Characterization and Fibrinolytic Properties of Human Omental Tissue Mesothelial Cells. Comparison With Endothelial Cells

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It has been reported that omental fat tissue is a good source of human microvascular endothelial cells. By characterization we demonstrate that the epithelioid cells isolated from omental tissue are not endothelial cells, but mesothelial cells. They contain abundant cytokeratins 8 and 18, which are absent in endothelial cells, and vimentin. No staining with the endothelial-specific antibodies EN-4 and PAL-E is observed. A faint and diffusely stained von Willebrand factor (vWF) in mesothelial cells is seen, whereas microvascular endothelial cells from subcutaneous fat display vWF in distinct granular structures. Human peritoneal mesothelium produces plasminogen activator-dependent fibrinolytic activity, which is essential in the resolution of fibrous exudates and may therefore be important in preventing the formation of fibrous peritoneal adhesions. This fibrinolytic activity is plasminogen activator-dependent, but has not been fully characterized. We report here that human omental tissue mesothelial cells in vitro produce large amounts of tissue-type plasminogen activator (t-PA), together with type 1 and 2 plasminogen activator inhibitor (PAI-1 and PAI-2). PAI-1 is predominantly secreted into the culture medium, whereas the major part of PAI-2 is found in the cells. No urokinase-type plasminogen activator is detected. On stimulation with the inflammatory mediator tumor necrosis factor (TNF), at least a threefold decrease in t-PA antigen is observed, together with an increase in both PAI-1 and PAI-2. TNF also induces a marked change in cell shape. Whereas TNF and bacterial lipopolysaccharide (LPS) have similar effects on the production of PA inhibitor by human endothelial cells, LPS has no or only a relatively small effect on the fibrinolytic properties of mesothelial cells. The decreased fibrinolytic activity induced by the cytokine TNF may impair the normal dissolution of fibrin deposits at the peritoneum in the presence of an inflammatory reaction.

MATERIALS AND METHODS

Materials. The specificities of our monoclonal antibodies (MoAbs) for cytokeratins 8 (M20) and 18 (M9), vimentin (V9), and desmin (D33) have been described previously. MoAb PAL-E was a gift of Dr R. Schlingemann (Department of Pathology, University of Nijmegen, The Netherlands). MoAb antibody EN-4 was obtained from Sandino B.V. (Uden, The Netherlands); polyclonal antibodies against human vWF were from the Central Laboratory of the Red Cross Blood Transfusion Service (Amsterdam, The Netherlands). Anti-tissue-type plasminogen activator (t-PA) immunoglobulin (Ig) G and anti-urokinase-type PA (u-PA) IgG were provided by Drs D.C. Rijken and G. Dooijewaard (Gaubius Institute TNO, Leiden, The Netherlands). FITC-labeled goat anti-mouse IgG was purchased from DAKO immunoglobulins (Glostrup, Denmark).

Human recombinant tumor necrosis factor (TNF) was a gift of Dr Jan Tavernier (Biogen, Gent, Belgium). The TNF preparation contained 2.45 x 10^7 U/mg protein and 40 mg LPS per milligram of protein. Bacterial LPS from Escherichia coli was purchased from Sigma (St Louis, MO).

Collagenase/dispace was purchased from Boehringer Mannheim (Mannheim, FRG); collagenase type II from Worthington (Freehold, NJ). Human serum was prepared from freshly collected blood of healthy donors and pooled (12 to 20 donors); newborn calf serum was purchased from Gibco (Grand Island, NY) and inactivated by treatment for 30 minutes at 56°C. A crude preparation of endothelial cell growth factor (EGF) was prepared as described by Maciag et al. Heparin was from Leo Pharmaceuticals (Copenhagen, Denmark). M199 medium supplemented with 20 mmol/L HEPES was from Flow Laboratories (Irvine, Scotland). [35S]Methionine was from New England Nuclear (Amherst, MA).

Cells. Human mesothelial cells were isolated from freshly obtained omental fat tissue according to the method of Kern et al. with minor modifications. Omental tissue from patients was obtained.
according to the guidelines of the Institutional Review Board of the University Hospital Leiden, The Netherlands. The tissue was collected in ice-cold buffer (140 mmol/L NaCl, 4 mmol/L KCl, 11 mmol/L D-glucose, 1% pyrogen-free human serum albumin, 10 mmol/L HEPES (pH 7.3), 100 IU/mL penicillin, and 0.10 mg/mL streptomycin) and immediately transferred to the laboratory. The tissue was rinsed, cut into pieces, and incubated in 0.1% pyrogen-free albumin and penicillin/streptomycin for 20 minutes at 37°C. After incubation, large pieces of tissue were removed and gently washed with M199 medium. The incubation medium and the washing fluid were sieved through a 0.40% gauze. After subsequent centrifugation for 5 minutes at 600 x g, the floating fat cells were carefully removed by aspiration, and subsequently the supernatant was removed. The pellet was resuspended in growth medium (M199 medium supplemented with 10% human serum, 10% heat-inactivated newborn calf serum, 150 µg/mL ECGF, 5 µU/mL heparin, 20 mmol/L HEPES, and penicillin/streptomycin) and the cells were seeded on fibronectin-coated dishes. The cells were propagated at 37°C under 5% CO₂/95% air atmosphere in growth medium, which was renewed every 2 to 3 days. When indicated, the serum was replaced by 20% human serum or 20% heat-inactivated newborn calf serum. When the cells had become confluent, they were detached by treatment with trypsin/EDTA and were passaged with a split ratio of 1:4. Cells were used after 1 or 2 passages.

Mesothelial cell-conditioned media were obtained by incubating confluent cells at 37°C for the indicated period of time (usually 24 hours) in M199 medium supplemented with 20% human serum, 20 mmol/L HEPES, and penicillin/streptomycin under 5% CO₂/95% air atmosphere, unless otherwise mentioned. The mesothelial cell-conditioned medium was immediately centrifuged and stored at -20°C until use. After two washes with phosphate-buffered saline (PBS), cell extracts were prepared in 0.5% Triton X-100 (Merck, Darmstadt, FRG) and stored frozen until use.

Immuno precipitation of radiolabeled PA inhibitor-1 from [125I]methionine-labeled 24-hour mesothelial cell-conditioned medium was performed as previously described. A small number of capillaries was isolated from human subcutaneous fat by the same procedure as given for omental tissue mesothelial cells. In contrast to the mesothelial cell preparation, the preparation of subcutaneous fat capillaries also contained fibroblastoid cells.

Endothelial cells from human umbilical artery and vein were isolated as previously described, and cultured in the same growth medium as described above.

Immunofluorescence microscopy. Immunofluorescence staining of mesothelial and endothelial cell antigens was performed on cell monolayers that had been grown on cover slips coated with crosslinked gelatin. The cells had been fixed in 80% (vol/vol) acetone at 4°C and rinsed with PBS. The cells were incubated with various MoAbs for 30 minutes at room temperature, washed twice with PBS, and subsequently incubated for 30 minutes with FITC-labeled goat anti-mouse Ig (1:50 in PBS). The cells were mounted with 1 mg/mL p-phenylenediamine in 10% (vol/vol) glycerol in PBS under glass coverslips, and examined with a Leitz epilluminisation system. The endothelial-specific antibodies showed no reactivity with cultured human fibroblasts or smooth muscle cells.

The uptake of Dil-acetylated low density lipoproteins (DLDL) was evaluated by microscopy as previously indicated. Assays. Levels of t-PA antigen were measured by an enzyme immunoassay (IMULYSE) purchased from Biopool (Umeå, Sweden) according to the manufacturer’s description. In this assay, t-PA and t-PA-inhibitor complexes are detected with similar efficiency.

The level of u-PA antigen was performed with an enzyme-linked immunosorbert assay (ELISA) for urokinase as described by Binnema et al. The assay detects both u-PA and u-PA:PA inhibitor complexes with equal efficiency.

Plasminogen activator activity in the conditioned medium was detected by fibrin autograph technique after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (9% gels) of the conditioned medium. To allow discrimination between t-PA and u-PA activity, parallel incubations were performed on fibrin gels containing either 120 µg/mL rabbit anti-human t-PA IgG or 80 µg/mL goat anti-human urokinase IgG.

PA inhibitor activity was measured by a modification of the quantitative assay for plasminogen activator activity as described by Verheijen et al. It was determined by titration with increasing amounts of t-PA of a fixed volume of mesothelial cell-conditioned medium or control medium. The amount of PA inhibitor activity was calculated from the intersection of the asymptote of the titration curve with the X-axis. This asymptote ran parallel to the control titration curve in all experiments. The curves were determined in duplicate (interassay variation less than 5%).

The levels of plasminogen activator inhibitor type-1 (PAI-1) antigens were determined by an enzyme immunoassay (IMULYSE, Biopool). According to the manufacturer, this assay determines mainly the unbound PAI-1 (both active and latent), whereas the t-PA:PAI-1 complex is recovered with about 10-fold lower efficiency. The levels of PAI-2 antigen were determined by an enzyme immunoassay (TintElize PAI-2, Biopool).

Cell protein content of the cell layers was assessed by the method of Lowry et al. Cell counts were made in triplicate with a hemocytometer.

RESULTS

Characterization of human omental tissue mesothelial cells (HOMC). A large number of epithelioid cells was obtained on isolation by collagenase treatment of omental tissue, most of them in small sheets of 10 to 200 cells (Fig 1a). The cells grew to confluence in 4 to 5 days; after reaching confluence, the diameter of the cells decreased by a continuation of cell division until a monolayer of closely apposed cells (Fig 1b) was formed. These cells could be propagated for several passages, usually 4 to 6 (split ratio 1:4). At the end of their life span the cells changed into multinucleated giant cells (Fig 1c). All further studies have been done with HOMC after 1 or 2 passages.

To establish the mesothelial nature of these cells, we examined whether they contain various types of intermediary filaments. With several MoAbs, the abundant presence of cytokeratins 8 and 18 was demonstrated in confluent and subconfluent HOMC (Fig 1d and e). These cytokeratins were consistently found in these cells (cells from four different donors tested). Their presence strongly suggests that these cells are mesothelial cells (further indicated as HOMC). Human umbilical vein endothelial cells did not contain cytokeratins 8 and 18 (Fig 1f). Vimentin was present in both HOMC and umbilical vein endothelial cells. Desmin was detected in highly confluent HOMC by the use of the MoAb D33, but a considerable variation in the desmin content of the cell was observed (not shown). No immunofluorescent staining was observed when the MoAbs were replaced by nonproducer Sp 2/0 myeloma cell supernatants (not shown) or by an anti-fibrin monomer MoAb (Fig 1g).

Confluent and subconfluent cultures of HOMC were examined for the presence of various endothelial cell mark-
ers. A faint and diffuse staining of vWF was observed in highly confluent HOMC by use of polyclonal anti-vWF antibodies (Fig 1h), whereas both confluent and subconfluent endothelial cells from umbilical vein and foreskin microvessels displayed vWF abundantly in granular structures. The MoAbs PAL-E and EN4 recognized endothelial cell-specific antigens on human umbilical vein and foreskin microvascular endothelial cells, as shown for EN4 in Fig 1i. No staining for EN4 and PAL-E was found in various HOMC cultures from four different donors (not shown). In only one of these cultures a few PAL-E positive cells were observed (less than 1 per 200 cells). A minute contamination of endothelial cells cannot be excluded in this culture. The uptake of acetylated-LDL has also been used to recognize endothelial cells and macrophages. Mesothelial cells from omental tissue ingested large amounts of Dil-labeled acetylated-LDL (Fig 1j). The immunofluorescence in HOMC after 4 hours of incubation with 20 μg/mL Dil-acetylated-LDL was significantly higher than in identically treated human umbilical vein endothelial cells (three independent experiments).

When subcutaneous fat was used to isolate endothelial cells, no sheets of mesothelial cells, but a limited number of small capillaries (Fig 2a), were obtained together with fibroblastoid cells. The endothelial cells of the capillaries adhered to the culture dish and spread. The cells were examined daily by phase contrast microscopy (Fig 2b) and their location was indicated on the culture dish. After 5 to 7 days the cells were fixed and examined. They displayed the presence of vWF in discrete granules (Fig 2c), similar to umbilical vein and foreskin microvascular endothelial cells.
Production of PA and PAI by HOMC. HOMC produced large amounts of t-PA. During a 24-hour incubation period in the presence of 10% human serum, they secreted \(148 \pm 98\) ng t-PA antigen/\(10^6\) cells (mean ± SD of six cultures from different donors). As is shown in Fig 3, the amounts of t-PA produced by HOMC markedly exceeded the amount produced by human umbilical vein endothelial cells. No u-PA activity was found in HOMC-conditioned medium by fibrin autography (Fig 3). Also, no u-PA antigen was found in the 24-hour conditioned media and the cell extracts of HOMC from three different donors (detection limit 0.5 ng/mg cellular protein).

Despite the production of large amounts of t-PA antigen, most HOMC cultures did not show spontaneous fibrinolytic activity due to the concomitant production of excess of PAI. In only 1 of 10 cell strains of HOMC we detected a fibrinolytic activity of 12 IU/mL in the 24-hour conditioned medium. In the other cultures an excess of PAI was found in the 24-hour conditioned medium; PAI activity ranged from 2 to 180 IU/mL. Both PAI-1 antigen and PAI-2 antigen could be demonstrated in the conditioned medium and the cellular extracts (see below).

Incubation of HOMC with TNF resulted in a dramatic change in cell morphology. The cells became spindle-shaped within 24 hours after addition of 500 U/mL TNF, and part of them detached (Fig 4). Cell counts showed that after this incubation period the cell number decreased to 82% ± 15% of that in control incubations (mean ± SD, \(n = 8\)). No change in cell morphology was observed in mesothelial cells treated for 24 hours with 10 \(\mu\)g/mL LPS (cell number 94% ± 8% of control, \(n = 8\)). The addition of TNF also resulted in at least a threefold decrease in the production of t-PA antigen, as is shown for two representative cultures in Table 1. This decrease was found in cells that had been cultured either in human serum or in newborn calf serum, notwithstanding the fact that large differences in the basal rate of t-PA production were observed under these conditions (Table 1). When TNF was inactivated by heating at 90°C for 15 minutes, it had lost its ability to alter the properties of HOMC (not shown). The addition of LPS (10 \(\mu\)g/mL) reduced t-PA production by only 20% or less. No production of u-PA was induced by incubation with either TNF or LPS. Concomitant with a decrease in t-PA antigen production, the PAI activity...
in the 24-hour conditioned medium was markedly higher than in their nontreated and LPS-treated counterparts (Table 1). In four cultures of different donors, the addition of 500 U/mL TNF to HOMC incubated with human serum resulted in a 5.8 ± 1.0-fold increase in PA1 activity, whereas only a 1.6 ± 0.6-fold increase was observed after addition of a high concentration of LPS (10 μg/mL) (mean ± SD).

To evaluate whether the increase in PA1 activity induced by TNF was due to an increased production of PA inhibitor(s) or to a decreased consumption of PA inhibitor(s) by the lower t-PA levels, PAL1 and PAI-2 antigens were assayed. The amount of PAI-I antigen measured in the 24-hour conditioned medium of LPS- and TNF-treated HOMC was 1.2 ± 0.3 and 2.5 ± 0.5-fold higher, respectively, than in the supernatant fluid of their untreated counterparts (mean ± SD, nine determinations with cells from three donors). Most of the PAI-1 synthesized by HOMC was secreted into the medium (Fig 5). Similar to the medium, the amount of PAI-1 antigen in TNF-treated cell extracts was 2.4 ± 0.4-fold higher than in that of nontreated cells. In contrast to PAI-1, the major part of PAI-2 remained in the cells (Fig 5). Very high concentrations of PAI-2 were observed under our standard culture conditions. Incubation for 24 hours with TNF resulted in a further increase in the PAL2 concentration in the cells as well as in the medium. LPS, even at a high concentration (100 pg/mL), was much less effective in enhancing the production of PAI-1 and PAL2 than TNF (Fig 5).

**Table 1. Effect of LPS and TNF on the Production of PA and PA Inhibitors by Human Omental Tissue Mesothelial Cells**

<table>
<thead>
<tr>
<th>Culture Medium</th>
<th>Addition</th>
<th>t-PA Antigen (ng/mg cell protein)</th>
<th>u-PA Antigen (ng/mg cell protein)</th>
<th>PAI Activity (IU/mg cell protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Culture 1</td>
<td>Culture 2</td>
<td>Culture 1 and 2</td>
</tr>
<tr>
<td>A</td>
<td>None</td>
<td>590 ± 48</td>
<td>154</td>
<td>≤0.5</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>505 ± 83</td>
<td>165</td>
<td>≤0.5</td>
</tr>
<tr>
<td></td>
<td>TNF</td>
<td>198 ± 28</td>
<td>77</td>
<td>≤0.5</td>
</tr>
<tr>
<td>B</td>
<td>None</td>
<td>449 ± 40</td>
<td>129</td>
<td>≤0.5</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>384 ± 7</td>
<td>96</td>
<td>≤0.5</td>
</tr>
<tr>
<td></td>
<td>TNF</td>
<td>108 ± 12</td>
<td>44</td>
<td>≤0.5</td>
</tr>
<tr>
<td>C</td>
<td>None</td>
<td>113 ± 13</td>
<td>25</td>
<td>≤0.5</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>120 ± 16</td>
<td>21</td>
<td>≤0.5</td>
</tr>
<tr>
<td></td>
<td>TNF</td>
<td>47 ± 1</td>
<td>3</td>
<td>≤0.5</td>
</tr>
<tr>
<td>D</td>
<td>None</td>
<td>116 ± 2</td>
<td>22</td>
<td>≤0.5</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>114 ± 18</td>
<td>25</td>
<td>≤0.5</td>
</tr>
<tr>
<td></td>
<td>TNF</td>
<td>35 ± 7</td>
<td>4</td>
<td>≤0.5</td>
</tr>
</tbody>
</table>

PA and PA inhibitor activity were assayed in the 24-hour conditioned medium of confluent mesothelial cells (cell density 1.4 to 1.5 × 10⁶ cells/cm²). The cells were grown and incubated in M199 medium containing 20 mmol/L HEPES and penicillin/streptomycin, and supplemented with (A) 20% human serum; (B) 20% human serum, 150 μg/mL ECGF and 5 U/mL heparin; (C) 20% newborn calf serum; or (D) 20% newborn calf serum, 150 μg/mL ECGF, and 5 U/mL heparin. The values of culture 1 represent the mean ± SD of three parallel cultures of cells from one donor grown on dishes coated with fibronectin, gelatin, or without coating. The data of culture 2 are the mean of duplicate fibronectin-coated dishes with HOMC from another donor. The experimental variation of duplicate dishes was less than 10%. Cellular protein content was determined in parallel wells at the start of the incubation period.
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addition of 10 pg/mL ECGF, and 5 U/mL heparin without (control) or with the incubation period in M199 medium supplemented with 20% human serum. The amount of PAI-2 antigen was determined in cell extracts after the incubation period (2 to 3 experiments). Significance from the t-test for paired data: (*) P = .05; *P < .02; **P < .01; ***P < .001.

Various serine proteases, like thrombin, activated protein C, and plasmin, can react with PAI-1 and subsequently degrade it into a 42-Kd degradation product. To evaluate whether consumption of PAI-1 occurred during incubation of HOMC with TNF, S-methionine was added to the incubation medium and metabolically labeled PAI-1 was immunoprecipitated after incubation. No 42-Kd PAI-1 degradation product was found in the conditioned medium of TNF-treated and untreated HOMC (not shown).

DISCUSSION

Omental fat tissue has been advocated as a convenient source of human microvascular endothelial cells. A marked contribution of mesothelial cells in these preparations might be anticipated, since the omentum contains four folded layers of mesothelial cells. We have demonstrated that the cell monolayers that are easily obtained from human omental fat adipose tissue are predominantly, if not all, mesothelial cells (HOMC). We also demonstrated that the inflammatory mediator TNF, but not bacterial LPS, markedly decreased the production of t-PA and hence the fibrinolytic activity by these mesothelial cells.

HOMC are identical in their morphology and in the diffuse and faint distribution of vWF to the cells described by Kern et al and Anders et al as fat microvascular endothelial cells. Similar to these cells, HOMC also produced large amounts of t-PA antigen. The two major arguments to characterize HOMC as mesothelial cells are: (1) the abundant presence of cytokeratins 8 and 18, which are known to be present in mesothelium in vivo and in vitro, and (2) the fact that most of the cells are isolated as sheets of cells, which excludes a microvascular origin. Furthermore, several endothelial cell markers, PAL-E, EN-4 and vWF, could not or only faintly be demonstrated by indirect immunofluorescence microscopy, under conditions that these markers could be clearly demonstrated in cultured umbilical vein and foreskin microvascular endothelial cells. From our experiments it remains uncertain whether the faint diffuse staining of vWF on HOMC originates from de novo synthesis by these cells or from the serum in the culture medium. In endothelial cells from subcutaneous fat capillaries, we found vWF in distinct granular structures, similar to its distribution in endothelial cells from arteries and veins.

Expression of cytokeratins 8 and 18 has been found in some peculiar endothelial cells from fetal tissue and rheumatoid arthritis tissue. Recently there have also been a few reports describing cytokeratin expression in epitheloid vascular tumors, such as epitheloid hemangioma and epitheloid hemangioendothelioma. However, abundant expression of cytokeratins, as found in human omental tissue mesothelial cells, was never reported for endothelial cells. In addition, triple expression of cytokeratin, vimentin and desmin, as seen in HOMC (Table 2), is in agreement with an earlier report that this type of intermediate-filament coexpression could be found in human fetal mesothelium as well as in mesothelial proliferations of pleura and peritoneum associated with inflammation. The characteristics of HOMC are summarized in Table 2; they are compared with those of human umbilical vein and foreskin microvascular endothelial cells.

By histochemical technique, PA activity has been demonstrated in mesothelial cells, but the type of PA was not determined. An inhibitor of fibrinolysis also has been demonstrated in extracts of bovine mesothelial cells, which probably contributes to the differences in the degree of fibrinolytic activity of the mesothelium reported by various investigators. Our data show that large amounts of two PA inhibitors, PAI-1 and PAI-2, are synthesized by cultured human mesothelial cells. Enhanced production of these inhibitors probably underlies the lack of detectable free inhibitors.

Table 2. Characteristics of Human Endothelial Cells and Omental Tissue Mesothelial Cells

<table>
<thead>
<tr>
<th></th>
<th>HUVEC</th>
<th>FMVEC</th>
<th>HOMC</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Intermediary filaments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokeratin 8</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>Cytokeratin 18</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>Desmin</td>
<td>—</td>
<td>ND</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>Vimentin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>Endothelial markers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vWF</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>Weibel Palade bodies</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>1,3</td>
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<tr>
<td>Angiotensin converting enzyme</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>1,3</td>
</tr>
<tr>
<td>Uptake Dil-acetylated enzyme</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>1,3</td>
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<tr>
<td>LDL</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3,7,8,A</td>
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<td>MoAb BMA 120</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>2,8</td>
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<tr>
<td>MoAb EN-4</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>A</td>
</tr>
<tr>
<td>MoAb Pal-E</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>A</td>
</tr>
<tr>
<td>t-PA production</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6,A</td>
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<td>Protein C activation</td>
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<td>2,6</td>
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<td>Prostacyclin production</td>
<td>+</td>
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<td>+</td>
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</table>

The presence of intermediary filaments and endothelial markers in human umbilical vein endothelial cells (HUVEC), human foreskin microvascular endothelial cells (FMVEC), and human omental tissue mesothelial cells (HOMC) is given. References for studies on HOMC are indicated.

Abbreviations: ND, not determined; +, abundantly present; +, present; —, absent/below detection level; A, this report.
fibrinolytic activity in most of our cultures, despite the presence of large amounts of t-PA antigen. t-PA activity probably becomes manifest only in the presence of fibrin deposits,\textsuperscript{43} which makes t-PA less accessible for inhibition by PA inhibitors.\textsuperscript{44} Our results suggest that the fibrinolytic activity of mesothelial cells in vivo is caused by the secretion of large amounts of t-PA, and not u-PA. The involvement of t-PA has also been suggested in a preliminary report by Carlin et al.\textsuperscript{45} This fibrinolytic quality of the mesothelium is an important factor in the resolution of fibrous exudates and fibrin deposits on the peritoneum by inadequate fibrinolysis may induce the ingrowth of fibroblasts and deposition of collagen, which result in the formation of intraperitoneal fibrous adhesions.\textsuperscript{9,12} A decrease of the fibrinolytic activity by mesothelial cells has been observed in peritoneal inflammation and ischemia in humans\textsuperscript{13} and can be induced experimentally by peritoneal injury.\textsuperscript{9,12} An important finding was that the addition of TNF, which is released from activated macrophages in areas of inflammation, resulted in a marked decrease in the production of t-PA by mesothelial cells. This finding suggests that inflammatory processes in the peritoneum may decrease mesothelial cell fibrinolytic activity not only by direct injury of the cells, but also by suppression of the cellular t-PA synthesis by inflammatory mediators, in particular TNF.

Concomitant with the decrease in t-PA production by TNF, a large increase in the production of PA inhibitor activity was observed in HOMC. The concentration of PAI-1 antigen increased two- to threefold during incubation of HOMC with TNF. However, this increase may be overestimated. The PAI-1 antigen assay measures unbound PAI-1, whereas the t-PA:PAI-1 complex is, according to the manufacturer's information, only measured at a 10% efficiency. It is likely that all t-PA in the HOMC-conditioned medium is present in the complex with PA inhibitors. If the TNF-induced decrease in t-PA antigen is reflected in a decrease in t-PA:PAI-1 complex, the ratio between free PAI-1 and complexed PAI-1 increases, and hence the efficiency of PAI-1 antigen measurements also increases in the conditioned medium of TNF-stimulated cells.

In addition to PAI-1, which is predominantly secreted, HOMC also synthesize a relatively large amount of PAI-2, which remains mainly within the cells. It has to be established whether the fibrinolytic inhibitor extracted from mesothelial cells by Pugatch and Poole\textsuperscript{46} represents this type of PA inhibitor. If a large amount of PAI-2 is also present in mesothelial cells in vivo, it may contribute to fibrinolysis inhibition on damage of these cells. Addition of TNF causes an increase in the PAI-2 level both in the cells and in the conditioned medium. Because TNF markedly affects the morphology of HOMC and even induces cell detachment, we cannot exclude that leakage of PAI-2 from the cells contributes to the increase in PAI-2 in the supernatant fluid of TNF-treated cells.

We have previously demonstrated that the inflammatory mediator TNF also increases PAI-1 production in human endothelial cells.\textsuperscript{47} However, in contrast to endothelial cells, which respond as potent to LPS as to TNF, HOMC responded much less to LPS than to TNF. HOMC also differed from human endothelial cells by the excessive production of PAI-2. The production of t-PA by HOMC, on the other hand, was in the same order of magnitude as that by large vein endothelial cells,\textsuperscript{17} which agrees well with a recent observation on tissue extracts of peritoneum and saphenous vein biopsies.\textsuperscript{15} The rate of t-PA production by HOMC markedly exceeds that by human umbilical vein endothelial cells.

In summary, our studies show that the epitheloid cells obtained from omental tissue are mesothelial in character and produce various regulators of fibrinolysis: t-PA, PAI-1, and PAI-2. The inflammatory mediator TNF can significantly decrease the fibrinolytic activity both by decreasing t-PA production and increasing PA inhibition.

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