

ROLE OF CELL SURFACE CARBOHYDRATE MOIETIES IN MONOCYTIC CELL ADHESION TO ENDOTHELIUM IN VITRO¹

PAUL E. DICORLETO² AND CAROL A. DE LA MOTTE

From the Department of Vascular Cell Biology and Atherosclerosis Research, Research Institute of the Cleveland Clinic Foundation, Cleveland, OH 44195

Monocyte adhesion to endothelium represents the first step in the emigration of this leukocyte from blood to tissue during such pathologic and physiologic processes as atherosclerotic plaque development, wound healing, and inflammation. We have examined the role of carbohydrate moieties in the binding of mononuclear cells to endothelium *in vitro*. Wheat germ agglutinin (WGA) completely inhibited binding of the human monocytic cell line U937 to pig or human endothelial cells (EC). The inhibition was abolished by the presence of *N*-acetyl glucosamine, a preferred ligand for WGA. This sugar itself, however, had no effect on monocytic cell binding to EC, suggesting that WGA is inhibiting the cell-cell interaction by binding to a distinct sugar moiety. We tested a series of simple and phosphorylated sugars for the ability to inhibit U937 cell binding to EC. Two phosphorylated disaccharides, lactose-1-phosphate and maltose-1-phosphate, but not 14 other sugars, caused complete suppression of monocyte adhesion to EC. Among the inactive sugars were mannose-6-phosphate and fructose-1-phosphate, which have been shown by others to markedly suppress lymphocyte adhesion to EC. A non-ionic detergent, *n*-octyl- β -D-glucopyranoside (octyl glucoside), which contains a sugar group as a hydrophilic moiety, also inhibited U937 cell or human monocyte binding to human or porcine EC. The inhibition was observed at a nontoxic concentration of octyl glucoside and appeared to be due to an effect on the monocytic cell rather than the EC. When suboptimal doses of WGA and octyl glucoside were added in combination to the U937 cell-EC adhesion assay, the level of inhibition was greatly reduced when compared with either of the inhibitors alone, suggesting an interaction between these two blocking agents. Lactose-1-phosphate, but not octyl glucoside or WGA, blocked neutrophil adhesion to EC. In summary, our results indicate that specific cell surface carbohydrate groups are required for

the adhesion of monocytes to the endothelium.

Involvement of monocytes and macrophages in atherosclerotic plaque formation is a well documented and highly investigated phenomenon. Within the last 10 yr many investigators have taken an interest in the early events of lesion development aided by several animal models; the porcine (1, 2), pigeon (3, 4), rat (5), and monkey (6) have been used to advantage in studying monocyte interactions with large vessels. An understanding of the regulation of monocyte adhesion to endothelium has advanced due to good *in vitro* systems (7, 8) which have permitted the highly controlled study of an *in vivo* event that is both localized and transient. Cultured EC³ may be stimulated to bind monocytes by plating at subconfluent density (7) by creating an *in vitro* wound in a confluent monolayer (7), or by treating with the multipotential modulators IL-1 (8, 9), LPS (10), phorbol esters (10), and TNF (11).

The molecular interactions involved in the binding of monocytes to the endothelium have been the object of much recent study. Bevilacqua et al. (8) have shown that cytokine-stimulated adhesion of neutrophils or monocytes to EC involves *de novo* protein synthesis. In addition, mAb have been employed by several investigators to identify adhesion molecules which may participate in leukocyte-endothelium interactions. Lewinsohn et al. (12) have shown that the 80- to 100-kDa glycoproteins identified by mAb MEL-14 are present on lymphocyte, neutrophils, monocytes and appear to be involved in EC recognition in post capillary venules. Rasmussen et al. (13) have identified an adhesion molecule expressed on lymphocytes that is specifically targeted to high endothelial venules of rat lymph nodes. More recently, Wallis and co-workers (14) have shown the importance of the glycoprotein leukocyte membrane complex CDw18 (CD11/CD18) by using the mAb 60.3 to block monocyte adhesion to cultured endothelium. ICAM-1 (mAb RR 1/1) described as a natural adherence molecule expressed on endothelial and various lymphoid cells including macrophages and U937 cells is a glycoprotein which may also participate in several cell adhesion interactions (15). Bevilacqua and co-workers have employed two mAb designated H4/18 and H18/7 to identify a transiently expressed surface protein, ELAM I (100 to 120 kDa) on EC that were stimulated with IL-1 or TNF (16, 17). This cell surface Ag which plays a role in neutrophil-endothelium recognition,

³ Abbreviations used in this paper: EC, endothelial cell(s); WGA, wheat germ agglutinin; ocytl glucoside, *n*-octyl- β -D-glucopyranoside; DME/F12, Dulbecco's modified Eagle's media; Ham's F12 media (1:1); NAG, *N*-acetyl glucosamine.

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² To whom correspondence and reprint requests should be addressed: Paul E. DiCorleto, Ph.D., Research Institute FF4, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195.

but probably not monocyte-EC binding, has been cloned and sequenced and appears to be a member of a family of cell surface molecules that are expressed during inflammation.

The carbohydrate components of the glycoproteins involved in monocyte or neutrophil adhesion to endothelium have not been described. Phosphomannan and fucoidin receptors, which have been demonstrated on the lymphocyte surface, may participate in the binding of this leukocyte to the endothelium (18). Stoolman and co-workers (19) have shown that mannose-6-phosphate and also fructose-1-phosphate specifically inhibit lymphocyte-high endothelial venule adhesion. In this study we demonstrate that carbohydrates may also play a crucial role in the adhesion of blood-borne monocytes to large vessel endothelium.

MATERIALS AND METHODS

Cell Culture

Porcine aortic EC were isolated by a modification (20) of the method of Schwartz (21). Briefly, thoracic aorta segments were split longitudinally, rinsed with serum-free media, and the exposed intimal surface digested with collagenase (2 mg/ml in serum-free media) for 15 min at 37°C. The detached EC patches were gently collected with a rounded spatula or cotton swab and placed in primary culture. At confluence the cells were subcultured at 1:3 or 1:5 ratios. Human umbilical vein EC were isolated by a technique similar to that described by Lewis et al. (22). Undamaged segments of 1- to 3-day-old umbilical cords were drained, and the remaining blood rinsed from the umbilical vein with PBS via a blunt cannula attached at one end of the vessel. The open end of the cord was sealed, and the cord distended with 0.06% trypsin-0.2% EDTA and incubated at 37°C for 15 min. The cord was massaged, cut, and the trypsin digest collected. The cells were washed and seeded into fibronectin-coated flasks (5 µg/ml rabbit fibronectin) under the conditions described by Thornton et al. (23). All EC were grown in DME/F12 media (Irvine Scientific Co., Irvine, CA) supplemented with 0.24% sodium bicarbonate, 0.1 mM MEM nonessential amino acids, penicillin (100 U/ml) and streptomycin (100 µg/ml) (supplements from Sigma Chemical Co., St. Louis, MO). The DME/F12 media was supplemented with 5% FCS (GIBCO, Grand Island, N.Y.) for the porcine cells and 15% FCS + 90 µg/ml heparin and 150 µg/ml EC growth supplement for the human cells. Cells were subcultured by trypsin-EDTA treatment; porcine EC were used between the third and twentieth passage, and human EC between the first and third subculture. U937 cells (ATCC, Rockville, MD) were grown in suspension culture in RPMI 1640 media (MA Bioproducts, Walkersville, MD) containing 5% FCS and routinely subcultured at a 1:5 ratio three times per week. All culture vessels were obtained from Costar, Cambridge, MA.

Separation of Human Leukocytes

Monocytes were separated from peripheral blood (100 U/ml heparin) by a modification of the method of Recalde (24). Briefly, total mononuclear cells were collected by Ficoll-Hypaque density gradient centrifugation (25), washed, and resuspended in FCS. The cells were then maintained at 37°C while 9% NaCl was added to three 10-min intervals (5 µl/ml, then 10 µl/ml, then another 10 µl/ml). After the last 10-min incubation, the cell suspension was mixed with two volumes of PBS (with 27 µl/ml 9% NaCl added) and underlayered with one volume of Ficoll-Hypaque (with 2.8 mg/ml NaCl added). The gradient was spun at 600 × *g* for 20 min, and the monocytes separated from the Ficoll interface, washed twice with cold PBS, and finally resuspended in DME/F12 + 5% FCS in a Teflon beaker to prevent attachment during labeling. Isolated populations were routinely 78 to 90% monocytes by differential count with buffered Wright-Giemsa stain, and viability was always greater than 90% by trypan blue dye exclusion.

Neutrophils were prepared according to the method of Stossel et al. (26) by using dextran sedimentation of the cells in the Ficoll pellet and hypotonic lysis of E. Cells isolated by this procedure are routinely greater than 95% neutrophils by differential counting.

Assay for Monocytic Cell Adhesion to EC

U937 cell adhesion to EC was measured as previously described (7). Briefly, EC were plated into 24- or 96-well plates in their appro-

prate medium 18 to 48 h before the assay, depending on the desired density. On the day of the assay, U937 cells or normal human monocytes were labeled for 90 min at 37°C with 100 µCi ⁵¹Cr as sodium chromate (NEN) in 1 ml culture media. The labeled cells were washed by centrifugation and 10⁶ viable cells added per well of EC after removal of the incubation media. This binding phase of the assay was performed at 4°C for 1 h, then the wells were washed and the cells lysed with 1% Triton X-100 and an aliquot removed for quantitation of radiolabel. The number of U937 cells or monocytes bound per well was calculated from the initial sp act (cpm/cell). Spontaneous release of chromium from the monocytic cells during the assay incubation was less than 5%.

The viability of EC and monocytic cells in the presence of octyl glucoside, lactose-1-phosphate, and WGA was monitored by dye exclusion methods and by reversibility. In addition, protein synthesis, as measured by [³H]leucine incorporation into TCA-precipitable material, was not significantly affected by any of the adhesion inhibitors.

Preparation of Crude Porcine IL-1

Conditioned medium containing IL-1 was generated from porcine mononuclear cells by a method similar to that of Schmidt (27) for preparing human IL-1. Whole porcine blood was collected into 20 units/ml heparin by sterile jugular puncture. The mononuclear cells were separated on a Ficoll-Hypaque gradient, washed twice with PBS, and resuspended at 5 × 10⁶ viable cells/ml (determined by trypan blue dye exclusion) in RPMI 1640 containing 20 mM HEPES, 4 mM L-glutamine, and 1 µg/ml PHA. The cells were incubated in culture for 4 days at 37°C in 5% CO₂ in a humidified chamber and subsequently harvested and spun at 400 × *g* for 20 min. The supernatant was collected, filtered through a 0.22 µ low protein binding filter (Gelman), and stored at 4°C. Porcine EC were maximally stimulated to bind U937 cells at 1/320 dilution of this supernatant designated crude porcine IL-1.

Reagents

Lectins. Dolichos biflorus agglutinin, Ulex europaeus I, Ricinus communis agglutinin, peanut agglutinin, soybean agglutinin, and Con A were purchased from Vector Labs. Wheat germ agglutinin, Limulus polyphemus agglutinin, PHA, and succinyl-Con A were from Sigma.

Other reagents. N-Acetylglucosamine, octyl glucoside, and all phosphorylated sugars were purchased from Sigma. Purified human monocyte IL-1β was obtained from Cistron Technology (Pine Brook, NJ) and Otsuka Pharmaceutical Co., Ltd. (Rockville, MD).

RESULTS

Effect of lectins and sugars on monocytic cell adhesion to EC. To begin to probe the importance of specific carbohydrate moieties in monocytic cell-EC binding, we screened a group of lectins, many of which serve as markers for leukocytes (27), for their ability to block the cell-cell interaction. Only WGA exhibited the ability to interfere with U937 cell binding to porcine EC in the concentration range tested (Table I). We subsequently demonstrated that WGA was also a potent inhibitor of U937 cell binding to bovine aortic and human umbilical vein EC (not shown). Several of the lectins (Con A, succinyl-Con A, soybean agglutinin, and peanut agglutinin) caused the agglutination of the U937 cells, demonstrating the presence of their specific target sugar moieties on the U937 cell surface. We could not determine whether these sugars were involved in the binding of U937 cells to EC because adhesion was in fact enhanced in the presence of these agglutinating lectins. The inhibitory activity of WGA was completely abolished by the presence of its preferred target sugar, NAG, indicating that WGA rather than a minor contaminant was responsible for the suppression of binding (Table II). The NAG alone, however, had no effect on cell-cell adhesion, suggesting that WGA may be inhibitory as a result of its binding to a distinct sugar for which it has lower affinity. This hy-

TABLE I
Effect of lectins on binding of U937 cells to EC^a

| Experiment | Lectin Treatment (100 µg/ml) | U937 Cells Bound (×10 ⁻⁴) | Reduction in Binding (%) |
|------------|--------------------------------------|---------------------------------------|--------------------------|
| I | None | 2.9 ± 1 | |
| | WGA | 1.2 ± 0.4 | 60 |
| | <i>Dolichos biflorus</i> agglutinin | 3.3 ± 1.0 | (-10) |
| | <i>Ulex europaeus</i> I | 3.2 ± 0.5 | (-10) |
| | <i>Ricinus communis</i> agglutinin | 4.6 ± 1.1 | (-60) |
| | Peanut agglutinin | 5.0 ± 1.0 | (-70) |
| | Soybean agglutinin | 23.0 ± 4.6 | (-780) |
| II | Con A | 25.0 ± 7.7 | (-850) |
| | None | 19.5 ± 1.0 | |
| | WGA | 3.1 ± 0.3 | 84 |
| III | <i>Limulus polyphemus</i> agglutinin | 22.2 ± 0.9 | (-14) |
| | None | 21.1 ± 3.8 | |
| | WGA | 3.2 ± 0.5 | 85 |
| | Succinyl-Con A | 35.7 ± 2.4 | (-70) |

^a Subconfluent porcine EC in 96-well (Expt. I) or 24-well (Expts. II and III) plates were treated for 1 h at 4°C with individual lectins in DME/F12 media + 5% FCS. ⁵¹Cr-labeled U937 cells (4 × 10⁶/ml) were similarly treated with the lectins. U937 cells were incubated with comparably treated EC and assayed for adhesion as described in *Materials and Methods*. Numbers are the average of five replicate (Expt. I) or triplicate wells (Expt. II and III) ± SD.

TABLE II
Effect of NAG on WGA inhibition of U937 cell-EC binding^a

| Pretreatment of U937 and EC | Addition to U937 and EC | U937 Cells Bound (×10 ⁻⁵) | Reduction in Binding (%) |
|-----------------------------|-------------------------|---------------------------------------|--------------------------|
| Media | Media | 3.1 ± 0.12 | |
| Media | NAG | 3.4 ± 0.30 | (-9) |
| WGA | Media | 0.1 ± 0.05 | 96 |
| WGA | NAG | 3.5 ± 0.26 | (-12) |

^a Subconfluent porcine EC cultured overnight in 24-well plates were treated for 1 h at 4°C with DME/F12 media + 5% FCS with or without 100 mg/ml WGA. ⁵¹Cr-labeled U937 cells (4 × 10⁶/ml) were treated with the same solutions for 1 h at 4°C, then media or 1 M NAG (to give a final concentration of 0.1 M for treatment) was added to the pretreated cells. The EC and U937 cells were further incubated 1 h at 4°C and then U937 cells (10⁶) were added to triplicate wells of similarly treated EC and adhesion measured as described in *Materials and Methods*. Number of cells bound is presented as the mean ± SD.

pothesis was supported by our finding that the inhibitory activity of WGA was readily reversed by rinsing the participating cells prior to the incubation; i.e., WGA had to be present during the adhesion assay to cause inhibition. In addition, the effective concentration of WGA between 10 and 100 µg/ml was consistent with a fairly low affinity process (Fig. 1). A series of sugars other than NAG, which were previously shown to bind with lower affinity to WGA (28, 29), had no blocking effect; *N*-acetylmannosamine, *D*-xylose, *D*-glucosamine at 100 mM, *N*-acetylneuraminic acid, and α -lactose at 50 mM and β -*D*-glucose and cellobiose at 30 mM showed no ability to block U937 cell-EC adhesion.

Because certain phosphorylated sugars have been shown to be potent inhibitors of lymphocyte-EC interactions (19), most notably mannose-6-phosphate and fructose-1-phosphate, we explored the possibility that phosphorylated carbohydrates were also involved in monocytic cell-endothelium interaction (Table III). None of the phosphorylated monosaccharides had a significant effect on binding, but two phosphorylated disaccharides, lactose-1-phosphate and maltose-1-phosphate, substantially inhibited adhesion. The inhibitory activity of lac-

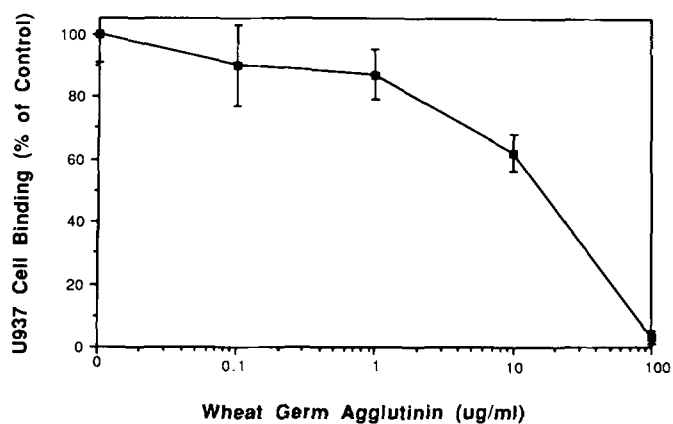


Figure 1. Effect of WGA on U937 cell binding to porcine EC. Subconfluent porcine EC and ⁵¹Cr-labeled U937 cells were treated with various concentrations of WGA for 2 h at 4°C. The U937 cells were then added to six replicate wells of the EC and incubated for 2 h at 4°C. Binding was calculated as described in *Materials and Methods* (values ± SD).

TABLE III
Effect of phosphorylated sugars on binding of U937 cells to EC^a

| Experiment | Sugar (10 mM) | U937 Cells Bound (×10 ⁻⁵) | Reduction in Binding (%) |
|------------|-----------------------|---------------------------------------|--------------------------|
| I | None | 2.8 ± 0.04 | |
| | Fructose-1-phosphate | 2.6 ± 0.44 | 9 |
| | Galactose-1-phosphate | 2.4 ± 0.11 | 15 |
| | Glucose-1-phosphate | 2.4 ± 0.17 | 14 |
| | Mannose-1-phosphate | 2.1 ± 0.41 | 24 |
| | Mannose-6-phosphate | 2.8 ± 0.19 | 1 |
| II | None | 2.1 ± 0.12 | |
| | Lactose-1-phosphate | 0.1 ± 0.03 | 95 |
| III | None | 1.2 ± 0.21 | |
| | Galactose-1-phosphate | 0.9 ± 0.11 | 26 |
| | Lactose-1-phosphate | 0.3 ± 0.07 | 77 |
| | Maltose-1-phosphate | 0.2 ± 0.03 | 81 |

^a Subconfluent porcine EC in 24-well plates were treated for 1 h at 4°C with 250 µl/well of phosphorylated sugar (10 mM) in DME/F12 + 5% FCS. ⁵¹Cr-labeled U937 cells (4 × 10⁶ cells/ml) were similarly treated. U937 cells were added to triplicate wells of comparably treated EC, then incubated and adhesion quantitated as described in *Materials and Methods*.

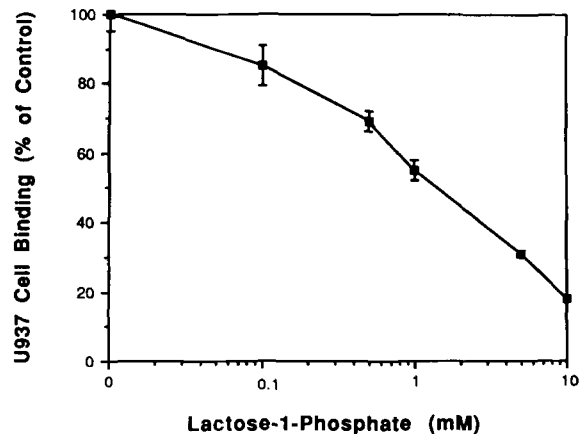


Figure 2. Effect of lactose-1-phosphate on U937 cell binding to porcine EC. Subconfluent porcine EC and ⁵¹Cr-labeled U937 cells were treated with varying concentrations of lactose-1-phosphate in DME/F12 media + 5% FCS for 1 h at 4°C. The treated U937 cells were combined with the treated EC and further incubated at 4°C for 1 h. The plates were washed and cells harvested as described in *Materials and Methods* (values ± SD).

tose-1-phosphate was detectable at 0.5 mM with maximal activity at 10 mM and half-maximal inhibition at 1 mM (Fig. 2). The inhibitory effect of lactose-1-phosphate could be washed away after incubation for a few hours,

but could not be reversed after 24 h incubation with U937 cells and EC. Adhesion was inhibited only slightly, if at all, by lactose-1-phosphate treatment of either cell type alone, but maximal inhibition of binding was observed following treatment of both the monocytic cell and EC (Table IV). The phosphorylated sugar blocked both basal binding of U937 cells to sparse EC as well as adhesion of the monocytic cells to LPS-stimulated EC (Table IV). Lactose-1-phosphate was also found to be an effective inhibitor of the binding of normal human peripheral blood monocytes to EC (Table V).

With the finding of a phosphorylated sugar that could block adhesion, as well as a lectin acting through a low affinity binding site, we investigated the additive properties of submaximal concentrations of lactose-1-phosphate and WGA. The effect of the combined inhibitors was neither additive, nor did they strongly block one another's action (data not shown). Rather, inhibition of cell binding occurred at the level of the more potent inhibitor in the particular assay. A lactose-phosphate containing carbohydrate therefore does not appear to be the ligand responsible for WGA inhibition of monocyte adhesion to EC.

Effect of octyl glucoside on monocyte adhesion to EC. During our attempts to solubilize the monocyte binding sites on the EC surface, we observed a striking effect on monocytic cell-EC adhesion by nondamaging concentrations of octyl glucoside (Table VI). Three other detergents, deoxycholate (0.75 mM), CHAPS (0.5 mM), and urea (0.01 M), had no effect on adhesion at maximal, nontoxic doses.

TABLE IV
Effect of extended lactose-1-phosphate treatment on U937 cell adhesion to EC^a

| LPS Stimulation of EC | Treatment with Lactose-1-Phosphate | | U937 Cells Bound ^b ($\times 10^{-5}$) | Reduction (%) |
|-----------------------|------------------------------------|------|--|---------------|
| | EC | U937 | | |
| | No | - | | |
| | + | - | 1.6 \pm 0.20 | 19 |
| | - | + | 1.7 \pm 0.37 | 14 |
| | + | + | 0.8 \pm 0.13 | 57 |
| Yes | - | - | 5.4 \pm 0.09 | |
| | + | - | 4.7 \pm 0.13 | 14 |
| | - | + | 5.2 \pm 0.42 | 4 |
| | + | + | 3.2 \pm 0.18 | 42 |

^a Porcine EC were plated at a subconfluent density in 24-well plates in DME/F12 + 5% FCS with or without 2 mM lactose-1-phosphate and incubated overnight. LPS (50 ng/well) was added to triplicate wells of untreated or lactose-1-phosphate-treated wells and incubated 4 h further. The wells were rinsed, and 10^6 ⁵¹Cr-labeled U937 cells, which had been precultured overnight (7×10^5 cells/ml) in the presence or absence of 2 mM lactose-1-phosphate, and washed three times, were added to wells of EC. Adhesion was quantitated as described in *Materials and Methods*.

^b Mean of triplicate wells \pm SD.

TABLE V
Effect of lactose-1-phosphate, octyl glucoside, and WGA on binding of normal human monocytes to EC^a

| Treatment of Monocyte and EC | Monocytes Bound ^b ($\times 10^{-4}$) | Reduction in Binding (%) |
|------------------------------|---|--------------------------|
| Media | 9.8 \pm 0.5 | |
| Lactose-1-phosphate | 1.4 \pm 0.1 | 86 |
| Octyl glucoside | 0.9 \pm 0.1 | 91 |
| WGA | 7.3 \pm 0.5 | 26 |

^a Subconfluent porcine EC and ⁵¹Cr-labeled monocytes in 24-well plates were individually treated for 1 h at 4°C with the various inhibitors (10 mM lactose-1-phosphate, 0.1% octyl glucoside, or 100 μ g/ml WGA) in 250 μ l of DME/F12 + 5% FCS. Monocytes (10^6) were then added to triplicate wells of comparably treated EC and assayed for adhesion as described in *Materials and Methods*.

^b Mean value \pm SD ($n = 3$).

Octyl glucoside inhibited nearly all monocytic cell binding to EC (94%) when both U937 cells and EC were pretreated with the reagent and the octyl glucoside remained during the adhesion assay. As shown in Figure 3, octyl glucoside was inhibitory in the concentration range of 0.03% (1 mM) through 0.1% (3.4 mM), less than one tenth the critical micelle concentration of the detergent. Octyl glucoside (0.1%) had no effect on the viability or morphology of either cell type, nor the spontaneous release of chromium from the U937 cells. The action of octyl glucoside seemed to be directed toward the monocytic cell since pretreatment of U937 cells with octyl glucoside, followed by rigorous washing of the cells still resulted in a substantial decrease in adhesion to EC, whereas pretreatment of EC followed by washing caused no decrease in cell-cell adhesion. When octyl glucoside remained present during the adhesion assay, pretreatment of the EC had no effect; however, the pretreatment of U937 cells caused a substantial increase in the level of inhibition. Octyl glucoside also inhibited U937 cell binding to bovine aortic EC and human umbilical vein EC, and fully inhibited normal human monocyte binding to EC (Table V).

With the finding of two potent inhibitors of monocytic cell binding to EC, we investigated the possible synergistic effect of WGA and octyl glucoside at suboptimal concentrations (Table VII). Surprisingly, their actions were not additive, but rather the two agents neutralized each other's inhibitory activity. The inhibition caused by the combination of submaximal concentrations of octyl glucoside and WGA was substantially less than either agent alone.

Effect of WGA, lactose-1-phosphate, and octyl glucoside on binding of monocytic cells and neutrophils to IL-1-stimulated EC. IL-1 has been shown by others to markedly stimulate monocytic cell adhesion to cultured EC in a process that requires protein synthesis. Because WGA, lactose-1-phosphate, and octyl glucoside had a significant effect on U937 cell binding to untreated, sparse EC (endogenous binding sites), we asked whether these agents would also inhibit the inducible binding seen in IL-1 stimulated EC (Fig. 4). The three inhibitors reduced U937 cell and monocyte adherence to IL-1-stimulated EC very effectively (Fig. 4, A and B), a finding confirmed with LPS-stimulated EC (data not shown). However, only lactose-1-phosphate inhibited neutrophil adhesion to EC. Octyl glucoside had little effect and WGA actually enhanced neutrophil adhesion to unstimulated EC (Fig. 4C).

Irreversibility of monocytic cell binding to EC. As we have noted previously, the adhesion of U937 cells to EC is not the result of weak or transient binding. The adhesive interaction, though formed at 4°C, is not reversed by a fairly rigorous washing procedure, and will not spontaneously reverse over the course of many hours after binding (7). The irreversibility of the cell-cell interaction was again demonstrated by the finding that even two potent inhibitors of binding, WGA and octyl glucoside, will not dissociate bound monocytic cells from the EC surface (Table VIII).

DISCUSSION

Monocyte binding to EC must be tightly regulated in vivo in order to allow for a rapid but transient influx of these pluripotent scavenger cells at sites of injury and

TABLE VI
Effect of octyl glucoside on U937 cell binding to porcine EC^a

| EC Pretreatment | U937 Pretreatment | Octyl Glucoside (0.1%) Present in Assay | U937 Cells Bound ($\times 10^{-5}$) | Reduction in Binding (%) |
|-----------------|-------------------|---|---------------------------------------|--------------------------|
| Media | Media | - | 1.1 \pm 0.06 | |
| Media | Media | + | 0.3 \pm 0.09 | 77 |
| Octyl glucoside | Media | - | 1.4 \pm 0.10 | 0 |
| Media | Octyl glucoside | - | 0.5 \pm 0.14 | 55 |
| Octyl glucoside | Media | + | 0.2 \pm 0.02 | 79 |
| Media | Octyl glucoside | + | 0.1 \pm 0.01 | 92 |
| Octyl glucoside | Octyl glucoside | + | 0.1 \pm 0.03 | 94 |

^a Subconfluent porcine EC in 24-well plates were treated for 1 h at 4°C with octyl glucoside in DME/F12 media + 5% FCS. ⁵¹Cr-labeled U937 cells (4×10^6 /ml) were similarly treated. U937 cells were incubated with comparably treated EC and assayed for adhesion as described in *Materials and Methods*. Numbers are the average of triplicate wells \pm SD.

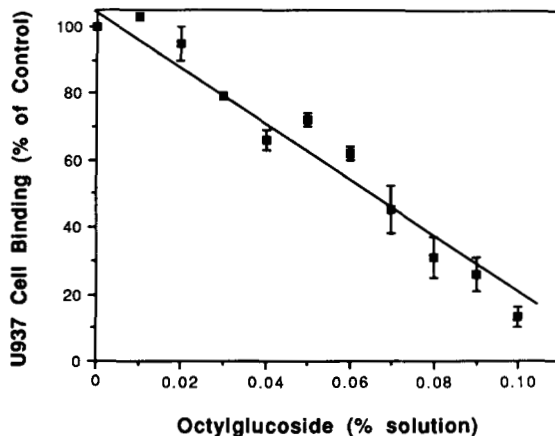


Figure 3. Effect of octyl glucoside on U937 cell binding to porcine endothelial cells. Subconfluent porcine EC and ⁵¹Cr-labeled U937 cells were treated with varying concentrations of octyl glucoside in DME/F12 media + 5% FCS for 1 h at 4°C. The treated U937 cells were combined with the treated EC and further incubated in the presence of octyl glucoside at 4°C for 1 h. The plates were washed and cells harvested as described in *Materials and Methods* (values \pm SD).

TABLE VII
Effect of submaximal doses of WGA and octyl glucoside on binding of U937 cells to EC^a

| Treatment of U937 and PAEC | U937 Cells Bound ^b ($\times 10^{-5}$) | Reduction in Binding (%) |
|----------------------------|--|--------------------------|
| Media | 6.1 \pm 0.47 | |
| WGA | 4.1 \pm 0.37 | 34 |
| Octyl glucoside | 2.3 \pm 0.27 | 61 |
| WGA + octyl glucoside | 5.9 \pm 0.33 | 3 |

^a Porcine EC and U937 cells were separately treated for 1 h at 4°C with DME/F12 + 5% FCS containing WGA (20 μ g/ml) or octyl glucoside (0.07%), or both. U937 cells (10^6) were added to triplicate wells of EC and further incubated for 1 h at 4°C and adhesion quantitated as described in *Materials and Methods*.

^b Value is the mean of three wells \pm SD.

inflammation. One *in vitro* method for enhancing monocyte adhesion to endothelium, and therefore allowing study of the adhesion process, is to plate the EC at subconfluence, such that the cells are actively migrating and proliferating, a situation perhaps analogous to a healing wound area *in vivo* (7). The adhesion step can be monitored after incubation of EC and monocytic cells at 4°C, a temperature which is conducive to separating the initial binding event from subsequent cellular interactions and biochemical "cross-talk." We and others have previously validated the use of human monocytic cell line U937 as a model for the blood-borne monocyte in studies of adhesion to EC. Because many cellular recognition events involve carbohydrate moieties either as part of a glycoprotein receptor or as a component of "target" molecules, we hypothesized that monocyte adhesion to EC

was mediated in part through specific sugar residues on the surface of either or both cell types. We wished to determine whether carbohydrate groups did in fact play a role in monocyte-EC adhesion and whether the sugars were the same or distinct from those critical to lymphocyte adhesion to high endothelial venules.

Several of the lectins tested caused U937 cell agglutination, indicating the presence of specific "target" sugars on the U937 cell surface. However, only WGA inhibited the binding of U937 cells to EC. Since the U937 cells were not agglutinated by WGA, one might infer that the sugar ligand of WGA was present solely on the EC. We were unable to confirm this hypothesis since the interaction of the WGA and its adhesion sensitive target is so readily dissociated. The reversibility of WGA inhibition and the lack of effect of NAG on adhesion suggest that this lectin is interacting with a sugar for which it has low affinity (29, 30). We were unable to identify the ligand to which WGA is binding via sugar competition studies, though we tested an extensive series of low affinity ligands for WGA. A second possibility is that WGA is binding to a carbohydrate site in close proximity to the actual adhesion protein and sterically interfering with monocyte-endothelial binding. This lectin is a dimer of 35,000 Da at neutral pH (28, 31). This latter possibility would account for the inability of WGA target sugars to interfere directly with binding between EC and monocytes.

Of the many neutral and phosphorylated sugars that were screened for the ability to block monocytic cells from binding to EC, including mannose-6-phosphate which has been shown to have a lectin-like receptor on several cell types including lymphocytes and fibroblasts, only lactose-1-phosphate and maltose-1-phosphate had any inhibitory activity. The phosphorylated monosaccharides, glucose and galactose, which represent the subunits of the active disaccharides, had little inhibitory activity and nonphosphorylated lactose had no effect. Thus, a combination of the disaccharide structure and the phosphate group is required to block monocyte-EC adhesion. Lactose-1-phosphate was active as an inhibitor in a concentration range comparable to that of mannose-6-phosphate and fructose-1-phosphate in the lymphocyte-high endothelial venule binding system; inhibition was seen at 0.5 mM and was maximal at 10 mM. The effect of phosphorylated lactose could be completely reversed by washing the involved cells after a few hours but not after overnight incubation. The target for the sugar appears both on the monocytic cell and the EC, and the long incubation time for a non-reversible effect

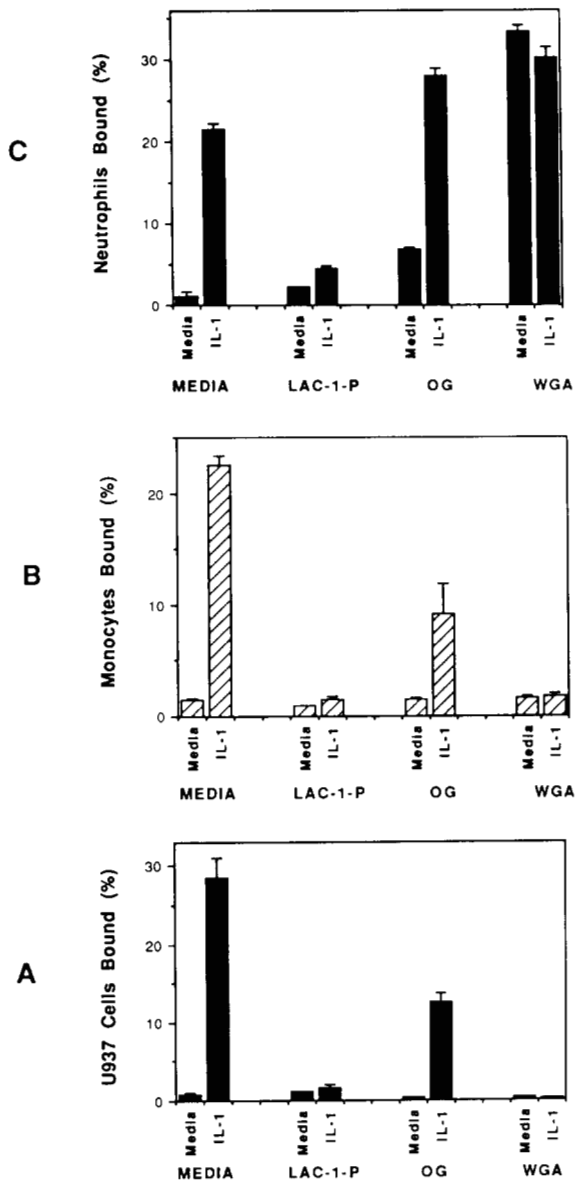


Figure 4. Effect of lactose-1-phosphate, octyl glucoside, and WGA on binding of U937 cells, normal human monocytes, and normal human neutrophils to unstimulated and IL-1-stimulated human EC. Confluent human umbilical vein EC were incubated for 4 h at 37°C with media (DME/F12 + 5% FCS) alone or media containing human IL-1 (10 ng/ml). Wells were rinsed and treated with either media alone or media containing 10 mM lactose-1-phosphate, 0.1% octyl glucoside, or 100 µg/ml WGA for 1 h at 4°C. ⁵¹Cr-labeled U937 cells, monocytes, and neutrophils were treated with each of the inhibitors for 1 h at 4°C, after which 10⁶-treated U937 cells or neutrophils, or 5 × 10⁵ monocytes were added to each well of comparably treated EC, and the cells again incubated at 4°C for 1 h. Adhesion was quantitated as described in *Materials and Methods*; (Values ± SD).

may reflect a lactose-1-phosphate-induced change at the membrane surface. A similar situation has been observed in studying the elastin receptor in which pretreatment of chondroblasts with 5 mM lactose for 18 h resulted in loss and redistribution of the 67-kDa component of the receptor (32). Lactose-1-phosphate did not interfere with the action of WGA, which suggests that the two inhibitors are acting via distinct mechanisms and that monocyte binding involves at least two distinct carbohydrate moieties.

We believe the ability of the detergent octyl glucoside to inhibit monocyte-EC binding was also related to carbohydrate involvement in the adhesion process. The de-

TABLE VIII
Effect of WGA and octyl glucoside on prebound U937 cell-EC complexes^a

| EC Pretreatment | Treatment of Bound U937-EC Complex | U937 Cells Bound ^b (×10 ⁻⁵) | Reduction in Binding (%) |
|--|------------------------------------|--|--------------------------|
| Media | Media | 3.9 ± 0.15 | |
| Media | WGA | 4.2 ± 0.38 | (-8) |
| Media | Octyl glucoside | 3.2 ± 0.05 | 19 |
| IL-1 | Media | 7.6 ± 0.11 | |
| IL-1 | WGA | 7.2 ± 0.20 | 5 |
| IL-1 | Octyl glucoside | 7.2 ± 0.33 | 5 |
| Octyl glucoside present during binding phase | | 0.7 ± 0.25 | 82 |

^a Subconfluent human EC were incubated at 37°C for 4 h with DME/F12 + 5% FCS in the presence or absence of IL-1 (5 U/ml) in DME/F12 + 5% FCS, and washed. ⁵¹Cr-labeled U937 cells (10⁶) were incubated with wells of pretreated EC for 1 h at 4°C. Wells were washed three times and then 0.5 ml/well of DME/F12 + 5% FCS alone or plus WGA (100 µg/ml) or octyl glucoside (0.1%) were added to triplicate wells, and incubated for 90 min at 4°C. Wells were again washed three times and harvested for radioactive determination as described in *Materials and Methods*.

^b Mean ± SD. (n = 3).

tergent did not change gross cell morphology or viability of either cell type, which is consistent with the concentration employed—i.e., below the critical micelle concentration of octyl glucoside (33). The amphipathic structure of octyl glucoside would cause it to partition into the plasma membrane of the cells with the substituted glucose ring accessible to the extracellular milieu. We therefore envision the action of this molecule to be that of a sugar rather than a detergent. The action of octyl glucoside was directed mainly toward the monocytic cell. The detergent may act either by binding to a specific site, perhaps due to similarities to an adhesion moiety on EC, and physically blocking the cell interaction, or less likely, by permanently altering the cell binding site on the monocyte nonspecifically, making adhesion impossible. The possibility that octyl glucoside is acting as a lipid-anchored sugar is further supported by our finding that WGA and octyl glucoside apparently interact in the binding assay to render each other inactive for inhibiting cell-cell adhesion. In addition, we have observed an inhibition of WGA-mediated human erythrocyte agglutination by phosphatidylcholine vesicles containing octyl glucoside but not by control phosphatidylcholine vesicles (our unpublished observation).

Enhanced expression of specific sugar moieties as a result of activation, differentiation and transformation has been shown by lectin typing experiments in functional subsets of T lymphocytes (28). When EC are stimulated to bind greater numbers of monocytic cells by sparse density or by the action of exogenous agents such as IL-1 and LPS, it is conceivable that the EC may also express greater numbers of specific sugar containing compounds. The three agents that block monocytic cell adhesion to sparse EC, WGA, lactose-1-phosphate, and octyl glucoside, also block adhesion to IL-1- and LPS-stimulated confluent EC. That the two different pathways of stimulated expression of monocyte binding sites on the EC surfaces lead to the exposure of similar carbohydrate moieties suggests a common path of activation. Recent sequencing data on known glycoprotein adhesion molecules, ELAM-1 and MEL-14, which are important in neutrophil and lymphocyte interactions with EC, have revealed lectin-like domains (17, 34). Another glycoprotein expressed on activated EC surfaces is

granule membrane protein 140 (GMP 140) which has been shown to contain greater than 28% carbohydrate (35), as well as carbohydrate binding sequence similar to the above mentioned adhesion molecules (17). The presence of specific sugar moieties or lectin-like molecules on the EC surface may serve as markers of the differing activated states of the endothelium.

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