Role of endothelial MCP-1 in monocyte adhesion to inflamed human endothelium under physiological flow

U. MAUS, S. HENNING, H. WENSCHUH, K. MAYER, W. SEEGER, AND J. LOHMEYER

Maus, U., S. Henning, H. Wenschuh, K. Mayer, W. Seeger, and J. Lohmeyer. Role of endothelial MCP-1 in monocyte adhesion to inflamed human endothelium under physiological flow. Am J Physiol Heart Circ Physiol 283: H2584–H2591, 2002. First published August 29, 2002; 10.1152/ajpheart.00349.2002.—Monocyte chemoattractant protein-1 (MCP-1) is an essential chemokine involved in monocyte traffic across endothelial and epithelial barriers both in vitro and in vivo. However, the contribution of endothelial MCP-1 signaling via its CCR2 receptor in monocyte adhesion to inflamed endothelium under flow is incompletely understood. A sensitive flow chamber assay was used to assess monocyte adhesion to TNF-α-activated primary human pulmonary artery endothelial cells (HPAEC) during physiological shear stress. Monocyte adhesion was markedly reduced (~45%) when HPAEC-derived MCP-1 was either neutralized with anti-MCP-1 mAb or inhibited by translational arrest of MCP-1 mRNA transcripts with MCP-1 antisense oligomers. Corresponding efficacy was observed for blockade of monocyte CCR2 receptor function by anti-CCR2 mAb or MCP-1 antagonists (9–76 analog). The impact of endothelial MCP-1 on monocyte-HPAEC adhesion occurred via β2-integrin but not via β1-integrin adhesion pathways. In this line, pretreatment of monocytes with MCP-1 but not RANTES provoked a rapid and transient neoepitope 24 expression on β2-integrin α-chains, as analyzed by increased reporter mAb24 binding. Collectively, our data show an important cross talk of endothelial MCP-1 with monocyte CCR2 effecting monocyte firm adhesion to inflamed HPAEC under physiological flow conditions.

monocytes/macrophages; adhesion molecules; chemokines; cell trafficking; monocyte chemoattractant protein-1

VARIOUS STUDIES in the past few years have demonstrated that monocyte chemoattractant protein (MCP-1) is the major chemoattractant for monocyte recruitment across endothelial cells (EC) as well as epithelial cells both in vitro and in vivo (9, 17, 25). Intra-alveolar overexpression of MCP-1 in pulmonary type II alveolar epithelial cells of mice was found to provoke a massive accumulation of monocytes within the alveolar air spaces (9). More recently, studies from our laboratory showed that intra-alveolar deposition of recombinant murine JE/MCP-1 in mice elicited substantial intra-alveolar accumulation of peripheral blood-derived monocytes. This further suggests that locally liberated low-molecular-weight chemokines like MCP-1 increase chemoattraction of circulating monocytes across biological barriers including EC and epithelial cell layers (17, 19).

There are, however, only a few studies with inconsistent results addressing the effects of MCP-1 on early monocyte recruitment events, such as adhesion to inflamed endothelium. MCP-1 was shown to be a major contributor to the robust arrest of monocytes to inflamed endothelium under shear stress conditions in vitro (5, 16). Furthermore, Palframan and co-workers (24) recently demonstrated a specific role of exogenous MCP-1 mediating monocyte arrest to high endothelial venule (HEV)-draining lymph nodes of inflamed skin in vivo. In contrast, another report (29) identified a specific contribution of growth-related oncogene (GRO)-α but not MCP-1 to the monocyte adhesion process to inflamed human umbilical vein EC (HUVEC) in vitro. Also, a recent report suggested a specific role of monokine induced by interferon-γ but not MCP-1 in the monocyte adhesion process to HEV draining inflamed lymph nodes in vivo (12).

The present study was designed to analyze a possible role of endothelium-derived MCP-1 on monocyte adhesion in the pulmonary vasculature. We specifically interrupted the cross-talk between MCP-1 and its receptor, CCR2, on the monocyte surface. MCP-1 antisense oligomers that result in translational arrest of endothelial MCP-1 synthesis, MCP-1 antagonists (9–76 analog) that block CCR2 downstream signaling, and neutralizing anti-MCP-1 or function-blocking anti-CCR2 mAbs were employed in a highly sensitive flow chamber assay of human pulmonary EC. The data show that MCP-1 liberated from inflamed human pulmonary artery endothelium is capable of effecting monocyte adhesion under physiological shear-stress conditions by promoting increased activation of β2-integrin adhesion pathways. These findings may be relevant for a further understanding of pulmonary artery remodeling processes, as observed in different types of pulmonary arterial hypertension and vasculitic processes involving the lung (13).

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MATERIALS AND METHODS

Monoclonal antibodies and reagents. The following adhesion function-blocking murine mAbs specific for human antigens were employed: clone 38 (anti-CD11a, R&D Systems; Wiesbaden, Germany), clone 44 (anti-CD11b, R&D Systems); MEM 48 (anti-CD18, R&D Systems), HP2/1 [anti-α4 chain of VLA-4 (CD49d), Serotec; Oxford, UK], and SIE4A4 [anti-major histocompatibility complex (MHC) class I, a generous gift from M. Hadam; Hannover, Germany]. The reporter major histocompatibility complex (MHC) class I, a generous gift from M. Hadam; Hannover, Germany]. The phosphorothioate-modified antisense oligodeoxynucleotides (PS-ODN) (MCP-1 AS2) and corresponding scrambled control oligodeoxynucleotides employed in the present study have been characterized recently (18, 20, 21). The phosphorothioate-modified antisense oligodeoxynucleotides (PS-ODN) (MCP-1 AS2) and corresponding scrambled control oligodeoxynucleotides employed in the present study have been characterized recently (18, 20, 21). The phosphorothioate-modified antisense oligodeoxynucleotides (PS-ODN) (MCP-1 AS2) and corresponding scrambled control oligodeoxynucleotides employed in the present study have been characterized recently (18, 20, 21).

Neutralization of endothelium-derived MCP-1 in response to TNF-α activation was performed by preincubating EC with saturating amounts of neutralizing anti-MCP-1 mAb (5 µg/ml, determined in pilot experiments) for 30 min at 37°C, 5% CO₂, as indicated. HPAEC monolayers were mounted in the flow chamber and washed to remove the neutralizing anti-MCP-1 mAb before being perfused with the appropriately pretreated monocytes.

Flow chamber assay. Monocyte adhesion to confluent monolayers of TNF-α-activated (2.5 nm/g, 6 or 12 h) HPAEC was investigated using a parallel plate flow chamber. Briefly, monocytes (1 × 10⁶ cells/ml) resuspended in RPMI-1% FCS were allowed to flow over HPAEC under physiological shear-stress conditions using a perfusion pump adjusted to a constant shear rate of 1.5 dyn/cm² at 37°C (model WPI-SP1001, World Precision Instruments; Berlin, Germany). Monocyte interaction with the endothelial monolayer was visualized by videomicroscopy (model TK-C1381, JVC digital charge-coupled device camera connected to an inverted microscope (Zeiss Axiovert S-100; Wetzlar, Germany)) using ×100 magnification. After a 5-min observation period, monocytes firmly adhered to the HPAEC monolayer were counted per high-power field (HPF) (mean ± SD of 5 randomly chosen HPF evaluated). Each experiment was repeated at least three to six times.

Flow cytometry of reporter mAb24 expression on monocytes in response to MCP-1, RANTES, or PMA and on lymphocytes in response to RANTES. Induction of 24 neoeptope expression was used to evaluate the different effects of MCP-1 and RANTES on the activation state of β₂-integrin α-chains. Monocytes were stimulated with Ca²⁺/Mg²⁺-free conditions (PBS without Ca²⁺/Mg²⁺ containing 5 mM EDTA (Seromed; Berlin, Germany), washed, and resuspended at 5 × 10⁶ cells/ml in Ca²⁺/Mg²⁺-free HBSS supplemented with 1 mM MgCl₂ (Sigma; Deisenhofen, Germany) and 0.5% human serum albumin (HSA; Baxter; Unterschleissheim, Germany) for 30 min at 37°C, 5% CO₂. Subsequently, incubation of cells with mAb24 (1:10 dilution of cell culture supernatant) or control IgG in microtiter plates (Becton-Dickinson; Heidelberg, Germany) was performed in the absence or presence of MCP-1, RANTES, or PMA for various time periods, as indicated. The microtiter plates were then placed on ice and the samples were washed once in ice-cold HBSS-1 mM MgCl₂-0.5% HSA, supplemented with 0.01% NaN₃, followed by incubation of the samples with phycoerythrin-conjugated rat anti-mouse reagent (Dako; Hamburg, Germany). In selected experiments, lymphocytes isolated under the same experimental conditions as described for monocytes were stimulated with recombinant RANTES for different time points. Analysis of monocyte or lymphocyte mAb24 expression was performed using a FACScan station and CellQuest software (Becton-Dickinson; San Jose, CA).

RESULTS

Effect of MCP-1 antisense oligomers or anti-MCP-1 monoclonal antibodies on endothelial adhesion of monocytes under physiological flow. Less than 5% of untreated monocytes adhered to resting HPAEC or HUVEC (2 ± 0.5 cells/HPF, mean ± SD, n = 7) under physiological shear-stress conditions. Inflammatory activation of HPAEC or HUVEC with TNF-α strongly increased firm adhesion of monocytes under these conditions (HPAEC: 97.7 ± 3 cells/HPF and HUVEC: 95.5 ± 2.5 cells/HPF, means ± SD, n = 7; Fig. 1, A and B). Depletion of endothelium-derived MCP-1 by pre-
Fig. 1. Effect of anti-monocyte chemotactrant protein (MCP)-1 mAbs or MCP-1 antisense (AS) oligomers on adhesion of monocytes to inflamed human pulmonary artery endothelial cells (HPAEC; A) or human umbilical vein endothelial cells (HUVEC; B) under physiological flow. HPAEC or HUVEC were grown to confluence on Thermanox membranes. The cells were then 1) left untreated; 2) stimulated with TNF-α (2.5 ng/ml, set 100%); 3) preincubated with either antisense or scrambled control phosphorothionate-modified oligodeoxynucleotide (PS-ODN) followed by TNF-α activation; or 4) incubated with anti-MCP-1 or anti-RANTES (regulated upon activation normal T cell expressed and secreted) mAb subsequent to activation with TNF-α, as indicated. In addition, reconstitution with exogenous MCP-1 (500–1,000 ng/10⁶ cells) was undertaken in the experiments with endothelial MCP-1 antisense pretreatment. Data are given as means ± SD of 7 independent experiments (HPAEC) and 5 independent experiments (HUVEC), respectively. * P at least <0.05 compared with monocyte adhesion to TNF-α- or AS2-treated endothelial cells without further intervention.

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- TNF + anti-MCP-1 mAb
- TNF + anti-RANTES mAb

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- TNF + anti-MCP-1 mAb
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Effect of endothelium-derived MCP-1 depletion on β₂ and β₁-integrin-dependent adhesion of monocytes to HPAEC under flow. To evaluate whether MCP-1 depletion differentially affected β₁- versus β₂-integrin-dependent pathways of monocyte adhesion to HPAEC, endothelium-derived MCP-1 depletion procedures were performed subsequent to blockade of one of these integrins. In agreement with a previous report (14), monocyte adhesion to TNF-α-activated HPAEC under physiological shear stress was dependent on β₂-integrins LFA-1 and Mac-1 and on β₁-integrin VLA-4. Inhibition of either integrin α-chain by specific adhesion function-blocking mAbs reduced monocyte adhesion by ~40%, whereas isotype-matched control antibody was without effect (Fig. 3A). Simultaneous blockade of both β₂-integrin α-chains CD11a and CD11b reduced monocyte adhesion by ~60%. The strongest inhibition of monocyte adhesion was ob-

Effect of anti-CCR2 monoclonal antibodies or MCP-1 antagonists on monocyte adhesion to HPAEC or HUVEC under physiological flow conditions. Preincubation of monocytes with a function-blocking anti-CCR2 mAb significantly reduced adhesion of monocytes to TNF-α-activated HPAEC by ~45% (Fig. 2A), with only a minor impact on HUVEC (Fig. 2B). Treatment of monocytes with either an isotypic control IgG or anti-MHC class I mAbs was ineffective, demonstrating that antibody binding to the cell surface per se did not interfere with monocyte-endothelium interaction under flow conditions. In addition, interruption of MCP-1 cross-talk with CCR2 by preincubation of monocytes with a 9–76 MCP-1 antagonist similarly reduced monocyte adhesion to inflamed HPAEC (~40%; Fig. 2A) with moderate effects on HUVEC (Fig. 2B). However, pretreatment of monocytes with heat-inactivated 9–76 analog was ineffective (Fig. 2, A and B).
Fig. 2. Effect of anti-CCR2 monoclonal antibodies or MCP-1 antagonists on monocyte adhesion to HPAEC or HUVEC under physiological flow. Monocytes were preincubated with 1) function-blocking anti-CCR2 mAb; 2) isotypic control IgG; 3) anti-MHC class I mAb used as an additional control mAb; or 4) 9–76 MCP-1 antagonist (active and heat inactivated), as indicated. Preincubation was followed by flowing the cells over TNF-α-activated HPAEC (A) or HUVEC (B). Data are given as means ± SD of 7 (HPAEC) and 5 (HUVEC) independent experiments, respectively, showing monocyte adhesion as a percentage of monocyte adhesion to TNF-α-activated EC without further intervention. *P at least <0.05 compared with TNF-α challenge alone.

Fig. 3. Effect of endothelial MCP-1 depletion on β2- and β1-integrin-dependent adhesion of monocytes to HPAEC under flow (inhibitory effects are given). Unstimulated monocytes were preincubated with either isotypic control IgG or saturating amounts of function-blocking mAb to β2- and/or β1-integrins (target antigens are indicated). Subsequently, monocytes were flowed over TNF-α-activated HPAEC (A) or MCP-1-depleted plus TNF-α-activated HPAEC (B). In another series of experiments, monocytes were treated with function-blocking mAb followed by stimulation with recombinant MCP-1 (rMCP-1) and subsequently flowed over MCP-1-depleted HPAEC, as indicated (C). The values (means ± SD of 4 independent experiments) are shown as the numbers of firmly adherent monocytes per high-power field (HPF) (means ± SD of 5 HPF evaluated) compared with monocyte adhesion to TNF-α-activated HPAEC. *P at least <0.05 (for columns in B compared with columns in A and for columns in C compared with the respective columns in B).
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served with simultaneous blockade of both β2-integrins LFA-1 and Mac-1 plus blockade of β1-integrin VLA-4 (~75%). These data demonstrate that monocyte firm adhesion to TNF-α-stimulated HPAEC under flow conditions is dependent on both β2- and β1-integrins (Fig. 3A), which only partially act as alternative adhesion pathways. Simultaneous blockade of both β2- and β1-integrin adhesion pathways plus additional depletion of HPAEC-derived MCP-1 did not further decrease monocyte firm adhesion observed with combined anti-CD11a plus anti-CD11b plus anti-VLA-4 blockade (Fig. 3B). This finding demonstrates that residual monocyte adhesion pathways independent from LFA-1, Mac-1, or VLA-4 mediating firm arrest on inflamed endothelium are not sensitive to endothelium-derived MCP-1. Similarly, β1-integrin (VLA-4)-dependent adhesion of anti-CD11a- plus anti-CD11b-pretreated monocytes was only marginally affected by depletion of endothelium-derived MCP-1, suggesting a minor role for MCP-1 in β1-integrin-mediated adhesion. In contrast, β2-integrin (CD11a and CD11b)-dependent adhesion of anti-VLA-4-treated monocytes was further markedly reduced by depletion of endothelium-derived MCP-1, which clearly suggests a primary effect of MCP-1 on β2-integrin (CD11a and CD11b) adhesion involved in monocyte arrest to inflamed endothelium. Moreover, depletion of endothelium-derived MCP-1 enhanced the inhibitory effect of anti-CD11a alone or anti-CD11b alone treatment of monocytes, demonstrating that MCP-1 exerts its effect on both β2-integrin α-chains LFA-1 and Mac-1.

When monocytes were reacted with anti-CD11a or anti-CD11b and subsequently stimulated with low doses of recombinant MCP-1 (500–1,000 pg recombinant MCP-1/10^6 cells) immediately (~30 s) before perfusion over MCP-1-depleted HPAEC, the inhibitory effect of endothelial MCP-1 depletion was fully reversed (Fig. 3C). Similar results were observed when monocytes were treated with anti-VLA-4 followed by MCP-1 stimulation and immediate perfusion over MCP-1-depleted HPAEC (P < 0.05; Fig. 3C). This again suggests a primary effect of MCP-1 on β2-integrin-dependent adhesion pathways (Fig. 3C). In contrast, when monocytes were treated simultaneously with anti-CD11a and anti-CD11b followed by incubation with recombinant MCP-1 before perfusion over MCP-1-depleted HPAEC, no significant increase in monocyte adhesion was observed, suggesting that depletion of MCP-1 primarily affects β2-integrin rather than β1-integrin monocyte adhesion pathways. Furthermore, stimulation of anti-CD11a plus anti-CD11b plus anti-VLA-4 treated monocytes with recombinant MCP-1 before perfusion over MCP-1-depleted HPAEC did not increase the numbers of arresting cells. This suggests that adhesion pathways other than β1- and β2-integrins mediating the observed residual adhesion of monocytes to MCP-1-depleted HPAEC (Fig. 3B) were not MCP-1 responsive.

Effect of MCP-1, RANTES, and PMA on activation-dependent 24 neoepitope expression on β2-integrin α-chains. To further investigate the effect of MCP-1 on monocyte β2-integrin α-chain activity, we analyzed the “reporter” expression of the 24 neoepitope on β2-integrin α-chains. Stimulation of monocytes with MCP-1 (Fig. 4, A–E) induced a rapid and transient expression of the 24 neoepitope on β2-integrin α-chains peaking between 30 and 150 s posttreatment, whereas stimulation of monocytes with RANTES was ineffective (Fig. 4, F–H). Stimulation of monocytes with PMA (Fig. 4, I–N), which is known to activate integrins independently of G protein-linked receptors, induced a sustained 24 neoepitope expression that was still detectable at 20 min posttreatment. Contrary to the results observed with monocytes, stimulation of lymphocytes with recombinant RANTES induced a rapid expression of the 24 neoepitope peaking at 150 s posttreatment with a decline thereafter, demonstrating that the recombinant RANTES preparations used were biologically active (Fig. 4, O–R).

**DISCUSSION**

Interrupting the MCP-1-based cross-talk between EC (MCP-1 synthesis) and monocytes (expression of the corresponding CCR2 receptor) profoundly reduced monocyte adhesion to inflamed HPAEC under physiological shear-stress conditions. Depletion of HPAEC-derived MCP-1 was found to primarily affect β2-integrin (LFA-1 and Mac-1)-mediated monocyte adhesion pathways. Importantly, pretreatment of monocytes with exogenously administered recombinant MCP-1 fully restored firm adhesion of monocytes to MCP-1-depleted HPAEC under physiological flow conditions. Moreover, MCP-1 rapidly and transiently induced the 24 neoepitope on monocytes, reflecting increased chemokine-induced β2-integrin α-chain activity. These data support the hypothesis that endothelium-derived MCP-1 primes monocytes for increased adhesion to inflamed endothelium under physiological shear-stress conditions, primarily by enhancing the activity of monocyte β2-integrin adhesion molecules.

The role of MCP-1 as a major chemoattractant for monocytes has been clearly established both in vitro and in vivo. Various mouse models have revealed a causal relationship between increased local release of MCP-1 and monocyte accumulation at extravascular sites (4, 8, 9). We recently used, in mice, a newly established technique for identifying monocytes recruited to the lung alveoli to demonstrate that local (intra-alveolar) deposition of recombinant JE/MCP-1 in mice provoked substantial and dose-dependent recruitment of circulating monocytes into the alveolar spaces (17, 19). This observation suggests that low-molecular-weight chemokines such as MCP-1 are capable of passing biological cell barriers, such as epithelial cells and EC, to establish a chemokine gradient capable of attracting monocytes to extravascular sites (8, 17). In fact, interruption of the MCP-1 cross-talk with its receptor, CCR2, has been previously shown to significantly affect leukocyte adhesion to cremaster muscle microvascular endothelium in knockout mice.
lacking the MCP-1 receptor CCR2, thereby decreasing the number of transmigrating monocytes (15).

To study the impact of endothelial MCP-1 on firm monocyte-endothelium adhesion in more detail, we used both immunologic and translation regulatory interventions to interrupt MCP-1/CCR2-based cross-talk in a flow chamber assay of monocyte arrest on TNF-α-activated HPAEC under physiological flow conditions.

We used a recently developed antisense-based approach that specifically and effectively (>85%) blocks TNF-α-induced MCP-1 synthesis in HPAEC by translational arrest of MCP-1 mRNA transcripts. This blockade does not affect endothelial ICAM-1 and VCAM-1 cell surface expression (18, 20). This molecular strategy markedly reduced monocyte arrest to TNF-α-activated HPAEC, which was reproduced by employment of anti-

Fig. 4. Effect of MCP-1, RANTES, or PMA on activation-dependent 24 neoepitope expression on monocyte β₂-integrin α-chains. Monocytes were stimulated with MCP-1 (A–E), RANTES (F–H), or PMA (I–N) for various time points in the absence or presence of mAb24 followed by flow cytometric analysis, as described in MATERIALS AND METHODS. In addition, in some experiments, lymphocytes were stimulated with RANTES for various time points, as indicated (O–R). Shaded histograms show mAb24 binding to the cell surface of unstimulated monocytes or lymphocytes, respectively. Open histogram overlays show mAb24 binding to the monocyte or lymphocyte surface after stimulation, as indicated. The x-axis shows fluorescence 2 emission (FL-2; F488/575, log scale). The y-axis shows relative cell numbers (linear scale). A representative fluorescence-activated cell sorting profile from four independent experiments is shown.
MCP-1 but not anti-RANTES monoclonal antibodies. Notably, the antisense-induced inhibition of monocyte firm adhesion was fully restored when monocytes were treated with low concentrations of recombinant MCP-1 immediately before perfusion through the flow chamber. Interestingly, a sensitive cell surface ELISA did not detect either TNF-α-induced endothelial MCP-1 or exogenously added recombinant MCP-1 immobilized on the HPAEC cell surface (data not shown). This suggests that immobilization on the EC surface is not a precondition for MCP-1 to promote monocyte adhesion to endothelium under flow.

In another study (10) investigating the adhesion of monocytes to atherosclerotic lesions of carotid arteries from ApoE−/− mice ex vivo, a significant contribution of KC/GRO-α but not JE/MCP-1 to monocyte arrest was observed. In contrast, we found that blockade of the CCR2 receptor on the monocyte cell surface with either anti-CCR2 mAbs (23) or a specific 9–76 MCP-1 antagonist (6, 7) significantly reduced monocyte adhesion to inflamed HPAEC to a similar extent as observed for anti-MCP-1 mAb or MCP-1 antisense pretreatment of HPAEC. However, both pretreatment of monocytes with the 9–76 MCP-1 antagonist or anti-CCR2 mAb as well as MCP-1 antisense or anti-MCP-1 mAb pretreatment of HUVEC was much less effective in reducing endothelial monocyte adhesion compared with HPAEC. Thus, from data currently available, it appears that both EC from different organs and their inflammatory activation (acute versus chronic inflammation) may considerably affect their inducible chemokine/cytokine profiles and related monocyte adhesion events. Against the background that HUVEC are often considered as a “standard” EC type, it is particularly relevant that there are actually many differences in phenotype between EC from different vascular beds (22, 26). Future studies with macro- and microvascular EC isolated from different organs should lead to a better understanding of endothelium-derived chemokine-dependent leukocyte arrest under flow conditions.

Characterization of molecular pathways of monocyte adhesion to TNF-α-activated HPAEC revealed that both β2-integrins LFA-1 and Mac-1 as well as the β1-integrin VLA-4 contributed equally to firm adhesion under physiological flow conditions, in agreement with a previously published report (14). Interestingly, blockade of LFA-1, Mac-1, or VLA-4 alone and subsequent perfusion of monocytes over MCP-1-depleted HPAEC further significantly reduced monocyte firm adhesion. In particular, anti-β1-integrin (VLA-4)-pretreated monocytes showed a significantly reduced adhesion to MCP-1-depleted HPAEC compared with control EC, and this was fully restored by exogenous administration of recombinant MCP-1. In contrast, anti-β2-integrin α-chain (CD11a and CD11b)-pretreated monocytes showed a comparable degree of firm adhesion to MCP-1-depleted HPAEC as to non-MCP-1-depleted HPAEC. Furthermore, such pretreated monocytes did not respond with increased firm adhesion to exogenous MCP-1 stimulation. These findings indicate that endothelium-derived MCP-1 predominantly affects β2-integrin-based monocyte adhesive interactions with HPAEC, whereas the contribution of VLA-4 to monocyte endothelium adhesion is largely MCP-1 independent.

Chemokine modulation of leukocyte integrin activity has been reported for various leukocyte subpopulations (1, 27, 28). Therefore, we tested whether MCP-1 was capable of increasing β2-integrin α-chain activity, using the 24 neoepitope expression as a “reporter” to monitor this α-chain activity (2, 3). Indeed, we found that stimulation of monocytes with MCP-1 but not RANTES induced a very rapid and transient activation of β2-integrin α-chains, occurring as early as ~30 s after induction. This MCP-1-induced 24 neoepitope expression on the monocyte cell surface corresponds well to the time frame necessary to restore reduced monocyte adhesion to MCP-1-depleted HPAEC by pretreatment of the monocytes with exogenous MCP-1 (~30 s).

With the use of several various translation regulatory and immunologic interventions to interrupt MCP-1 cross-talk with its receptor on the monocyte cell surface, we demonstrated that endothelial-derived MCP-1 primes monocytes for increased firm adhesion to TNF-α-activated HPAEC under physiological flow conditions. Inhibition of HPAEC-derived MCP-1 synthesis only weakly affected β1-integrin (VLA-4) but strongly diminished β2-integrin (LFA-1 and Mac-1) adhesion-dependent pathways, which was fully rescued by exogenous recombinant MCP-1. Together with the analysis of monocyte β2-integrin activity changes in the presence of MCP-1, these data suggest that endothelial MCP-1 exerts its effect on firm monocyte adhesion to inflamed endothelium via modulation of β2-integrin α-chain activity.

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REFERENCES


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