Essential role of ICAM-1 in mediating monocyte adhesion to aortic endothelial cells

CHRISTOPHER G. KEVIL,1 RAKESH P. PATEL,2 AND DANIEL C. BULLARD1
Departments of 1Genomics and Pathobiology and 2Pathology, University of Alabama at Birmingham, Birmingham, Alabama 35294-0019

Received 4 May 2001; accepted in final form 10 July 2001

Kevil, Christopher G., Rakesh P. Patel, and Daniel C. Bullard. Essential role of ICAM-1 in mediating monocyte adhesion to aortic endothelial cells. Am J Physiol Cell Physiol 281: C1442–C1447, 2001.—Monocyte-endothelial cell interactions are thought to be critical for the initiation and progression of many inflammatory vascular diseases that target arterial and aortic endothelium, including atherosclerosis. Many different adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, are thought to mediate monocyte binding to endothelial cells during the development of these diseases. However, conflicting results have been reported regarding the specific role of ICAM-1 in these events. In this study, we used a genetic approach to determine the contribution of ICAM-1 in mediating monocyte adhesion to mouse aortic endothelial cells (MAEC) derived from both wild-type and ICAM-1−/− mice. Treatment of wild-type MAEC with oxidized low-density lipoprotein significantly induced both WEHI 274.1 and whole blood monocyte adhesion, whereas similarly treated ICAM-1−/− MAEC showed a complete inhibition of monocyte binding. Dose-response treatment with tumor necrosis factor-α also increased monocyte adhesion to wild-type MAEC, but significant adhesion was only observed at higher doses for ICAM-1−/− MAEC. These data demonstrate a crucial role for ICAM-1-mediated monocyte-endothelial cell interactions in response to specific stimuli involved in inflammatory vascular diseases.

atherosclerosis; oxidized lipids; inflammation; gene targeting; leukocyte; intercellular adhesion molecule

Monocyte-endothelial cell interactions are thought to be critical for the initiation and progression of many inflammatory vascular diseases that involve arterial and aortic endothelium, such as atherosclerosis and vasculitis. An early event in atherosclerotic lesion development is the recruitment of monocytes into subendothelial segments of arteries, which ultimately leads to the formation of foam cells (6, 36). Similarly, monocyte infiltration of arteries and arterioles is observed during vasculitic lesion formation in diseases such as Wegener's granulomatosis, polyarteritis nodosa, and giant cell arteritis (15). Many different adhesion molecules are thought to facilitate monocyte-endothelial cell interactions in these diseases, including the selectins, intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM)-1, and the integrin ligand VLA-4 (very late antigen). However, the specific interactions and individual contributions of these adhesion molecules involved in monocyte binding remain to be fully determined.

ICAM-1 is a member of the immunoglobulin superfamily of adhesion molecules and is expressed on many different cell types including endothelium (10, 41). This adhesion molecule, through interactions with its β2-integrin ligands LFA-1 (lymphocyte function-associated antigen) and Mac-1, is thought to play key roles in inflammatory and immune responses by mediating leukocyte firm adhesion, transendothelial migration, and lymphocyte costimulation. Several different pieces of experimental evidence implicate ICAM-1 interactions in monocyte/arterial adhesion events during the development of atherosclerosis and vasculitic disorders (15, 36). For example, increased ICAM-1 expression has been correlated with increased infiltration of monocytes in disease specimens and in animal models (7, 13, 23, 28, 33, 36, 37). In addition, many of the inflammatory mediators associated with endothelial cell activation in these diseases, such as oxidized lipids and cytokines, can significantly increase ICAM-1 expression on cultured endothelial cell lines (12, 14, 46). Finally, loss or inhibition of ICAM-1 expression has been reported to decrease both atherosclerotic and vasculitic lesion formation in animal models (3, 4, 27, 29, 32). Although these reports suggest that ICAM-1 may be an important mediator of monocyte adhesion during these diseases, the examination of monocyte adhesion to ICAM-1-deficient endothelium has not been determined.

In vitro endothelial model systems have been used to investigate the contribution of ICAM-1 to monocyte adhesion (19–21, 26). Although it appears from these studies that ICAM-1 is at least partially involved in monocyte binding in response to interleukin-1β, high glucose, and endotoxin, conflicting reports exist regarding the role of ICAM-1 in response to other inflammatory stimuli, such as oxidized low-density lipoproteins (oxLDL) and tumor necrosis factor (TNF)-α (9, 12, 17, 26, 38, 42). For example, Erl et al. (9) have shown...
that Mac-1-ICAM-1 interactions contribute to monocyte adhesion in response to oxLDL, whereas Shih et al. (39) have reported that ICAM-1 was not involved in oxLDL-stimulated monocyte binding. These findings may be due to differences in the experimental reagents used, the source of endothelial and monocyte cell populations, and the methods used to inhibit adhesion molecule interactions.

This study employs a genetic approach to specifically examine the role of ICAM-1 in initiating monocyte adhesion to aortic endothelium in response to TNF-α or oxLDL. Aortic endothelium from normal and gene-targeted ICAM-1-deficient (ICAM-1−/−) mice were isolated and cultured, and adhesion assays were performed with the use of both a monocyte cell line and whole blood monocytes. We report that monocyte adhesion to ICAM-1−/− mouse aortic endothelial cells (MAEC) was completely inhibited in response to oxLDL. In contrast, loss of ICAM-1 resulted in a significant, but incomplete, attenuation of monocyte binding following TNF-α treatment. These data clearly demonstrate that ICAM-1 is critical for monocyte adhesion to aortic endothelium in response to two different inflammatory stimuli implicated in the development of cardiovascular disease. Moreover, these findings illustrate that the requirement for ICAM-1-mediated adhesion can vary depending on the type and strength of the inflammatory stimulus.

MATERIALS AND METHODS

Mice. Wild-type C57BL/6J mice were obtained from Jackson Laboratory (Bar Harbor, ME). The ICAM-1 mutant mice used in these studies were backcrossed 12 generations onto the C57BL/6J strain background (40).

Endothelial isolation procedure. Endothelial cells were isolated as previously described (16). Isolated cells were plated onto 0.5% gelatin-coated six-well plates in medium containing MCDB-131 (pH 7.2), 10% fetal bovine serum (GIBCO), 1 mg/ml penicillin-streptomycin (GIBCO), 1 μg/ml hydrocortisone (Sigma), 10 U/ml heparin (Sigma), and 50 μg/ml endothelial mitogen (Biomedical Technologies). Cell passages 3 and 5 were used for these studies with no differences noted between passage numbers.

Monocyte cell culture. The mouse monocyte cell line WEHI 274.1 was purchased from American Type Culture Collection and maintained in suspension culture as previously described (43). Cells were labeled with 1 μM 2',7'-bis-2-carboxyethyl-5-carboxyfluorescein acetoxyethyl ester for adhesion assays (Molecular Probes).

Monocyte adhesion assay. Endothelial monolayers were treated with 0, 1, 5, 10, or 25 ng/ml TNF-α or 100 μg/ml LDL, which had been oxidized to differing degrees, for 6 h. WEHI 274.1 adhesion was determined as previously reported (14, 16). Whole blood monocyte adhesion assays were performed as previously described (35). Briefly, mouse whole blood was diluted 1:10 in Hanks' balanced salt solution and added to endothelial cell monolayers at 37°C for 15 min. Nonadherent cells were removed, and endothelial monolayers were washed. Adherent monocytes were determined by differential staining and counted. Some assays were also performed with 5 μg/ml of the ICAM-1-blocking antibody YN1 (11). Increased monocyte adhesion is reported as a percentage or the amount of normalized adhesion. Normalized adhesion was used to clearly demonstrate the differences in monocyte binding in response to YN1.

Isolation and oxidative modification of LDL. Human LDL was isolated from plasma of healthy donors as previously described (30, 31). LDL was oxidatively modified by using copper ions, and the reaction was stopped by the addition of diethylenetriaminepentaacetic acid (50 μM). The degree of oxidative modification of LDL was determined by measuring the relative electrophoretic mobility (REM) using lipogel electrophoresis (Paragon). The specific oxidation products in oxLDL that are responsible for stimulation of monocyte-endothelial cell interactions have been previously shown to reside in a mildly oxidized or minimally modified LDL (2). OxLDL preparations with REM values between ~1.2 and 2.1 were used because these encompass the range in which minimally modified LDL resides.

Statistical analysis. TNF-α or oxLDL-mediated monocyte adhesion data were analyzed by one-way ANOVA with Bonferroni posttest vs. control. Direct comparison of monocyte adhesion between endothelial cell types was determined by using unpaired t-test. All data are expressed as means ± SE.

RESULTS

WEHI 274.1 monocyte adhesion to oxLDL-stimulated mouse aortic endothelium. Initial adhesion studies were performed with the mouse monocyte cell line WEHI 274.1. Both wild-type and ICAM-1−/− MAEC were treated with LDL that had been oxidatively modified (oxLDL) to varying degrees (Fig. 1). Oxidative modification was determined by REM, which reflects oxidative damage to the protein component of LDL that directly correlates with formation of oxidized lipids (30). Figure 1 shows that 100 μg/ml oxLDL at both REM 1.2 and REM 1.6 significantly increased WEHI 274.1 adhesion to wild-type MAEC (11.21 ± 6 and 11.61 ± 1.36%). Importantly, native LDL treatments did not increase WEHI 274.1 adhesion to ICAM-1−/− or wild-type MAEC (data not shown). Direct comparison between wild-type and ICAM-1−/− MAEC revealed a
significant overall attenuation of monocyte adhesion for all oxLDL treatments. These data clearly demonstrate that ICAM-1 is essential for monocyte adhesion to aortic endothelium in response to oxidized lipids.

**WEHI 274.1 monocyte adhesion to TNF-α-activated mouse aortic endothelium.** Figure 2 shows the amount of WEHI 274.1 adhesion to TNF-α-stimulated wild-type or ICAM-1−/− MAEC. Treatment of wild-type MAEC with various doses of TNF-α caused a significant increase in WEHI 274.1 adhesion (Fig. 2A). Stimulation with 1, 5, 10, and 25 ng/ml of TNF-α caused 22.27 ± 0.54, 35.12 ± 1.59, 38.12 ± 1.53, and 39.2 ± 2.08% adhesion of WEHI 274.1, respectively. These data demonstrate that TNF-α stimulates a dose-response increase in WEHI 274.1 adhesion to wild-type MAEC that is maximal at 5 ng/ml TNF-α. ICAM-1−/− MAEC were also treated with various doses of TNF-α (Fig. 2B). Doses of 1 and 5 ng/ml TNF-α did show a trend toward increased WEHI 274.1 adhesion, but this was not statistically significant compared with 0 ng/ml TNF-α. However, treatment of ICAM-1−/− MAEC with 10 or 25 ng/ml TNF-α did result in a significant increase in WEHI 274.1 adhesion (17.17 ± 3.44 and 17.87 ± 4.23%, respectively). Direct comparison of the 5 and 10 ng/ml TNF-α treatments between wild-type and ICAM-1−/− MAEC showed a significant reduction in the amount of WEHI 274.1 adhesion to ICAM-1−/− MAEC (Fig. 2C). Adhesion to ICAM-1−/− MAEC was reduced an average of 72 and 55% in response to 5 and 10 ng/ml TNF-α, respectively.

**YN1 inhibition of monocyte adhesion.** We next investigated for potential differences in ICAM-1 function on wild-type endothelium by using the ICAM-1-blocking antibody YN1 (11). Figure 3 shows the effects of 5 μg/ml YN1 on WEHI 274.1 adhesion after TNF-α or oxLDL treatment. Figure 3A shows that YN1 significantly decreased monocyte adhesion to TNF-α-stimulated wild-type MAEC by 30%; however, adhesion was still significantly greater than in unstimulated endothelium. Treatment of ICAM-1−/− MAEC with YN1 did not further attenuate WEHI 274.1 adhesion in response to TNF-α (data not shown) (18). Comparison of TNF-α-mediated WEHI 274.1 adhesion data from ICAM-1 mutant endothelium and wild-type endothelium treated with YN1 revealed substantial differences in that the functional lack of ICAM-1 expression further decreased adhesion compared with YN1 (55% vs. 30%). In contrast, administration of YN1 completely prevented 100 μg/ml oxLDL (REM 2.1)-mediated WEHI 274.1 adhesion to wild-type MAEC (Fig. 3B), paralleling the findings with ICAM-1−/− MAEC.

**Whole blood monocyte adhesion.** Adhesion assays were performed with circulating murine monocytes to determine whether any specific differences in adhesion mechanisms exist between continuous monocyte cell lines and peripheral blood monocytes (34, 35, 43). Figure 4 shows that whole blood monocyte adhesion to resting ICAM-1−/− endothelial cells was significantly reduced compared with wild-type MAEC. TNF-α or oxLDL both significantly increased adhesion of whole blood monocytes to wild-type MAEC. Dot-blot analysis

![Fig. 2. WEHI 274.1 adhesion to tumor necrosis factor (TNF)-α-stimulated MAEC. Percent WEHI 274.1 adhesion to wild-type (A) and ICAM-1−/− MAEC (B) is shown in response to 0, 1, 5, 10, or 25 ng/ml TNF-α for 6 h. C: direct comparison of TNF-α-mediated monocyte adhesion between wild-type and ICAM-1−/− MAEC. *P < 0.05; n = 8.](image-url)
also revealed an increase in ICAM-1 protein levels in wild-type MAEC (data not shown). TNF-α (10 ng/ml) stimulated adhesion to ICAM-1−/− MAEC, yet monocyte binding was significantly reduced compared with wild-type MAEC at this dose. Loss of ICAM-1 completely abrogated monocyte adhesion in response to oxLDL and was not significantly different from vehicle treatments. These data confirm results obtained with WEHI 274.1 monocytes and further demonstrate that ICAM-1 is important for monocyte adhesion to oxLDL- or TNF-α-stimulated endothelial cells.

**DISCUSSION**

We used a genetic approach to specifically examine the molecular interactions involved in monocyte adhesion to aortic endothelium. This approach has several advantages in that it allows analysis of primary endothelium from tissues directly involved in atherosclerosis and several vasculitic diseases, eliminates the requirements for monoclonal antibodies or other inhibitors, and allows for adhesion studies of specific leukocyte populations. Using this system, we have previously shown that basal WEHI 274.1 monocyte adhesion is significantly greater to wild-type MAEC compared with ICAM-1−/− MAEC (16). Here we have demonstrated further that ICAM-1 plays a major role in mediating monocyte adhesion to either oxLDL- or TNF-α-stimulated endothelium.

Oxidized lipids have been reported to increase adhesion molecule expression, including ICAM-1 on aortic endothelial cells, contributing to atherosclerotic plaque formation (36). Moreover, specific oxidized lipid products can stimulate monocyte-endothelial cell interactions in vitro (44, 45). We found that stimulation of wild-type MAEC with oxLDL induced a significant increase in WEHI 274.1 and whole blood monocyte adhesion that was absent in ICAM-1−/− MAEC. These observations are in contrast to previously reported investigations of monocyte adhesion to human umbilical vein endothelial cells or human aortic endothelium (9, 17, 25, 39). In these studies, ICAM-1 was shown to have a minimal role or was not involved in monocyte adhesion in response to oxidized lipid preparations. These differences may be due to the amount or degree of oxidized lipids used to stimulate endothelial cells and the methods used to inhibit monocyte adhesion. It is also possible that ICAM-1 may act as a central adhesion molecule to facilitate other adhesive interactions and that the genetic loss of ICAM-1 affects the efficiency or ability of other adhesion pathways. Nonetheless, our data clearly demonstrate that endothelial ICAM-1 is critical for monocyte adhesion in response to...
oxLDL and further implicates this adhesion molecule in monocyte-endothelial cell interactions during the initiation and progression of atherosclerosis.

TNF-α is widely recognized as a powerful cytokine involved in stimulating monocyte adhesion in many inflammatory vascular diseases (15). We observed that TNF-α stimulated a dose-response increase in monocyte adhesion to wild-type MAEC, whereas only higher concentrations of TNF-α (10 and 25 ng/ml) induced significant adhesion to ICAM-1−/− MAEC. This finding suggests that monocyte adhesion in response to lower doses of TNF-α may be largely ICAM-1 dependent, whereas higher concentrations may stimulate expression of other adhesion molecules. Other adhesive interactions that could be involved include VLA-4/VCAM-1, VLA-4/β1 integrin (CS-1), or E-selectin. VCAM-1 and E-selectin are both upregulated in response to TNF-α and have been previously shown to participate in monocyte-endothelial cell interactions (3, 14, 24). Further examination of these and other adhesion molecules is necessary to fully understand ICAM-1-independent mechanisms of monocyte adhesion.

We also compared the use of blocking monoclonal antibodies to gene-targeted loss of ICAM-1 in inhibiting monocyte adhesion in response to inflammatory stimuli. We have previously reported that coinoculation of wild-type MAEC with the ICAM-1-blocking antibody YN1 significantly reduced basal adhesion of WEHI 274.1 monocytes and did not further reduce basal monocyte binding to ICAM-1−/− MAEC (16). Treatment of TNF-α-stimulated wild-type MAEC with YN1 significantly attenuated monocyte adhesion (30%); however, this effect was not as large as that observed using ICAM-1−/− MAEC (55%). This observation may result from many factors, such as incomplete blocking of all ICAM-1 molecules, competitive inhibition of YN1 by soluble ICAM-1, intracellular processing of the antibody by the endothelium or monocytes (through Fc receptor binding), or through the inability of blocking antibodies to similarly influence intracellular signaling pathways mediated by endothelial cell adhesion molecules (e.g., immunoglobulin superfamily members) (1, 22). Although it is not certain as to which of these explanations is responsible, these data clearly demonstrate that the ICAM-1−/− MAEC provide a more direct, unambiguous method of analyzing protein function.

In summary, we have identified a potential molecular mechanism by which ICAM-1 mutant mice show reduced atherosclerotic and vasculitic lesion formation. ICAM-1 also may be involved in the recruitment of other leukocyte populations associated with these diseases, such as T lymphocytes and dendritic cells. Our data further demonstrate the differential requirement for ICAM-1 in mediating monocyte adhesion in response to various inflammatory mediators. These observations suggest that oxLDL and TNF-α stimulate different adhesion pathways, possibly due to activation of distinct signaling cascades (5, 46).

We thank Dr. Klaus Ley for the gift of the YN1 antibody. This work was supported by National Institutes of Health Grants HL-10312 (to C. G. Kevil) and AR-46404 (to D. C. Bullard) and American Heart Association Grant 000632B (to R. P. Patel).

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