously. Only one viral stock of each construct was used in the course of these studies. Stock titer was 10^9 pfu/mL for both vectors, with a particle/pfu ratio of ~10^7.

Infection of HUVECs With Adenoviral Vectors
HUVECs were plated at confluence and infected 18 hours later. Infection was performed in 96-well plates (Costar) or on 25-mm-diameter glass coverslips by the addition of virus diluted in 0.05 or 0.8 mL, respectively, of infection medium (DMEM with 2% FCS) for 1 to 1.5 hours at 37°C. At that time, 0.15 or 1.5 mL of growth medium was added to each well, and the cells were incubated for 48 hours before evaluation.

Monoclonal Antibodies
The following mAbs have been described previously: H18/7 and H4/18 (to human E-selectin), Hu5/3 (to human ICAM-1), E1/16 (which recognizes both human and rabbit VCAM-1), Hu8/4 (which recognizes only human VCAM-1), B1/9 (which is specific for rVCAM-1), HP2.1 (to α-integrin, Immunotech), HPDG2/3 (to P-selectin, Genetics Institute), TSI/18 (to CD18, American Type Culture Collection clone HB203), HUTS21 (which recognizes an epitope on β₂-integrin that is induced by cell activation), K16/16 (IgG, nonbinding control), W6/32 (IgG, binding control), LAM1-4 and LAM1-14 (to L-selectin, functional blocking and nonblocking, respectively), and KPL-1 (function blocking mAb to PSGL-1, the best-characterized ligand for P-selectin).

Surface Immunofluorescence Assays
HUVECs monolayers in 96-well plates were incubated on ice with the indicated primary mAb in RPMI/1% FCS at 10 µg/mL for 45 minutes. Wells were washed three times with RPMI/1% FCS and then incubated with a FITC-conjugated goat anti-mouse polyclonal F(ab’)2 antibody (Caltag) diluted 1:100 in Dulbecco’s PBS on ice. After 45 minutes, wells were washed twice with Dulbecco’s PBS/20% FCS and twice with Dulbecco’s PBS alone. Cells were lysed with 0.01% NaOH in 0.1% SDS (pH 9.6), and fluorescence was quantified using a CytoFluor 2350 FACS (Becton Dickinson) plate reader set at 485 nm (excitation)/535 nm (emission). For each flow experiment, a 96-well plate was cultured and infected in parallel at the same multiplicity of infection. On the day of the flow adhesion assay, a fluorescence immunoassay was performed on this plate to document rVCAM-1 expression and to rule out nonspecific activation of the endothelial monolayer.

Leukocyte Isolation and Flow Cytometric Analysis
Human monocytes were purified from single donor human platelet pheresis residues by Ficoll-Hypaque density gradient centrifugation at 15°C (LSM, Organon Teknika), followed by counterflow centrifugation elutriation. Monocyte suspensions were >91% pure with >98% to 8% lymphocyte, <2% granulocyte, and essentially no platelet contamination, as determined by light scatter and cell surface antigen analysis with mAbs directed to CD14, CD41, CD61, and P-selectin as detailed previously. Flow cytometric analysis of monocyte cell surface antigens was subsequently performed. Binding of murine mAbs recognizing various monotypic surface antigens was detected with indirect immunofluorescence using goat FITC-labeled secondary mAb (1:100 dilution) in RPMI 1640/5% FCS. The fluorescence of 10^4 cells was measured on a FACScan (Becton Dickinson). Nonspecific fluorescence was corrected by subtracting the mean
channel fluorescence of mAb K16/16 (nonbinding control) from the mean channel fluorescence for each mAb used. The number of binding sites for the antibodies on Jurkat T cells and monocytes was estimated as follows: A mixture of 8-μm microbeads (Flow Cytometry Standards Corp) with different predetermined binding sites per bead for mouse IgG was used. The microbeads were stained with each mAb and analyzed using the same protocol for the cells as described above. A standardized curve for each mAb was generated by using the ratio of the mean channel fluorescent to binding sites. The number of binding sites for the respective mAb on the cells was then estimated. To normalize the number of binding sites for the different cell surface areas, cell diameters were measured from the image on the video monitor, the ratio of the surface areas was calculated (Jurkat T cell:monocyte surface area ratio, 1.54:1), and the number of T-cell binding sites was adjusted accordingly.

**Adhesion Assays Under Flow: Apparatus Design and Experimental Application**

The parallel-plate flow chamber used in the present study has been described previously in detail. Endothelial monolayers on coverslips were incubated with culture medium for 48 to 72 hours after viral infection. Where indicated, monolayers were stimulated with TNF-α (200 U/mL) for 4 hours. For blocking experiments, immediately before the assay, HUVEC monolayers were incubated with culture medium containing the indicated mAb or culture medium alone for 30 minutes at 37°C. The coverslip was positioned in the flow chamber, and the entire chamber was then mounted on an inverted microscope equipped with 10×, 20×, and 40× phase-contrast objectives. Leukocytes were incubated with a saturating concentration of the indicated mAb for 20 minutes at 4°C and diluted with perfusion medium to 10^6 cells/mL. The mAb concentration was adjusted to saturating levels in perfusion medium, and leukocytes were drawn through the chamber at controlled flow rates. The cells were perfused at an estimated shear stress of 1.5 dyne/cm² (flow rate, 0.78 mL/min) for 3 minutes, and when indicated, the flow rate was then decreased by 0.5 dyne/cm² every 3 minutes to 0.5 dyne/cm². The entire period of perfusion was recorded on videotape using a video recorder equipped with a time-date generator with a millisecond clock. Leukocyte adhesion was quantified as follows: Total accumulation of leukocytes was determined by counting the number of T cells or monocytes in five to seven randomly selected 20×40 fields during the final 2 minutes at each level of flow. To allow comparison between experiments, all data obtained with the 40 objective were multiplied by the ratio of the mean channel fluorescence to binding sites. The number of binding sites for the respective mAb on the cells was calculated (Jurkat T cell:monocyte surface area ratio, 1.54:1), and the number of T-cell binding sites was adjusted accordingly.

**Statistical Analysis**

Data are expressed as the mean±SD or mean±SEM, as indicated. Statistical comparison of means was performed by two-tailed unpaired Student t test or ANOVA, as indicated. The null hypothesis was considered rejected at P<0.05.

**Results**

AdRSVrVCAM-1–Transduced HUVECs Support Monocyte Adhesion Under Flow

As previously detailed, AdRSVrVCAM-1 mediated specific and homogenous expression of rVCAM-1 on endothelial cells without global activation. Surface expression of the endothelial markers of activation, E-selectin, ICAM-1, and endogenous VCAM-1, was not increased compared with parallel uninfected cultures as measured by fluorescence immunoassay, consistent with our previous report. Because preliminary experiments revealed no monocyte adhesion on transduced monolayers at flow rates of ≥2.0 dyne/cm², further studies were performed at flow rates of ≤1.5 dyne/cm². For the initial 3 minutes of the experiment, the cells were perfused at a shear stress of 1.5 dyne/cm². This was subsequently decreased by 0.5 dyne/cm² at 3-minute intervals to the final shear stress of 0.5 dyne/cm². As seen in Figure 1, AdRSVrVCAM-1–infected HUVECs supported high levels of monocyte adhesion under laminar flow between 1.5 and 0.5 dyne/cm². In contrast, control (AdRSVβ-gal–infected) HUVECs supported little monocyte adhesion under these conditions.

To verify the specificity of the observed interactions, monoclonal blocking experiments were performed (Figure 1). A mAb (HP2.1) to the VCAM-1 counterligand (the α4-integrin) ablated monocyte adhesion to AdRSVrVCAM-1–transduced HUVECs. Monocyte adhesion was also blocked by Rb1/9, a function-blocking mAb specific for VCAM-1. Therefore, adhesion was mediated by the adenovirally expressed rVCAM-1 rather than endogenous human VCAM-1 or fibronectin, which can also support α4-integrin–dependent adhesion. In contrast, mAbs to the β2-integrins (mAb TS1/18), the best characterized counterreceptor for endothelial ICAM-1, or to P-selectin (mAb HPDG 2/3) did not block adhesion.
Monocyte Adhesion Exceeds Jurkat T-Cell Adhesion

The JS-10 Jurkat T-cell line, which expresses high levels of \( \alpha_4 \)-integrins, was used to compare monocyte and T-cell adhesion. We have previously shown that VCAM-1 supports the same level of adhesion of the JS-10 Jurkat T-cell line and memory CD4\(^+\)CD45RO\(^+\) T lymphocytes.\(^7\) In contrast, Jurkat T-cell adhesion to AdRSVrVCAM-1–transduced HUVECs was significantly less than monocyte adhesion (Figure 2). This was most notable at the highest level of shear examined (1.5 dyne/cm\(^2\)), where monocyte adhesion was 4-fold higher than Jurkat T-cell adhesion. We again verified that Jurkat T-cell adhesion was entirely abolished by anti–VCAM-1 mAb and by a mAb directed against \( \alpha_4 \)-integrins (data not shown).

Role of Monocyte-Monocyte Interactions

In addition to interacting primarily with endothelial cells, leukocytes can also bind secondarily to leukocytes already adherent to the endothelium. Such secondary attachment has been reported to occur through an L-selectin–dependent mechanism and has been best characterized for neutrophils and lymphocytes.\(^23\) Therefore, we next examined monocyte-monocyte interactions and their impact on overall monocyte adhesion to AdRSVrVCAM-1–transduced monolayers. Monocyte adhesion over 5 minutes at 1.5 dyne was reduced by either a blocking mAb to L-selectin or a blocking mAb to PSGL-1 (a P-selectin ligand) or both (Figure 3a). In contrast, adhesion was not influenced by a control antibody, by a nonblocking mAb to L-selectin, or by a combination of both compared with medium alone. We next determined whether the decrease in adherent monocytes seen with blocking antibodies to L-selectin and PSGL-1 was due to a diminution in monocyte-monocyte interactions or primary interactions with the endothelium. In Figure 3b, we quantified the number of cells entering and adhering to the endothelium in a \( \times 10 \) field, differentiating whether the initial interaction was leukocyte-leukocyte or leukocyte-endothelial. Blocking mAbs LAM1-4 and KPL-1 (or their combination) markedly reduced secondary adhesion of monocytes, whereas primary adhesion...
remained relatively unaffected. Jurkat T cells did not form secondary adhesions at any level of shear tested.

Analysis of Monocyte and Jurkat T-Cell Surface Antigens
Flow cytometric analysis of monocyte and Jurkat T-cell surface antigens was performed to gain insight into quantitative and qualitative differences in adhesion (Table). Jurkat T cells and monocytes exhibited similar levels of \( \alpha_v \)-integrins. Expression of the \( \beta_2 \)-integrin activation epitope, as detected by mAb HUTS21, was present, albeit low, on both cell types. Of note, the levels of L-selectin and PSGL-1 were significantly greater on monocytes. These data suggest that both Jurkat T cells and monocytes express activated \( \alpha_v \)-integrins but that the levels of L-selectin and PSGL-1 are lower on Jurkat cells compared with monocytes. Thus, both cell types can use \( \alpha_v \)-integrins to initially attach and arrest on VCAM-1. However, monocytes are also able to accumulate through monocyte-monocyte interactions; this accumulation of monocytes appears to be functionally related to their increased surface expression of L-selectin and PSGL-1 (Figure 3 and Table).

Monocyte Adhesion to TNF-Stimulated Versus AdRSVrVCAM-1–Transduced HUVECs: Differences in Initial Attachment Rates
As previously described, adhesion of either memory T cells or the Jurkat JS-10 cell line under flow to VCAM-1–transduced HUVECs was not significantly different from adhesion to TNF-activated HUVECs. In contrast, overall monocyte adhesion to AdRSVrVCAM-1–transduced monolayers was far less than that seen with TNF-activated HUVECs (Figure 4). To gain insight into these differences, we quantified monocyte initial interaction rates by counting the number of monocytes that entered a 40 field and were captured (either transiently or permanently) by adherent monocytes or endothelial cells (Figure 5). The interaction rates of monocytes to TNF-activated HUVECs significantly exceeded the rate of attachment to AdRSVrVCAM-1–transduced HUVECs at only the highest level of shear examined. Interestingly, in both cases the number of interactions between monocytes and the endothelial monolayer decreased over time as the shear level was decreased. This may be due to monocyte transmigration that transpires over the time it takes to complete the experimental protocol of progressively lower shear rates. Although monocytes transmigrating across the monolayer are counted as firm adhesions, they can no longer serve as the substrate for secondary adhesions.

As noted in our previous study, T cells initially attached but subsequently did not roll downstream at any flow level examined. Monocytes predominantly exhibited a similar phenotypic interaction with AdRSVrVCAM-1–transduced HUVECs. However, some monocyte rolling was evident at all flow levels tested. This phenotype was not affected by antibodies to ICAM-1 or P-selectin. Monocyte but not lymphocyte transmigration was noted after attachment to AdRSVrVCAM-1–transduced HUVECs (Figure 6). Monocyte rolling, firm adhesion, and transmigration were abolished by antibodies to VCAM-1 or its counterligand. Together, these data suggest VCAM-1 can mediate rolling, firm attachment,
and transmigration under flow conditions, when expressed in the context of otherwise unactivated vascular endothelium.

Discussion

The experiments presented here examine the molecular mechanisms of monocyte-endothelial and monocyte-monocyte interactions under defined flow conditions in vitro. They use an adenosine expression system that achieves effective transgene expression of rVCAM-1 with minimal nonspecific perturbation of the endothelial cells. AdRSVrVCAM-1–infected HUVECs support the adhesion of monocytes under defined laminar flow conditions. This adhesion was blocked by an antibody (HP2.1) to the VCAM-1 counterligand (the α4-integrin) or to rVCAM-1 but was unaffected by mAb blockade of P-selectin or β2-integrins. These results demonstrate that VCAM-1 alone is sufficient to support adhesion and transmigration of monocytes across vascular endothelial cells under laminar flow at shear levels up to 1.5 dyne/cm².

The present study is the first, to our knowledge, to compare quantitative differences between T-cell and monocyte adhesion to endothelium expressing VCAM-1 in the absence of overt endothelial activation and the expression of other inducible adhesion molecules. We have previously shown that VCAM-1 supports the same level of adhesion of memory T cells and the JS-10 Jurkat T-cell line. In contrast, monocyte adhesion to VCAM-1–transduced endothelial cells significantly exceeds T-cell adhesion at all levels of shear examined and was as much as 4-fold greater at 1.5 dyne/cm². The greater monocyte adhesion was explained, at least in part, by leukocyte-leukocyte interactions (secondary adhesions), which were not seen with either the Jurkat cell line or primary memory T cells. The difference between monocyte and T-cell adhesion was greatest at the highest level of shear examined, where secondary adhesion may contribute more to overall adhesion than it does at lower levels of shear. Secondary adhesion of monocytes was dependent on L-selectin and the P-selectin ligand, PSGL-1. The lack of lymphocyte secondary tethering may reflect either the absence of L-selectin ligand on primary T cells or the greatly reduced expression of L-selectin and PSGL-1 as seen in the JS-10 Jurkat T-cell line. In either case, the absence of secondary tethering appears to be an important qualitative and quantitative difference between T-cell and monocyte adhesion under flow in vitro.

Interestingly, although VCAM-1 supported only firm adhesion of lymphocytes in this system, it was able to mediate monocyte rolling, firm adhesion, and transmigration when expressed in the context of otherwise unactivated vascular endothelium. Although adhesion molecules are sometimes categorized as mediating only one kind of adhesive interaction, these data suggest that VCAM-1 can mediate or contribute to multiple phenotypic interactions of monocytes with vascular endothelium. Lymphocytes appear to require additional signals present in activated endothelium for transmigration. Monocytes, in contrast, will progress to transmigration after initial VCAM-dependent tethering even in the absence of global activation of endothelial cells. VCAM-1 is more effective at mediating the initial attachment of monocytes than of lymphocytes. In addition, it is sufficient to initiate events culminating in monocyte, but not lymphocyte, transmigration. Together, these data suggest that stimuli inducing vascular VCAM-1 expression in vivo may initiate events leading to monocyte accumulation in the vessel wall and contribute to the predominance of monocytes in atherosclerotic lesions. Of note, the prevalence of monocytes and memory T cells in peripheral blood is comparable. In addition, differences in monocyte-versus-lymphocyte survival, proliferation, or egress may also influence the relative numbers of these cells in lesions.

We have also shown previously that memory T-cell adhesion to AdRSVrVCAM-1–transduced endothelium is quantitatively similar to lymphocyte adhesion to TNF-α–stimulated endothelium (4 hours, 200 U/mL). Therefore, the majority of memory lymphocyte adhesion to TNF-α–stimulated endothelium can be quantitatively accounted for by VCAM-1–dependent adhesion. In contrast, monocyte adhesion to TNF-α–activated endothelium was much greater than to AdRSVrVCAM-1–transduced HUVECs. Insight into these differences in the level of adhesion can be found by comparing the rates of initial monocyte interaction (Figure 5). Of note, the initial rate of monocyte attachment to TNF-α–activated HUVECs significantly exceeds attachment to AdRSVrVCAM-1–transduced HUVECs only at relatively high shear (1.5 dyne/cm²). At this flow level, VCAM-1 alone supports initial attachment of monocytes less effectively than does VCAM-1 in concert with the other adhesion molecules expressed by activated HUVECs. It seems likely that the selectins, absent from AdRSVrVCAM-1–transduced monolayers, contribute significantly to the initial attachment of monocytes to TNF-α–stimulated HUVECs at this level of shear. At 1.0 and 0.5 dyne/cm², there was no significant difference between the attachment rates of monocytes to TNF-α–activated compared with AdRSVrVCAM-1–transduced
HUVECs. This suggests that at 1.0 and 0.5 dyne/cm², VCAM-1 alone is able to support initial monocyte attachment as effectively as all the adhesion molecules expressed by TNF-α-activated HUVECs, including the selectins. Interestingly, at these lower flow levels, TNF-α-stimulated HUVECs still support significantly greater overall firm adhesion of monocytes than do AdRSVrVCAM-1–transduced HUVECs. Therefore, at these lower flow rates, the smaller number of firmly adherent monocytes seen on AdRSVrVCAM-1–transduced endothelium primarily reflects the failure of initially interacting monocytes to remain adherent. On the basis of these data, we would hypothesize that coexpression of E- or P-selectin with VCAM-1 might further enhance monocyte accumulation at 1.5 dyne/cm² or even higher flow but have less effect at lower shear, where initial attachment is already comparable to TNF-α-activated endothelium. At 0.5 to 1.0 dyne/cm², the addition of molecules that further enhance firm adhesion, such as ICAM-1, would more likely increase monocyte accumulation. Finally, because the majority of T-cell adhesion to TNF-α-stimulated endothelium can be quantitatively accounted for by VCAM-1–dependent adhesion, we further hypothesize that concomitant expression of other adhesion molecules or cytokines in our system will augment even more dramatically the differences between monocyte and lymphocyte adhesion.

The in vitro experiments presented here demonstrate monocyte adhesion at shear levels up to 1.5 dyne/cm², a level comparable to those used in other in vitro flow studies. In vivo, multiple physical and biological factors not present in our flow chamber are thought to augment leukocyte adhesion at higher levels of shear. In addition, the shear forces used in the present study are comparable to those found in intimal neovascular microvessels present in atherosclerotic plaques. These microvessels may provide an important portal of entry for leukocytes into the arterial wall. VCAM-1 expression is more prevalent on the neovascularization than on the luminal endothelium in human atherosclerosis. Moreover, neovascular VCAM-1 expression correlates with increased intimal lymphocyte and macrophage accumulation. Extrapolation of our in vitro results to the in vivo setting will require further investigation in animal models of leukocyte trafficking. Adenoviral gene transfer in animal models that use intravital microscopy could be used to directly address possible causal relationships between VCAM-1 expression and monocyte recruitment in vivo.

These data demonstrate that VCAM-1 supports the adhesion of the primary leukocyte populations present in atherosclerotic plaque and may contribute to the quantitative predominance of monocytes over lymphocytes. Adenoviral gene transfer can serve as the basis for a useful model to reconstitute and thus define the endothelial signals important in monocyte recruitment. Dissecting the role of these molecules in monocyte recruitment may have important clinical implications.

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