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Human monocyte–endothelial cell interaction *in vitro*

(endothelium/smooth muscle cell/neutrophil/platelet/lymphocyte)

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ABSTRACT **We have examined the interaction of freshly isolated human blood monocytes with cultured human umbilical vein endothelial cells *in vitro*. Purified monocytes incubated with confluent primary or passaged endothelial cells (EC) for 90 min at 37°C bound at maximal densities of 6.5–7.0 × $10^3/mm^2$ (8 or 9 per EC) without causing disruption of the monolayer. Monocyte–EC binding proceeded in the presence of plasma proteins or optimal phagocytic doses of opsonized zymosan particles. The avidity of attachment was not diminished by alternative monocyte isolation techniques. Monocyte attachment to EC was dependent upon the presence of divalent cations (magnesium > calcium) and was inhibited at 4°C. Monocytes selectively bound to EC when incubated with monolayers composed of smooth muscle cells and EC. Neither EC monolayer confluence nor a variety of EC culture conditions affected the high levels of monocyte binding. In contrast, human neutrophils (<1 per EC) and lymphocytes (<2–3.5 per EC) bound at lower maximal densities under the same conditions, while platelet reactivity remained minimal. The distinctively higher affinity of human blood monocytes relative to other circulating white cells for binding to cultured human EC may have relevance to their function *in vivo*.**

The adherence of leukocytes to endothelium is an early component of the inflammatory response, which precedes their emigration into the extravascular compartment. Although the interaction of neutrophils (polymorphonuclear leukocytes; PMN), lymphocytes, and platelets with endothelium has received considerable attention (reviewed in ref. 1), less is known about the circulating human blood monocyte. Through their diverse secretory repertoires (2), the monocyte and its tissue macrophage counterpart may play important roles in modifying cellular and matrix components located at intravascular and extravascular sites. Atherosclerosis is an important example in which the binding of circulating monocytes to prelesional areas of endothelium destined to become plaques appears to be an early event (reviewed in refs. 3 and 4).

We are now examining the interaction of highly purified populations of freshly isolated human monocytes with endothelium *in vitro*. By using a homologous system of human cell types, the binding of monocytes to cultured human umbilical vein endothelial cells (EC) was compared to PMN, lymphocyte, and platelet binding under similar conditions. Human blood monocytes exhibited much higher levels of attachment to EC than did the other cell types. Monocyte binding was selective for EC over smooth muscle cells (SMC) and was not significantly altered in the presence of plasma proteins. This high affinity of monocytes for EC under these *in vitro* conditions may have consequences for both physiological and pathological states of migration out of the vascular tree.

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MATERIALS AND METHODS

Human Cell Sources. Peripheral blood cells were isolated from leukocyte concentrates (CPDA-1 anticoagulant) (The New York Blood Center) or whole blood (20 units of heparin per ml or acid citrate/dextrose solution; ref. 5) from healthy volunteers after informed consent. Human umbilical cords were stored for 24 hr or less.

Reagents. Media for EC culture (medium 199) and monocyte isolation (RPMI 1640) were both supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml) (KC Biological, Lenexa, KS). For EC culture, collagenase (CLS II) and trypsin (TR3) from Cooper Biomedical (Malvern, PA), human fibronectin (FN) from The New York Blood Center or Collaborative Research (Waltham, MA), EC growth supplement from Collaborative Research, and collagen (Vitrogen 100) from Collagen (Palo Alto, CA) were used as specified. Opsonized zymosan (OpZ) and human serum were prepared as described (6).

Isolation and Culture of EC. EC were isolated by collagenase treatment of human umbilical veins as previously described (7, 8) and cultured in medium 199 plus 20% heat-inactivated (HIA) human serum. The cultures routinely used were derived from pooled primary cultures (4–7 days) that were passaged with collagenase or trypsin onto FN-treated plastic dishes (35- or 16-mm diameter) and grown to confluence (4–7 days) unless otherwise specified. EC cultures showed typical morphology under phase-contrast microscopy and homogeneous staining for factor VIII antigen (Miles). The use of FN as a culture substrate provided EC monolayers that were stable to incubations at 4°C or in the absence of divalent cations. SMC-contaminated EC monolayers were prepared by the following modifications of routine methods: umbilical cords were clamped prior to collagenase treatment, and the isolated cells were cultured for prolonged periods (3 wk) before and after a single passaging with trypsin (8).

Monocyte Isolation. Purified peripheral blood monocytes depleted of platelet contaminants were prepared as described (6). The procedure involved the following steps: (*i*) removal of platelet-rich plasma by centrifugation, (*ii*) conventional density gradient separation of whole mononuclear cell fraction (9), (*iii*) incubation in fresh human serum containing EDTA (5 mM) to remove monocyte-associated platelets (6), (*iv*) incubation of mononuclear cells on plastic in RPMI 1640 medium plus 20% human serum to isolate monocytes by adherence, and (*v*) after 5–6 hr of culture, during which most adherent granulocytes and lymphocytes spontaneously detached, the monolayer was washed and the monocytes were detached by incubation in phosphate-buffered saline contain-

Abbreviations: HUVEC, human umbilical vein endothelial cell(s); EC, endothelial cell(s); PMN, polymorphonuclear leukocyte(s); OpZ, opsonized zymosan; SMC, smooth muscle cell(s); FN, fibronectin; HIA, heat-inactivated.

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ing 0.2 mM EDTA. Purified cells were >90% viable and >90% monocytes by Wright's, peroxidase, and esterase stains (6). As indicated in *Results*, some experiments used the total mononuclear cell fraction isolated when using only steps 1 and 2 to eliminate exposure to serum and prior adherence. Similar results were obtained using monocytes isolated by steps 1–3 followed by separation of Nycodenz monocytes (Nyeggard, Oslo), which eliminated prior adherence.

Isolation of Other Blood Cells. PMN were prepared as described (10). Lymphocytes were isolated as the nonadherent cell fraction after two successive 2-hr incubations on plastic (>90% purity by Wright's stain). Platelets were isolated from platelet-rich plasma. After centrifugation, platelet pellets were washed twice in phosphate-buffered saline containing 0.5% human serum albumin prior to use.

Adhesion Assay. Confluent EC monolayers were first washed with 4 vol of appropriate incubation buffer or medium 199. Suspensions of monocytes (specified numbers per mm^2 of EC monolayer surface area), PMN (routinely 5×10^3 per mm^2), lymphocytes ($10–15 \times 10^3$ per mm^2), and platelets ($25–100 \times 10^3$ per mm^2) were incubated in 1-ml vol (per 35-mm- or 16-mm-diameter vessel) for 90 min at 37°C in 5% CO_2/95% air with EC-monolayered and untreated plastic (control) unless otherwise noted. Cultures were agitated every 15–20 min to insure even dispersal of suspended cells over the EC monolayers. After two vigorous washes (and microscopic examination to confirm removal of nonadherent cells), monolayers were scraped into 0.05% Triton X-100 and assayed for total protein (11) as described in *Results*. In some experiments, comparisons of the densities of monocytes bound to EC monolayers and to plastic (control for optimum binding) were performed by direct counting after fixation. The results confirmed determinations using protein assays. Lymphocyte binding was also quantitated by increments in added cell protein. For all experiments, the protein content (mean ± SD) of confluent EC monolayers remained consistent for 35-mm (190 ± 12 μg)- and 16-mm (36 ± 4 μg)-diameter culture surfaces.

RESULTS

Monocyte Attachment to Cultured EC. Initial studies showed that freshly isolated human peripheral blood monocytes bound avidly to cultured human umbilical vein EC (HUVEC) in the presence of serum-free medium 199 at 37°C. Within 10–20 min after initial contact, monocytes began to spread on the surfaces of EC, and by 60–90 min elongated monocytes were uniformly associated with the EC monolayers. Monocytes added to plastic culture dishes showed more rapid (5–10 min) and circumferential spreading. Fig. 1 compares the appearance of monocytes attached to either EC or a plastic dish.

Incubation of confluent EC monolayers with serial dilutions of purified monocytes indicated that as many as 7×10^3 monocytes per mm^2 could attach and spread under these conditions. Monocyte binding could be quantitated by assaying the additional cell protein as compared to EC alone. Fig. 2 shows the results from a representative experiment ($n = 4$) in which $0.65–5.0 \times 10^3$ monocytes were added per mm^2 of confluent EC. This range reflected approximate monocyte/EC ratios of 1:1 to 8:1. The binding of increasing

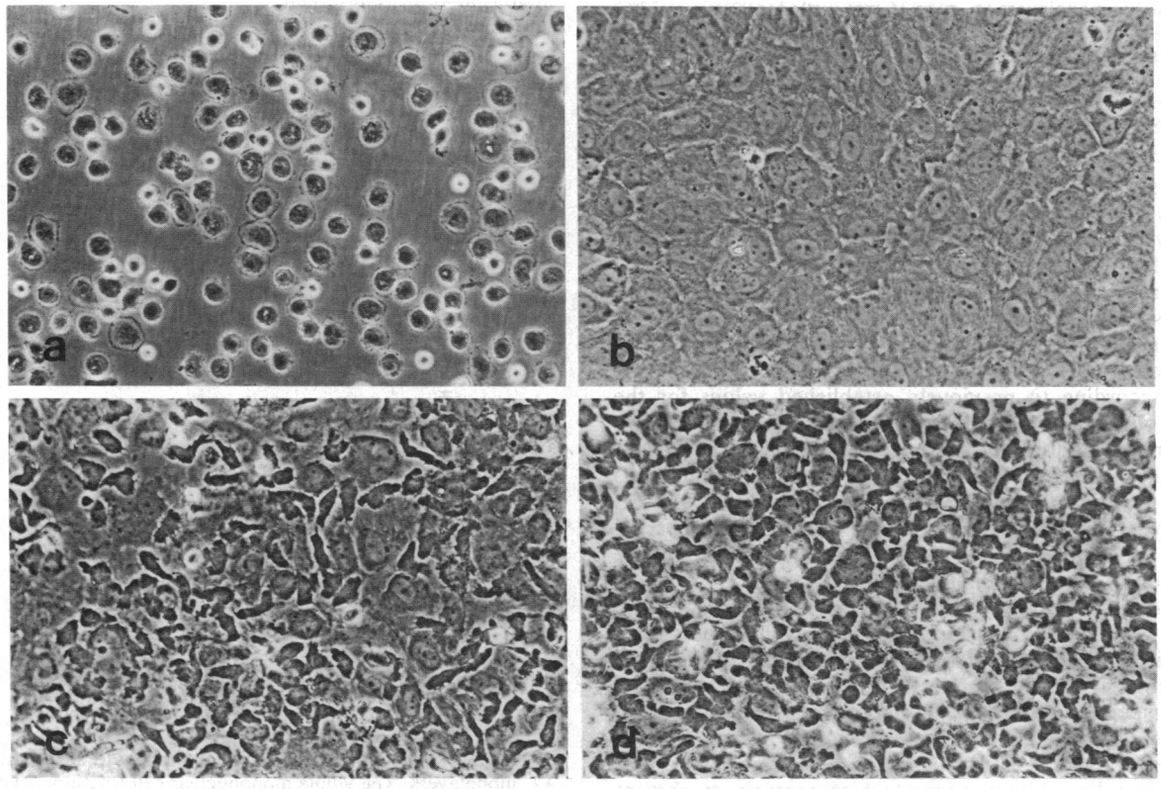

FIG. 1. Phase-contrast photomicrographs of purified monocytes spread upon plastic or EC monolayer. (*a*) Monocytes suspended in medium 199 and incubated in plastic dishes at a density of 3×10^3 per mm^2 promptly bound and maintained circumferential spreading for 90 min. (*b*) Appearance of washed EC monolayer incubated in medium 199 for 90 min. (*c*) Monocytes (3×10^3 per mm^2) added in medium 199 to confluent EC monolayers bound in 10–15 min and spread more slowly. At 90 min, monocytes exhibited spreading and elongation suggestive of migration over, and likely through, the EC monolayer. Direct counting of fixed preparations confirmed the apparent equal density of monocytes bound to plastic (*a*) and EC (*c*). (*d*) The appearance of an EC monolayer after incubation for 90 min with a 2-fold greater number of monocytes (6×10^3 per mm^2) under otherwise similar conditions is shown. (×400.)

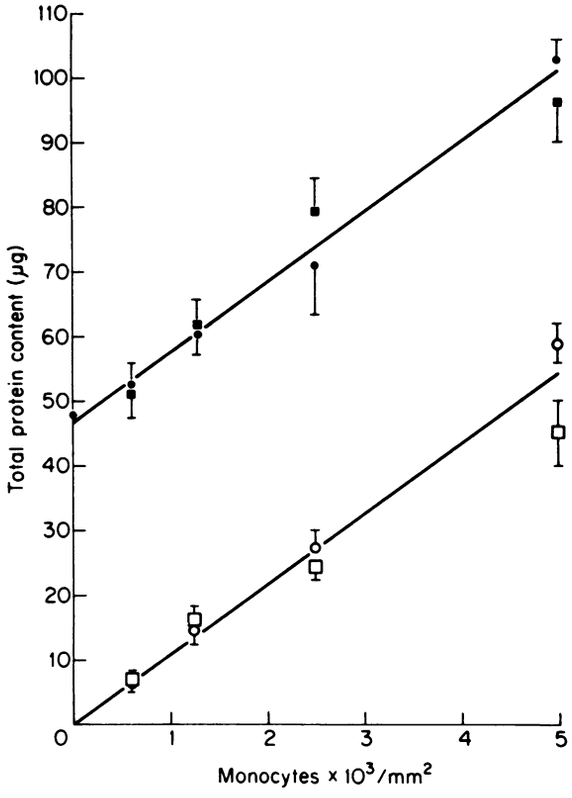

FIG. 2. Quantitation of monocyte attachment to EC monolayer and to plastic surfaces. Serial dilutions of purified human monocytes in medium 199 were incubated with either untreated plastic (○, □) or confluent EC monolayers (●, ■) in 16-mm wells for 90 min at 37°C. Monocytes were added either alone (○, ●) or with optimal doses of OpZ (□, ■). After vigorous washout of nonadherent cells, the protein contents of individual wells were determined. The results for each point represent quadruplicate determinations in this representative experiment (n = 4).

numbers of monocytes remained linear up to at least 6.0×10^3 monocytes per mm^2. This indicated that more than one monocyte can bind to an individual EC. The increments in protein found in monocyte–EC incubations were >85% of the protein recovered from incubations of monocytes with plastic surfaces under conditions of maximal adherence. The increments in adherent monocyte protein recovered also correlated well (>85%) with the numbers of purified monocytes added according to previously established values for the protein content of 10^6 monocytes (50 ± 5 μg; ref. 6). The simultaneous addition of maximal phagocytic doses of OpZ during the monocyte attachment phase (multiplicity of >10:1) did not significantly inhibit monocyte adherence to the EC monolayer (Fig. 2). The binding of maximal numbers of monocytes (± OpZ) over 90 min did not result in the disruption of the EC monolayer.

Factors Influencing Monocyte Attachment to EC. Monocyte binding to EC also occurred efficiently in the presence of heparinized, platelet-depleted AB^+ plasma. Results from incubations of EC for 90 min with monocytes (3×10^3 per mm^2) suspended in either medium 199 or plasma indicated that levels of adherence in the presence of plasma were at least 75% of those in medium alone (n = 2). This implies that the receptor–ligand interaction(s) which controls attachment is not easily saturated by plasma constituents. Further experiments examined the divalent-cation requirements for monocyte–EC binding. Quantitation of adherence was assessed by assays of adherent cell protein and was confirmed by microscopy. By using Earle's balanced salt solution without divalent cations, monocyte adherence to plastic remained optimal, but binding to EC was inhibited by >90%. With the addition of 1 mM Mg^{2+}, monocyte–EC binding reached 85% of maximal levels, whereas calcium alone was somewhat less supportive (45–60% maximal at 1 to 2 mM) (n = 2). Monocyte adherence to EC in medium 199, however, was completely inhibited at 4°C.

Effect of EC Culture Conditions. The EC monolayers routinely used in these studies were first-passage cultures derived from pooled primary cultures and were grown to confluence in 20% HIA A^+ serum in medium 199. We questioned whether culture conditions might affect the intensity of monocyte attachment. Monocytes in medium 199 bound avidly to both confluent and subconfluent monolayers. Other variables of HUVEC culture that did not alter monocyte attachment and spreading included: (*i*) primary versus passaged cultures, (*ii*) time after confluence (2–12 days), (*iii*) type of serum supplementation (fresh versus HIA AB^+ serum, HIA AB^+ serum versus heparinized AB^+ plasma, and maternal AB^+ serum), (*iv*) type of substrate provided for EC growth (plastic ± FN or collagen ± FN), and (*v*) culture with endothelial cell growth factor on a matrix of FN (12).

Monocyte–EC binding also was tested in an experiment in which serum for culture (6-wk prepartum), HUVEC, and maternal mononuclear cells (12 days postpartum) were all available from a single AB^+ donor. Maximal densities of monocytes bound to passaged EC when the whole mononuclear cell fraction of peripheral blood was added in either medium 199 or fresh, heparinized, autologous plasma. These results indicate that monocyte–EC binding takes place *in vitro* despite the compatibility of serum protein constituents and cell-surface antigenic characteristics defined by the maternal–fetal relationship.

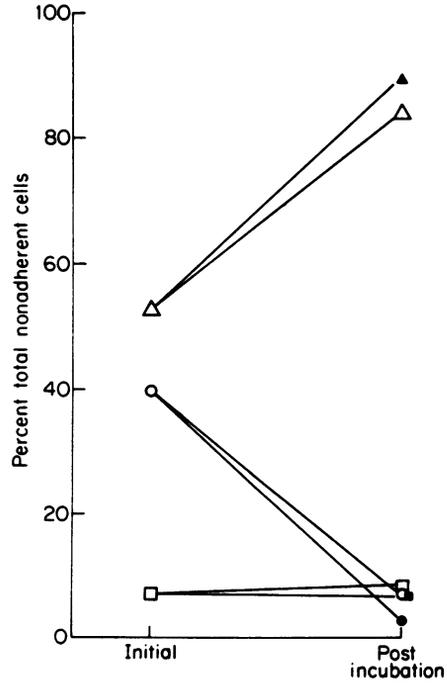

FIG. 3. Incubation of monocyte–lymphocyte suspensions with EC monolayers. The whole mononuclear cell fraction isolated from peripheral blood was incubated at densities of 8×10^3 total cells per mm^2 of confluent EC in the presence of either medium 199 (○, □, △) or autologous plasma (●, ■, ▲). After 90 min of incubation at 37°C, the percentages of granulocytes (□, ■), monocytes (○, ●), and lymphocytes (△, ▲) recovered as nonadherent cells after vigorous washing were compared to initial percentages by differential counts of stained cytocentrifuge preparations.

Comparison of Attachment by Other Circulating Inflammatory Cells. A selective binding of monocytes versus lymphocytes to cultured EC monolayers was demonstrated by using whole mononuclear cell fractions separated by conventional density gradients (9) from fresh whole blood or stored (8–12 hr) leukocyte concentrates. Mononuclear cell suspensions (monocyte/lymphocyte ratios between 1:4 and 1:1) were suspended in medium 199 to yield 8–9 $\times 10^3$ total cells per mm^2 (approximately 2–4 $\times 10^3$ monocytes per mm^2). After incubation for 60–90 min with confluent EC monolayers, >90% of the bound cells were identified as monocytes by their intense spreading and peroxidase staining; <8% of the nonadherent cells recovered after washing were monocytes (n = 3). Fig. 3 shows the results of another experiment comparing the relative adherence of monocytes and lymphocytes to confluent EC when added as a whole mononuclear cell population in either medium 199 or autologous, platelet-depleted plasma. In this case, monocyte adherence was monitored by removal of cells from the nonadherent population. These results further demonstrate the selectivity of monocyte binding to cultured EC in the presence of complex cellular and protein mixtures. It was of interest that autologous plasma preferentially inhibited monocyte adherence to plastic but not to EC (not shown). The lower affinity of lymphocytes for EC binding was confirmed in experiments in which purified lymphocytes were incubated with EC. When added in medium 199 to confluent EC at 10–15 $\times 10^3$ per mm^2, lymphocytes bound at lower maximal densities (<2.2 $\times 10^3$ per mm^2) (n = 2) than did purified monocytes (up to 7.0 $\times 10^3$ per mm^2).

Incubation of purified human PMN (<15% eosinophils) suspended in medium 199 or plasma with confluent EC monolayers at 5 $\times 10^3$ per mm^2 for 90 min yielded low levels of attachment. At 15–20 min, direct microscopic examination indicated that less than one PMN bound per EC (<0.65 $\times 10^3$ per mm^2). Beyond 20-min incubation in the absence of plasma, the PMN caused a gradual disruption of the EC monolayer. The simultaneous addition of maximal phagocytic doses of OpZ accelerated the disruption of EC monolayers. PMN failed to degrade the EC monolayer in the presence of plasma, however, suggesting a role for their neutral proteases (13).

Washed suspensions of human platelets in medium 199 (25–100 $\times 10^3$ per mm^2) also showed minimal attachment to confluent EC monolayers. Light microscopy indicated that platelet binding was limited to areas containing debris, the margins of EC monolayers in areas of subconfluence, or to the dish surface. Neither the addition of fibrinogen at concentrations optimal for platelet aggregation (14) nor thrombin or collagen altered the minimal platelet binding. Similar information with platelets and cultured HUVEC have been reported (15). This contrasted with the uniform distribution of adherent monocytes, which included the upper EC surfaces to which platelets did not bind.

Lack of Monocyte Binding to Venous SMC. Cultures of HUVEC were established by using conditions that promote the growth of contaminating SMC (8), which could be distinguished from EC by their relative size, morphology (light and electron microscopy), and lack of factor VIII antigen content. Purified monocytes suspended in medium 199 or heparinized plasma failed to show significant binding to SMC, whereas they bound avidly to adjacent EC. Fig. 4 shows the appearance of a typical monolayer containing large SMC surrounding discrete islands of confluent EC and the selective binding of monocytes to areas containing EC. The similar results observed in four separate preparations dem-

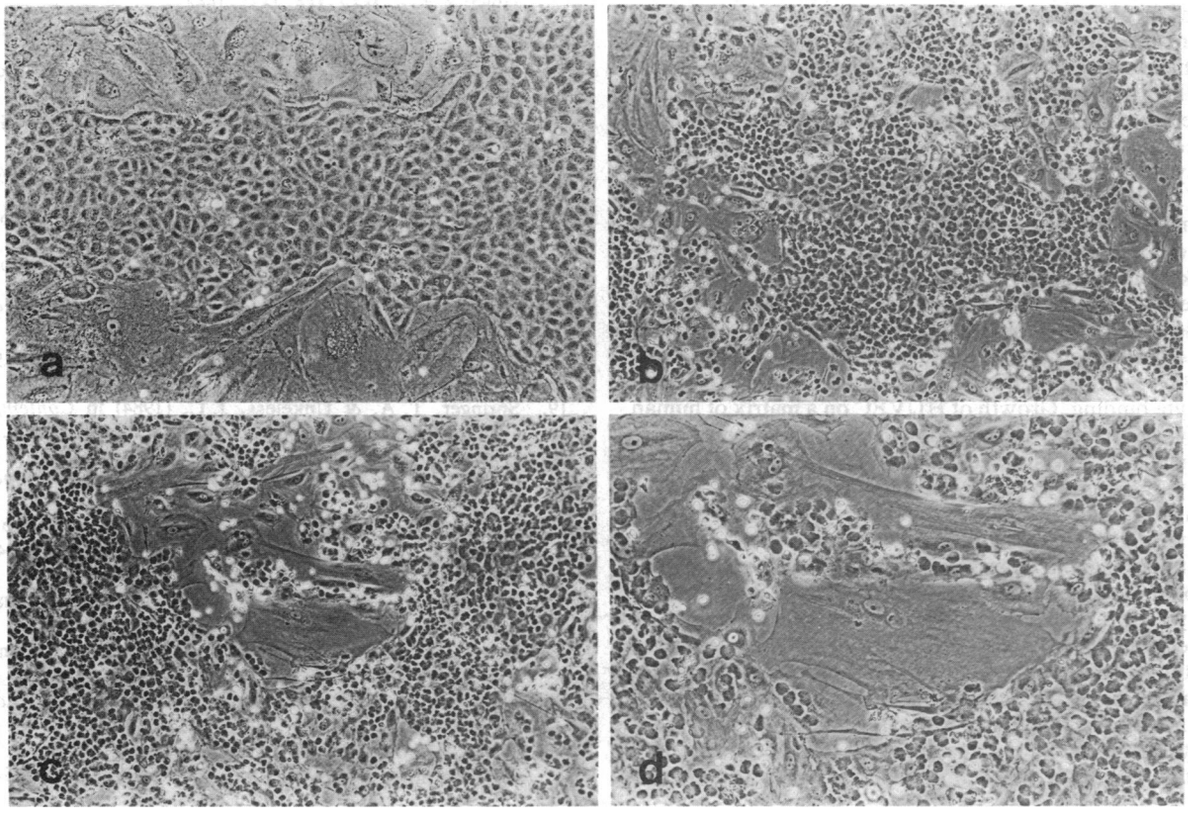

FIG. 4. The selective interaction of monocytes with EC and not SMC was observed under phase-contrast microscopy. EC monolayers containing SMC contaminants were prepared as described in the text. (*a*) Discrete islands of EC surrounded by SMC. (×100.) (*b*) After incubation with monocytes in medium 199 (5 $\times 10^3$ per mm^2) for 90 min with frequent agitation followed by vigorous washing, bound monocytes were found on EC islands. (×100.) (*c* and *d*) Areas lacking bound monocytes (*c*) (×100) contain SMC (*d*) (×200).

onstrate the considerable selectivity of monocyte binding to cultured EC. However, monocyte binding to EC was not inhibited in the presence of conditioned medium (3 day) from pure cultures of venous SMC.

DISCUSSION

Human peripheral blood monocyte interaction with endothelium was examined *in vitro* using HUVEC cultured in the presence of human serum supplementation to provide an entirely homologous experimental setting. In the presence of medium 199 alone, the binding of highly purified (>95%) populations of freshly isolated and platelet-depleted human blood monocytes was extremely avid, reaching a maximum density of $6.5–7.0 \times 10^3$ cells per mm^2 of confluent EC (8–10 monocytes per EC) after incubation for 60–90 min at 37°C. In contrast, human PMN, lymphocytes, and platelets all showed much lower levels of binding. The low levels of PMN adherence (approximately one PMN per EC) observed with the EC culture conditions we have used are similar to those determined by others (1, 16–18). Interestingly, in the cited studies, the enhancement of PMN binding achievable by a variety of chemotactic factors was only 2- to 3-fold greater (16–18). This is still considerably lower than the numbers of monocytes binding in the absence of an apparent exogenous stimulus.

The conditions routinely used for monocyte isolation in these studies included exposure to human serum to deplete surface-bound platelets and a prior adherence step to separate monocytes from lymphocytes and basophils (6). The high level of monocyte reactivity was not likely related to these factors, since monocytes within whole mononuclear cell fractions separated without exposure to serum or prior adherence also avidly bound and spread upon EC monolayers. Monocyte adherence also remained largely unaffected by the presence of autologous plasma. This indicated that the mechanisms responsible for binding are neither dependent upon the absence of normal plasma constituents nor saturated by their presence. However, binding to EC was inhibited completely at 4°C and was diminished by 90% at 37°C in the absence of Ca^{2+} and Mg^{2+}. The cation dependence of monocyte–EC binding ($Mg^{2+} > Ca^{2+}$) was similar to that for PMN (16) and for the newly described Mac-1/LFA-1 series of membrane glycoproteins that function in phagocyte adherence (19).

Leukocyte binding to cultured EC appears to be enhanced relative to binding to EC *in situ* (20). The variety of culture conditions aimed at optimizing EC growth toward more native organization of extracellular matrix and surface characteristics were all ineffective in altering the high avidity of monocyte binding. Growth of HUVEC on a matrix of human amnionic membrane also appears not to influence monocyte adherence (21). The surface ligands and receptors of EC and monocytes leading to *in vitro* recognition remain unclear as does the difference in surface characteristics of cultured cells from their *in vivo* counterparts.

Light microscopic observations during the 90-min incubations of monocytes with EC suggested that, once bound, monocytes spread and then migrate through the monolayer as observed for PMN (22, 23). Preliminary results of a comparative study of monocyte versus PMN migration through EC monolayers have been described (21). It is clear that in addition to the significantly lower levels of EC binding by PMN, these cells do not spread upon EC surfaces but attach via small segments of their membrane surfaces (23). In contrast, the intense spreading by monocytes indicates that these cells maintain considerably larger areas of surface contact with EC plasma membranes (Fig. 1).

In separate studies, we have found that incubation of EC with monocytes alone resulted in stimulation of EC prostacyclin synthesis. Upon monocyte stimulation with OpZ, a more pronounced stimulation of prostacyclin synthesis by EC took place. This arachidonic acid metabolite is a potent mediator of vasodilation and may be implicated in alteration of vascular permeability (24, 25). Therefore, the stimulated secretion of prostacyclin may have a functional role during monocyte emigration under steady-state or inflammatory conditions.

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1. Harlan, J. M. (1985) *Blood* **65,** 513–525.
2. Nathan, C. F., Murray, H. W. & Cohn, Z. A. (1980) *N. Engl. J. Med.* **303,** 622–626.
3. Faggioto, A., Ross, R. & Harker, L. (1984) *Arteriosclerosis* **4,** 323–340.
4. McGill, H. C., Jr. (1984) *Arteriosclerosis* **4,** 443–451.
5. Aster, R. H. & Jandl, J. (1964) *J. Clin. Invest.* **43,** 843–855.
6. Pawlowski, N. A., Kaplan, G., Hamill, A. L., Cohn, Z. A. & Scott, W. A. (1983) *J. Exp. Med.* **158,** 393–412.
7. Cramer, E. B., Pologe, L., Pawlowski, N. A., Cohn, Z. A. & Scott, W. A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4109–4113.
8. Jaffe, E. A. (1984) in *Biology of Endothelial Cells*, ed. Jaffe, E. A. (Nijhoff, Boston), pp. 1–3.
9. Boyum, A. (1976) *Scand. J. Immunol.* **5,** Suppl. 5, 9–15.
10. Pawlowski, N. A., Scott, W. A., Andreach, M. & Cohn, Z. A. (1982) *J. Exp. Med.* **155,** 1653–1664.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. J. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
12. Gospodarowicz, D., Vlodavsky, I. & Savion, N. (1980) *J. Supramol. Struct.* **13,** 339–372.
13. Harlan, J. M., Killen, P. D., Harker, L. A., Striker, G. E. & Wright, D. G. (1981) *J. Clin. Invest.* **68,** 1394–1403.
14. Polley, M. J. & Nachman, R. L. (1983) *J. Exp. Med.* **158,** 603–615.
15. Zetter, B. R., Johnson, L. K., Shuman, M. A. & Gospodarowicz, D. (1978) *Cell* **14,** 501–509.
16. Charo, I. F., Yuen, C. & Goldstein, I. M. (1985) *Blood* **65,** 473–479.
17. Zimmerman, G. A., Wiseman, G. A. & Hill, H. R. (1985) *J. Immunol.* **134,** 1866–1874.
18. Gimbrone, M. A., Jr., Brock, A. F. & Shafer, A. I. (1984) *J. Clin. Invest.* **74,** 1552–1555.
19. Springer, T. A. & Unkeless, J. C. (1984) in *Contemporary Topics in Immunobiology*, eds. Adams, D. O. & Hanna, M. J., Jr. (Plenum, New York), Vol. 1, pp. 1–31.
20. deBono, D. P. & Green, C. (1984) *Br. J. Exp. Pathol.* **65,** 145–154.
21. Migliorski, G., Pawlowski, N. A., Scott, W. A., Fulop, A. & Cramer, E. (1984) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **43,** 845 (abstr.).
22. Beesley, J. E., Pearson, J. D., Carleton, J. S., Hutchings, A. & Gordon, J. L. (1978) *J. Cell Sci.* **33,** 85–101.
23. Beesley, J. E., Pearson, J. D., Hutchings, A., Carleton, J. S. & Gordon, J. L. (1979) *J. Cell Sci.* **38,** 237–248.
24. Wedmore, C. V. & Williams, T. J. (1981) *Nature (London)* **289,** 646–650.
25. Davies, P., Bailey, P. J., Goldenberg, M. M. & Ford-Hutchinson, A. W. (1984) *Annu. Rev. Immunol.* **2,** 335–357.