Basic Science Reports

Effect of Endothelin-1 on Neutrophil Adhesion to Endothelial Cells and Perfused Heart

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Background. Based on recent evidence showing that endothelin-1 stimulates several activation mechanisms on neutrophils, the aim of the present study was to analyze the effects of endothelin-1 on neutrophil adhesion to endothelial cells and neutrophil accumulation in the heart.

Methods and Results. The experiments included (1) adhesion of 51Cr-labeled human neutrophils to bovine endothelial cells in culture both in the presence and absence of monoclonal antibodies against the α- and β-subunits of integrins; (2) surface expression of the α- and β-integrin antigens; (3) accumulation of 3H-thymidine-labeled neutrophils on the isolated perfused rabbit heart; (4) in vivo accumulation of autologous neutrophils in the heart, as assessed by myeloperoxidase activity. Endothelin-1 stimulated neutrophil adhesion to endothelial cells (increase of 1×10^4±1×10^4 neutrophils per well). The endothelin-1-induced adhesion was blocked (83±6%) by the anti-CD18 antibody TS1/18 and by several anti-α-subunit antibodies. The expression of CD18 and CD11b on the neutrophil surface was also increased by endothelin-1. Endothelin-1 enhanced neutrophil accumulation in the isolated rabbit heart by 4.2 times throughout a TS1/18-inhibitable mechanism. Myeloperoxidase activity increased by 4.2 times in hearts infused in vivo with endothelin-1.

Conclusions. Endothelin-1 stimulates neutrophil adhesion to endothelial cells by an effect on the expression of adhesive molecules on the neutrophil surface. Endothelin-1 stimulates neutrophil accumulation in vivo and in vitro in the heart. Antibodies against the integrin complex block the endothelin-1-dependent neutrophil adhesion. These findings have potential importance in the pathophysiology of endothelin-1-induced states. (Circulation. 1993;88:1166-1171.)

Key words • endothelin • neutrophils • integrins • ischemia • reperfusion

The interaction of circulating neutrophils with vascular endothelium is a critical phenomenon in several pathological circumstances.1,2 Neutrophil accumulation on the endothelial surface of blood vessels may lead to alterations in blood flow, changes in vascular permeability, and endothelial injury. However, a whole picture of the mechanisms involved in such phenomena is still unavailable, and several relevant aspects remain to be clarified.

Endothelin-1 is a potent vasoconstrictor peptide synthesized by endothelial cells3; it is highly expressed in several pathological circumstances, eg, myocardial and renal ischemia4-5 and atherosclerosis,6 which also involve interaction between polymorphonuclear or mononuclear leukocytes and the endothelium. Recently, studies from several laboratories including ours have found a significant effect of endothelin-1 on neutrophils by increasing intracellular free calcium mobilization,7 N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)–mediated superoxide anion production,8 or aggregation.9

Based on the above-mentioned data about the effects of endothelin-1 on neutrophils, we hypothesized that a significant effect of endothelin-1 on neutrophil adhesiveness was likely to occur. The general mechanisms for the adhesion of neutrophils to endothelial cells have been extensively studied in recent years (see References 10 and 11 for review). Briefly, neutrophil activation results in the expression or activation of surface adhesive proteins, which are members of the glycoprotein-adhesive complex CD11/CD18 (see References 12, 13, and 16 for review). The CD11/CD18 adhesion complex is a family of three heterodimers, each consisting of a common β-polypeptide chain and a variable α-chain. These heterodimers have been called LFA-1, Mac-1, and p150,95.17 In the present study, we first addressed the effect of endothelin-1 on neutrophil adhesion to endothelial cells in culture. After observing that endothelin-1 induced neutrophil adhesion to the endothelial cells, we tested the implication of the adhesive molecules LFA-1, Mac-1, and p150,95. Further studies were done to assess the actual validity of these findings in entire organs both under in vitro and in vivo conditions; we have chosen the heart as a probe, based on the particular importance of neutrophils on myocardial ischemia.

Methods

Chemicals

Ficoll-hypaque medium was obtained from Flow Laboratories. fMLP and phorbol 12-myristate-13-acetate were purchased from Sigma Chemical (St Louis, Mo). N0-monomethyl L-arginine (L-NMMA) was obtained

Received September 29, 1992; revision accepted May 3, 1993.
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from Calbiochem (La Jolla, Calif) and endothelin-1 from Peninsula Laboratories, Inc (London). Na$_{18}$CrO$_{4}$ was obtained from Amersham (Buckinghamshire, England).

The monoclonal anti-integrin antibodies were generously provided by Prof F. Sánchez Madrid (Hospital de la Princesa, Universidad Autónoma, Madrid). All other chemicals were from the highest commercially available quality from Sigma.

**Neutrophil Preparation**

Human or rabbit neutrophils were obtained from peripheral blood by Ficoll-hypaque centrifugation, as previously described.7 Neutrophils (95% pure, 98% viable by trypan blue exclusion) were resuspended in 3 mL of HEPES buffer (approximately $5 \times 10^7$ cells/mL) containing in mmol/L: NaCl 131, KCl 4.7, glucose 5, and HEPES 20, pH 7.4 and 2 $\mu$Ci/mL Na$_{18}$CrO$_{4}$. After incubation at $37^\circ$C for 60 minutes, the cells were washed three times in HEPES buffer and resuspended in Krebs-Henseleit buffer (in mmol/L: NaCl 118, KCl 4.7, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, Cl$_2$Ca 2.5, and NaHCO$_3$ 25, pH 7.4) at a density of $6 \times 10^6$ cells/mL.

A similar labeling procedure was performed for erythrocytes, which were used as a control of the specificity of neutrophil accumulation in the isolated heart experiments (see below).

**Endothelial Cell Culture**

Endothelial cells were isolated from bovine aortas, obtained from a local slaughterhouse. In brief, the aortic lumen was filled with 0.5 mg/mL collagenase (type II, Sigma), and the vessel was incubated at $37^\circ$C for 20 minutes. Endothelial cells were harvested in RPMI medium supplemented with 10% fetal calf serum, 5 mmol/L glutamine, 200 U/mL penicillin, and 200 $\mu$g/mL streptomycin and seeded into 24-well plates. Cells were used at confluency and between 1 and 2 passages.

Human endothelial cells were isolated from the umbilical vein by treatment with 0.5 mg/mL collagenase (type II, Sigma) (30 minutes, $37^\circ$C). These cells were seeded on gelatin-coated multiwell plates in RPMI medium containing 20% fetal calf serum and supplemented with 5 mmol/L glutamine, 20 $\mu$g/mL heparin, and 50 $\mu$g/mL endothelial cell growth factor. The cells were characterized as endothelial by the typical cobblestone appearance and by indirect staining immunofluorescence for factor VIII–related antigen.

**Adhesion and Flow Cytometry Assays**

Endothelial cells were washed three times with sterile Krebs buffer containing 0.2% bovine serum albumin, and $^{51}$Cr-neutrophil suspension (500 $\mu$L, $3 \times 10^6$ cells) was added. The agents to be tested were then added, and the cells were incubated for 30 minutes at $37^\circ$C under static conditions. Loosely adherent or unattached neutrophils were washed twice with 0.5 mL of Krebs buffer, and the endothelial monolayer plus the adherent neutrophils were lysed in 0.5 mL of 5 mol/L NaOH. For antibody inhibition experiments, either specific or nonspecific antibodies were diluted in assay buffer and added to the $^{51}$Cr-labeled neutrophils 10 minutes before initiating the adhesion assay. The number of neutrophils in each experiment was estimated from the radioactivity of a control sample. Results were expressed as the number of neutrophils adhered by well of endothelial cells. Each well contained a mean of $3.5 \times 10^6 \pm 10^6$ endothelial cells, corresponding to a mean of $80 \pm 10$ $\mu$g of protein.

To further address the individual role of neutrophil-related or endothelium-related mechanisms in the adhesion phenomenon, two additional experiments were performed: (1) neutrophil adhesion to the endothelium was examined after preincubation (10 minutes) of the endothelial cells with endothelin-1 ($10^{-7}$); endothelin-1 was washed off with fresh Krebs buffer ($37^\circ$C, 5 times), and the assay was performed in Krebs containing no endothelin-1; (2) similar neutrophil adhesion experiments were carried out on a plastic surface (tissue culture dish $35 \times 10$ mm, Costar, Cambridge, Mass) both in the presence and absence of endothelin-1. Furthermore, adhesion experiments were done in the presence of other pressor hormones, namely, arginine vasopressin ($10^{-7}$ mol/L) or angiotensin II ($10^{-7}$ mol/L), to examine the specificity of the observed endothelin-1 effect.

For immunofluorescence flow cytometry assays, the neutrophils were incubated with the monoclonal antibodies against the CD18 or CD11 antigens followed by a secondary incubation with a fluorescein isothiocyanate–conjugated goat anti-mouse IgG (The Binding Site, Birmingham, England).18,19 The fluorescence was measured by using an EPICS flow cytometer/ sorter (Coulter) at 480-nm excitation and 520-nm emission. A minimum of 6000 cells were analyzed for each sample. The data were displayed as one-parameter histograms plotting the logarithm of the green fluorescence vs cell number.

**Monoclonal Antibodies**

The monoclonal antibodies used in these studies have been described elsewhere.17 These antibodies recognize different epitopes on the $\alpha$- and $\beta$-integrin chains as follows: the anti-CD18 was TS1/18, the anti-LFA-1-$\alpha$ (CD11a) was TS1/11 and TP1/32, the anti-Mac-1 (CD11b) was Bear-1, and the anti-p150,95 (CD11c) was HC1/1. P3X63 myeloma culture supernatant was used as negative control.

**Isolated Heart Experiments**

Healthy male rabbits weighing approximately 1.9±0.6 kg were anesthetized with sodium pentobarbital (100 mg/kg body wt IP). The chest was opened, and the sternum and ventral portions of the ribs were removed to expose the heart and lungs. The aorta was cannulated with a 21-gauge needle, and the tip of the cannula was placed approximately 3 mm above the aortic valve. The heart was quickly removed and placed into a thermostatted ($37^\circ$C), oxygenated (95% O$_2$, 5% CO$_2$), siliconized glass beaker, which contained Krebs solution supplemented with 5 mol/L glucose and 5% bovine serum albumin. The heart was externally and internally washed, and the coronary circulation was perfused until a uniform pale color was obtained. Thereafter, endothelin-1 solution (0.027 $\mu$g/min in Krebs solution) was perfused during 30 minutes at a rate of 0.1 mL/min. The endothelin-1 dose was selected according to previous reports showing that doses lower than 0.3 $\mu$g did not produce a significant in vivo coronary vasoconstrictor effect.20 Two minutes after the beginning of the endothelin-1 infusion, $^{51}$Cr-labeled rabbit neutrophils in sterile Krebs buffer ($8 \times 10^6$ in 0.5 mL, 79 995±10 354 cpm/8$\times 10^6$ cells) were injected as a bolus. Additional
experiments were performed by preincubating (10 minutes) the $^{51}$Cr-labeled neutrophils with the monoclonal antibody TS1/18 or with the nonspecific antibody P3X63.

The perfusion system was closed by a catheter connected from the perfusion Krebs solution to the aortic cannula, using a flow pump. Through this catheter, the perfusate from the heart returned continuously to the organ during the whole experiment. The experiment was stopped by washing out the nonadherent neutrophils with fresh Krebs buffer. The hearts were homogenized in 10% TCA (6 mL), divided into polystyrene tubes, and counted in a gamma counter. As a control of the specificity of neutrophil accumulation, additional experiments were done using $^{51}$Cr-labeled erythrocytes (packed cell volume, 30% in the Krebs buffer; 295 035±36 550 cpm) instead of neutrophils. As a further control for the role of neutrophil isospecificity or heterospecificity in the observed phenomena, additional experiments were done using human instead of rabbit neutrophils.

**In Vivo Experiments: Myeloperoxidase Activity Measurement**

Under ketamine (10 mg/kg body wt) and xilazine (20 mg/kg body wt IV) anesthesia and mechanical ventilation (Harvard miniature animal ventilator, Edenbridge, Kent, England), tracheostomized rabbits with characteristics similar to those described above were surgically prepared for exposure of the left atrium, which was used as the mixing chamber. A 22-gauge needle connected to an infusion pump was inserted into the left atrium and tied up. A continuous infusion of physiological saline solution (PSS; in mmol/L: NaCl 137, KCl 2.6, KH$_2$PO$_4$ 1.5, NaH$_2$PO$_4$ 8, glucose 5.6) (0.1 mL/min, 37°C) was then administered during a 30-minute equilibration period. At the end of the equilibration, either endothelin-1 (0.027 μg/min) or PSS alone was infused for an additional 30 minutes. Once this second infusion was finished, the animals were given an overdose of anesthetic, and the heart, lungs, and kidneys were perfused and washed with PSS. Immediately after, the organs were frozen in liquid nitrogen for myeloperoxidase activity determination.

Myeloperoxidase activity measurements were performed after homogenizing the tissues in 50 mmol/L of potassium phosphate buffer, pH 6.0, containing 0.5% hexadecyltrimethylammonium bromide. The homogenized tissue was freeze-thawed three times and centrifuged; after centrifugation, the supernatant (0.2 mL) was mixed with 2.9 mL of 50 mmol/L of potassium phosphate buffer, pH 6.0, containing 0.167 mg/mL of o-dianisidine dihydrochloride and 0.5% hydrogen peroxide. The myeloperoxidase activity was measured as the change in absorbance at 460 nm. One unit of myeloperoxidase was defined as the amount of sample degrading 1 μmol of hydrogen peroxide per minute at 25°C. Mean arterial pressure was continuously registered during the whole experimental procedure by a catheter in the femoral artery connected to a pressure recorder (Leticia, Scientific Instruments, Madrid).

**Statistical Methods**

Results are expressed as mean±SEM. Unless otherwise stated, each value corresponds to a minimum of six experiments done in triplicate. Comparisons were done by ANOVA and Scheffé’s multiple comparisons test and unpaired Student’s $t$ test when appropriate.

**Results**

**Stimulation of Neutrophil Adhesion by Endothelin**

Endothelin-1 ($10^{-7}$ mol/L) stimulated human $^{51}$Cr-neutrophil adherence to bovine endothelial cells (Fig 1). The endothelin-1-induced neutrophil adhesion was equivalent to that provoked by the known chemoattractant peptide fMLP ($10^{-7}$ mol/L) and smaller than the adhesion induced by a full dose of the phorbol ester PMA ($10^{-4}$ mol/L) (Fig 1). The dose of endothelin-1 was chosen based on previous studies in which $10^{-7}$ mol/L endothelin-1 consistently provoked a peak of cytosolic free calcium. In addition, an increased neutrophil adhesion was observed by using $10^{-8}$ and $10^{-7}$ mol/L endothelin-1 (increase of neutrophil adhesion: 9.06±7.13×10$^4$ neutrophils per well, $P<.01$). Endothelin-1 $10^{-10}$ mol/L or lower did not consistently induce neutrophil adhesion (5.23±4.6×10$^4$ neutrophils per well, $P=NS$). No significant increase of adhesion was observed when the endothelial cells alone were preincubated with endothelin-1 ($10^{-7}$ mol/L, 10 minutes) (increase of neutrophil adhesion: 2.7×10$^4$±8.1×10$^4$ neutrophils per well, $n=9$, $P=NS$). No adhesion experiments were done with neutrophils alone preincubated with endothelin-1 since endothelin-1 may provoke neutrophil aggregation; therefore, interpretation of the results was difficult. On the other hand, an increased adhesion in the presence of endothelin-1 was observed on the plastic surface (increase of neutrophil adhesion: 1.94×10$^4$±1.2×10$^4$ neutrophils per well; $n=6$, $P<.01$). A significant effect of endothelin-1 on the adhesion of human neutrophils to human umbilical vein endothelial cells was also observed (increase of adhered neutrophils: 1.2×10$^4$±1×10$^4$ neutrophils per well; $n=5$, $P<.01$), therefore ruling out a nonspecific effect related to species heterogeneity between human neutrophils and bovine endothelial cells. Further support for the specific effect of endothelin-1 was obtained in the in vivo experiments (see below), in which accumulation of autologous rabbit neutrophils was observed on rabbit hearts.
Results

Neutrophil

reproduces

effect

significant

CD11c/CD18

presence

CD11b/CD18 (TP1/32), and the CD18 antigen (TS1/18). Results are represented as mean±SEM. *P<.05 with respect to endothelin-1-induced adhesion.

Similar adhesion experiments were performed in the presence of other pressor peptides, ie, angiotensin II (10−7 mol/L) and arginine vasopressin (10−7 mol/L). No significant effect was observed (increase of neutrophil adhesion with angiotensin II: 1.83×104±3.2×103; with arginine vasopressin: 6.77×103±1.8×103 neutrophils per well, P=NS), therefore suggesting that the adhesive effect was quite specific for endothelin-1.

Characterization of the Neutrophil Adhesion Proteins

The implication of the CD11/CD18 complex in endothelin-1-stimulated neutrophil adhesion was initially examined by performing the adhesion assay in the presence of the specific monoclonal antibody TS1/18, directed against the common β-subunit. As shown in Fig 2, the blockade of the β-chain of the integrin complex blunted the endothelin-1-stimulated neutrophil adhesion. No effect of the nonspecific antibody P3X63 was detected on endothelin-1-induced neutrophil adherence (2±1.4% blockade, P=NS).

Once the CD11/CD18 complex was identified as the putative mediator of the endothelin-1-induced neutrophil-endothelium interaction, we examined which of the α-subunits was possibly involved in this process. As shown in Fig 2, a significant inhibition of the endothelin-1-induced neutrophil adhesion to endothelial cells was observed with anti-CD11a, anti-CD11b, and anti-CD11c monoclonal antibodies. The inhibition of binding caused by anti-CD11a, anti-CD11b, and anti-CD11c was partial. The inhibition observed by TS1/18 was nearly complete (about 80% to 90%), suggesting that CD11a/CD18, CD11b/CD18, or CD11c/CD18 has a function in adhesion of endothelin-1-stimulated neutrophils to endothelial cells. This was confirmed by the observation that a mixture of monoclonal antibodies against LFA-1, Mac-1, and p150,95 had an additive inhibitory effect on the neutrophil adhesion (Fig 2).

Analysis by flow cytometry demonstrated that endothelin-1 (n=4) increased the expression of the CD18 β-integrin common subunit in a similar degree to that induced by fMLP (Fig 3A). An increased expression of CD11b was also observed in the presence of endothelin-1 (Fig 3B), whereas no significant effect of endothelin-1 was detected on the surface expression of CD11a and CD11c (data not shown).

Fig 2. Bar graph shows inhibition of endothelin-1-induced neutrophil adhesion (%) by monoclonal antibodies against CD11a/CD18 (TP1/32 and TS1/11), CD11b/CD18 (Bear-1), CD11c/CD18 (HC1/1), and the CD18 antigen (TS1/18). Results are represented as mean±SEM. *P<.05 with respect to endothelin-1-induced adhesion.

Fig 3. A, Flow cytometry analysis of the expression of CD18 antigen on the neutrophil surface, unstimulated (1) or stimulated with 10−7 mol/L endothelin-1 (2) or 10−7 mol/L N-formyl-l-methionyl-l-leucyl-l-phenylalanine (fMLP) (3). The figure reproduces the real trace of one typical experiment. B, Flow cytometry analysis of the expression of CD11b/CD18 antigen on the neutrophil surface, unstimulated (1) or stimulated with 10−7 mol/L endothelin-1 (2) or 10−7 mol/L fMLP (3). The figure reproduces the real trace of one typical experiment.
Myeloperoxidase Activity (U/100 mg Tissue) in Heart From Rabbits With Intracardiac Endothelin-1 (0.027 µg/min) or Vehicle Infusion

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<tr>
<th></th>
<th>Heart</th>
<th>Kidney</th>
<th>Lung</th>
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<tr>
<td>Control</td>
<td>0.024±0.001</td>
<td>0.0013±0.0003</td>
<td>0.083±0.02</td>
</tr>
<tr>
<td>Endothelin-1 (n=5)</td>
<td>0.102±0.032±</td>
<td>0.0074±0.001*</td>
<td>0.087±0.01</td>
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*p<.01, †p<.001.

Neutrophil Adhesion to Perfused Heart

Endothelin-1 infusion markedly increased the accumulation of rabbit neutrophils on the isolated perfused rabbit heart (unstimulated neutrophil accumulation: 1.5x10^4±1.6x10^3; endothelin-1-stimulated neutrophils: 4.2x10^6±3.8x10^5 neutrophils; n=5, P<.01). No changes in the coronary perfusion pressure were detected with the dose of 0.027 µg/min endothelin-1 (perfusion pressure, 100±21 and 104±26 mm Hg in the absence and presence of endothelin-1, respectively, P=NS). Moreover, in order to discard a nonspecific trapping of neutrophils induced by any undetected vasoconstrictor effect of endothelin-1, two additional experiments were carried out: (1) Neutrophils were incubated with the monoclonal antibody against the CD18 antigen TSI1/18 from 10 minutes before and throughout the infusion into the coronary circulation. TSI1/18 inhibited endothelin-1-induced neutrophil accumulation by 65±15% (P<.01), therefore indicating a significant involvement of the CD11/CD18 complex. No inhibition of the endothelin-1-stimulated neutrophil accumulation was found by preincubating the neutrophils with the nonbinding monoclonal antibody P3X63 (percent inhibition, 1.2±0.8; P=NS). (2) 51Cr-labeled erythrocytes were infused both in the presence (n=3) and the absence of endothelin-1 (n=3). No differences were detected in the accumulated 51Cr radioactivity, depending on the absence or presence of endothelin-1 (total 51Cr, 20 313±1300 and 23 110±1810 cpm per gram of heart, respectively; P=NS). In all of the comparative experiments, cells from the same batch of 51Cr-labeled neutrophils or erythrocytes were used.

To rule out any possible nonspecific interaction between TSI1/18 and rabbit neutrophils, additional experiments were carried out by preincubating human neutrophils with TSI1/18 before infusing them into the rabbit heart (n=3). Endothelin-1 stimulated human neutrophil accumulation in the isolated rabbit heart in a degree similar to that observed by using rabbit neutrophils (increase of human neutrophil accumulation, 3.01x10^4±3.01x10^5 neutrophils; n=4, P<.05). This effect was inhibited (62±12% inhibition; n=3, P<.05 with respect to endothelin-1 alone) by coinfusion with TSI1/18.

In Vivo Neutrophil Accumulation in Endothelin-1-Infused Rabbits

The intra-atrial administration of endothelin-1 induced a significant increase of the activity of the neutrophil-specific enzyme myeloperoxidase in the rabbit heart (Table). A significant albeit smaller increase of myeloperoxidase activity in the rabbit kidneys and no changes of myeloperoxidase activity in the rabbit lungs also were observed (Table). These results confirmed that endothelin-1 was capable of inducing neutrophil accumulation in in vivo conditions. No changes in systemic arterial pressure were detected with the intra-cardiac administration of this dose (0.027 µg/min) of endothelin-1 (increase in mean arterial pressure, 4±1.8 mm Hg; P=NS).

Discussion

The complete in vivo functions of endothelin-1 are still insufficiently defined. Several studies have shown that circulating immunoreactive endothelin-1 levels are elevated in conditions in which the interaction between neutrophils and endothelium is especially relevant, eg, myocardial ischemia. However, no reports have been published to date about the possible relation between the high endothelin-1 levels and the functional properties of the neutrophils, eg, adhesiveness. The present results may contribute to the understanding of the actual pathophysiological role of endothelin-1. For the first time, endothelin-1 and organ accumulation of neutrophils are shown to be related.

First we found that endothelin-1 induced neutrophil adhesion to the endothelium. The endothelin-1 concentrations that provoked neutrophil adhesion were higher than the reported endothelin-1 plasma levels. However, the local tissue levels of endothelin-1 during ischemia are necessarily much higher than those measured in blood samples in ischemic conditions. Therefore, local concentrations of endothelin-1 in the range of 10^9 mol/L or higher could probably be reached in pathological circumstances. Subsequently, it was found that endothelin-1-induced neutrophil adhesion was blocked by TS1/18, therefore implicating the CD11/CD18 integrins in the observed phenomenon. This was further supported by the flow cytometric studies, which showed that endothelin-1 induced an increased surface expression of the CD18 and CD11b antigens, as previously shown with agents such as fMLP. Our findings using monoclonal antibodies to each particular α-chain suggested that CD11a/CD18, CD11b/CD18, or CD11c/CD18 may have a significant albeit partial participation in endothelin-1-stimulated neutrophil adhesion. Specifically, the anti-CD11b/CD18 antibody Bear-1 appeared to be more efficient as a neutrophil adherence inhibitor, a result that is consonant with the finding of an increased surface expression of CD11b by endothelin-1. The almost total inhibition observed by either blocking the CD18 common antigen or by combining the monoclonal antibodies against the three different α-chains suggested that a positive interaction of the three members of the CD11/CD18 integrin family mediates the endothelin-1-induced neutrophil adhesion by a mechanism that may include either activation or overexpression of the integrins.

Further experiments are necessary to precisely identify the specific endothelial ligands involved in endothelin-1-induced adhesion. However, these putative ligands should be basally expressed on the endothelial surface and probably are not induced by endothelin-1, as suggested by the experiments preincubating endothelial cells alone with endothelin-1. In this regard, some integrin ligands, eg, ICAM-1 and ICAM-2, are constitutively expressed on endothelial cells. The effect on the neutrophil side of the adhesion mechanism similar to that reported in the present study has been demonstrated by Goldman et al in the presence of thromboxanes.

Several other aspects of the endothelin-1-induced neutrophil adhesion were clarified in the present series of experiments. The adhesion to plastic surfaces, the lack of effect of endothelial cell preincubation with
endothelin-1, and the adhesive effect of endothelin-1 using human neutrophils and human endothelium illustrated the predominant effect of endothelin-1 on the neutrophil side of the adhesion phenomenon and the absence of a nonspecific effect due to species heterogeneity. The specificity of endothelin-1 as an adhesion-inducing agent was additionally substantiated by the experiments showing the absence of action of either angiotensin II or arginine vasopressin.

More information was obtained from the isolated heart perfusion and intra-atrial in vivo infusions. In this regard, both the blockade of neutrophil accumulation in the perfused heart by TS1/18 and the absence of labeled erythrocyte accumulation suggested that endothelin-1-induced neutrophil accumulation on the heart corresponds to a specific integrin-mediated phenomenon. Moreover, an effect of endothelin-1 on neutrophil accumulation in the kidney was detected in the in vivo experiments. This finding is further supported by isolated kidney perfusion experiments done in our laboratory showing an increased neutrophil accumulation in the presence of endothelin-1.26 Of further interest, an increased myeloperoxidase activity, which was in- habitable by the administration of anti-endothelin-1 antibody, has been detected in the hearts of rabbits submitted to 1 hour of renal ischemia (Espinosa et al., unpublished observations), therefore opening the possibility of heart effects of ischemic phenomena occurring in distant organs. The lack of effect of endothelin-1 on neutrophil accumulation in the lungs in the present experimental conditions could be due to either a decreased amount of endothelin-1 reaching the lungs or to the high endothelinase activity found in the pulmonary circulation.27 Further experiments are necessary to reveal the complete mechanisms and in vivo pathophysi- ological consequences of these findings. In particular, the question should be raised about the actual role of endothelin-1 in neutrophil accumulation during the reperfusion phase that follows coronary ischemia.

Acknowledgments

This work was supported by grants 229/90, 234/90, and 239/93 from Fondo de Investigaciones Sanitarias de la Seguridad Social (F.I.S.S.), PM 92/0041 of Dirección General de Investigación Científica y Tecnológica, and by Fundación Ramón Areces and Fundación Renal. Dr López Farré is an investigator of Fundación Jiménez Díaz and Boehringer Mannheim. The authors wish to thank Prof Francisco Sánchez Madrid for providing the monoclonal antibodies and for help with the interpretation of the results and Drs Carlos Castilla for help with the surgery.

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Circulation. 1993;88:1166-1171
doi: 10.1161/01.CIR.88.3.1166
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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