Expression of Adhesion Molecules and Chemotactic Cytokines in Cultured Human Mesothelial Cells

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Summary

The mesothelium is a flat epithelial lining of serous cavities that could gate the traffic of molecules and cells between the circulation and these body compartments. The present study was designed to elucidate the capacity of mesothelial cells to express adhesion molecules and chemoattractant cytokines, two fundamental mechanisms of regulation of leukocyte recruitment. Cultured human mesothelial cells express appreciable levels of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), and these were increased by in vitro exposure to tumor necrosis factor (TNF), interferon γ (IFN-γ), or TNF and IFN-γ. Interleukin 1 (IL-1) was a less consistent stimulus for adhesion molecule expression in vitro. Unlike endothelial cells, used as a reference cell population, resting or stimulated mesothelial cells did not express E-selectin and ICAM-2, as assessed by flow cytometry. Analysis of VCAM-1 mRNA by reverse transcriptase and polymerase chain reaction using appropriate primers revealed that mesothelial cells expressed both the seven- and the six-Ig domain transcripts, with predominance of the longer species. Monocytes bound appreciably to "resting" and, to a greater extent, to stimulated mesothelial cells. Monocytes exposed to IFN-γ and lipopolysaccharide, used as prototypic activation signals, showed increased capacity to bind mesothelial cells. Anti-CD18 monoclonal antibody significantly inhibited binding of monocytes to mesothelial cells, and this blocking effect was amplified by anti–very late antigen 4. Mesothelial cells were able to express the chemotactic cytokines IL-8 and monocyte chemotactic protein 1 at the mRNA and protein levels. These results indicate that mesothelial cells can express a set of adhesion molecules (ICAM-1 and VCAM-1) overlapping with, but distinct from, that expressed in vascular endothelium (ICAM-1, ICAM-2, VCAM-1, E-selectin), and that these are functionally relevant for interacting with mononuclear phagocytes. The regulated expression of adhesion molecules and chemotactic cytokines by mesothelial cells is probably important in inflammatory and immune reactions that involve serous cavities, such as the long-known macrophage appearance and disappearance reactions.

The mesothelium is a flat epithelial lining of the peritoneal, pleural, and pericardial cavities (1). It provides a nonadhesive, slippery surface and gates the traffic of molecules and cells between the circulation and these body compartments.

Serous cavities can be involved by infectious, immune, and carcinogenic processes. Among serous surfaces, the peritoneal cavity has been most extensively studied as a site of immune and inflammatory reactions. Upon local application of infectious, inflammatory, or immune stimuli, leucocytes are rapidly recruited in the peritoneum. Conversely, intraperitoneal challenge with antigen causes rapid disappearence of mononuclear phagocytes from inflammatory exudates (macrophage disappearence reaction [MDR][1] 2–7). The MDR can be mimicked by intraperitoneal injection of lymphokines that have not been molecularly defined (4, 7).

Given its strategic location, it is likely that the mesothelial lining participates actively in regulating leucocyte traffic between the circulation, the peritoneal fluid, and the walls of the peritoneal cavity. The present study was designed to define the capacity of mesothelial cells to express adhesion mole-

[1] *Abbreviations used in this paper:* EC, endothelial cell; MCP-1, monocyte chemotactic protein 1; MDR, macrophage disappearance reaction; VLA-4, very late antigen 4.

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cules and chemoattractant cytokines, two fundamental mechanisms of regulation of leukocyte recruitment and activation (for recent reviews see references 8 and 9).

Materials and Methods

Cell Culture Media and Reagents. The following reagents were used for culture of cell lines, separation of effector cells, adhesion, and cytotoxicity assays: pyrogen-free saline for clinical use (S.A.L.F., Bergamo, Italy); pyrogen-free distilled water (S.p.A. Laboratorio Farmacologio); RPMI 1640 (10×; Biochrom KG, Berlin, Germany); IMDM; glutamine (Gibco Laboratories, Grand Island, NY); penicillin and streptomycin for clinical use (Farmitalia, Milan, Italy); gentamycin (Gibco Laboratories); aseptically collected FCS (HyClone Laboratories, Logan, UT). The routinely used tissue culture medium was RPMI 1640 with 2 mM glutamine, 50 μg/ml of gentamycin, 15% or 20% FCS, hereafter referred to as complete medium. All reagents contained <0.125 EU/ml of endotoxin as checked by the limulus amebocyte lysate assay (Microbiological Associates, Walkersville, MD).

Cytokines and Antibodies. Human rIL-1β (sp act, 8×10^6 U/ml) was obtained from Sclavo (Siena, Italy). Human rIFN-γ was obtained from Roche (Basel, Switzerland) or from Roussel Uclaf (Paris, France). Human rTNF (sp act, 8.1×10^6 U/mg) was a kind gift of BASF/Knoll (Germany). mAbs used in this study were obtained through the courtesy of the following persons or produced in our laboratory by hybridomas obtained from American Type Culture Collection (ATCC; Rockville, MD): two mAbs directed against the common β2 subunit (CD18) of leukocyte integrins, clone 10F12 (Dr. J. Ritz, Dana Farber Cancer Research Institute, Boston, MA) and clone TS1/18 (ATCC); mAb anti-VLA-4, clone HP2/1 (IgG1) (Dr. F. Sanchez-Madrid, Hospital de la Princesa, Madrid, Spain); mAb anti-ICAM-1, clone LB2 (IgG2b) (Dr. N. Hogg, Imperial Cancer Research Fund, London, UK); mAb anti-VCAM-1, clone 4B9 (IgG1) (Dr. J. Harlan, University of Washington, Seattle, WA); mAb anti-ELAM-1 (E-selectin), clone H4/18 (IgG1) (Dr. M. Bevilacqua, Boston, MA); mAb anti-ICAM-2, clone 6D5 (IgG1) (Dr. C. G. Gahmberg, University of Helsinki, Finland) (10); mAb directed against functional epitopes of the α chains of β2 integrins, clone CLB-LFA1/2 (CD11a) (Dr. R. van Lier, CLB, Amsterdam, The Netherlands), clone 44a (CD11b) (Dr. R. Todd, Ann Arbor, MI); and clone L29 (CD11c) (Dr. L. Lanier, Becton Dickinson & Co., Mountain View, CA). Appropriate irrelevant antibodies, including anti-IFN-γ and anti-CD8, were used for flow cytometric analysis and functional assays, as detailed in previous reports from this laboratory (11–13).

Mesothelial Cells. A total of 15 mesothelial cell cultures were used for the present study from December 1988 to January 1992. Only two of these were generated from pleural or peritoneal fluids from nonneoplastic conditions and the remaining ones were derived from ovarian cancer ascites. The culture conditions, growth factor requirements, and characteristics of mesothelial cells are described in greater detail elsewhere (Lanfrancone et al., manuscript in preparation). Ficoll-Hypaque–separated mononuclear cells were resuspended in RPMI 1640 with 15% FCS and plated in a 75-cm^2 culture flask. After overnight incubation at 37°C, the medium containing nonadherent cells (mostly erythrocytes, mononuclear leukocytes, and tumor cells) was removed and cells were cultured in IMDM with 15% FCS and 10% PHA-conditioned medium. We subsequently found that RPMI 1640 could substitute for IMDM, and this medium, supplemented with 15% FCS, 10% conditioned medium, and 50 μg/ml endothelial cell growth factor (Collaborative Research, Inc., Lexington, MA) was employed for mesothelial cell culture. The conditioned medium was obtained by culturing human PBMC (10^6/ml) with 1 μg/ml PHA for 3 d. Under these conditions, tumor cells and macrophages did not grow appreciably and were rapidly lost from the cultures. The medium was changed every 2–3 d, and the cultures, detached by trypsin/EDTA, were split 1:2. In these conditions they could be maintained for six to seven passages before they become senescent. Mesothelial cell cultures were characterized on the basis of: expression of cytokeratins (mAb CAM 5.2; Becton Dickinson & Co.; DAKO-CK1 and CK-19; DAKOPatts, Glostrup, Denmark), carcinoembryonic antigen (polyclonal anti-CEA antiserum; DAKOPatts), OC125 and Mov18 (markers of ovarian carcinoma cells; mAbs were gifts of Dr. R. C. Bast, Duke University, Durham, NC, and Dr. M. I. Colnaghi, Istituto Nazionale Tumori, Milan, Italy), factor VIII and caderin 5 (polyclonal antiserum and mAb 7B4, gifts of Dr. E. Dejana, of this Institute), vimentin (DAKOvimentin, DAKOpatts), CD31 (anti-PECAM-1, clone 9611, British Bio-Technology Ltd., Oxford, England), and CD45 (mAb 9.4, NEN-DuPont Co., Wilmington, DE) by immunocytochemistry; expression of leukocyte differentiation antigens (CD2, CD13, CD14, CD16, CD20, HLA-DR) and DNA content by flow cytometry; nonspecific esterase by cytochemistry. Mesothelial cells had no karyotypic abnormality, did not form colonies in soft agar, and were not tumorigenic in nude mice. For adhesion assays, care was taken to use cultures within the third to fourth passage, as these grew vigorously and formed confluent monolayers. Cell cultures were grown in medium without PHA-conditioned medium for 24–72 h (in three experiments, 7 d, see Results) before assays.

Phenotyping of mesothelial cells was performed by indirect immunofluorescence. Briefly, cells were exposed to mAb (5 μg/ml in saline with 2% human serum) specific for different surface molecules for 30 min at 4°C, washed, and incubated with fluoresceinated affinity-purified goat anti-mouse IgG F(ab')$_2$ (Technogenetics, Turin, Italy) for 30 min at 4°C. The cells were washed and fixed with PBS containing 1% paraformaldehyde. Fluorescence was measured on a FACStar Plus® apparatus (Becton Dickinson & Co.).

Endothelial Cells. (EC). Human EC obtained from umbilical veins served as a reference cell population for these studies. Their culture and use have been described previously (11–13).

Leukocyte Populations. PMN were isolated by Ficoll-Hypaque gradient centrifugation and gelatine sedimentation (Biochrom KG, Berlin, Germany), while lymphocytes and monocytes were obtained from Ficoll-Hypaque–separated mononuclear cells by centrifugation on a discontinuous (46%) gradient of isosmotic (285 mOsmol) Percoll (Pharmacia, Uppsala, Sweden) (14). Monocytes were activated by culturing 3–7 ml of a cell suspension containing 3×10^6 cells/ml complete medium in 5-cm-diameter Petriperm hydrophobic dishes (Heraeus, Vienna, Austria) for 20 h in the presence of 100 U/ml of IFN-γ and 100 ng/ml of LPS.

Adhesion. Adhesion of leukocytes to mesothelial cells was studied as described previously for binding of leukocytes to endothelial cells with minor modifications (11–13). Mesothelial cells were grown to confluence in flat-bottomed 96-well trays. Leukocytes (10^7/ml in complete medium) were incubated at 37°C for 1 h with 100 μCi ^{51}Cr (sodium chromate; Amersham, Buckinghamshire, UK). At the end of the incubation, the cells were washed three times with medium and were resuspended at 10^6/ml. Cells in 0.2 ml medium were added to mesothelial cell monolayers and incubated at 37°C for 30 min, unless otherwise specified. When mAbs directed against adhesion molecules were used, they were added to leukocytes or mesothelial cells 30 min before the adhesion test. After the adhesion assay, the wells were carefully washed and adherent

cells were solubilized with 0.2 ml of 0.025 M NaOH and 0.1% SDS. Results are presented as mean (± SD) percentage of adherent cells with three replicates/group.

Cytokine Production. IL-6 was measured as hybridoma growth factor using 7TD1 cells as described (15). IL-8 was measured by a commercially available immunometric assay (British Biotechnology Ltd.). Monocyte chemotactic protein (MCP-1) activity was evaluated as the capacity of mesothelial cell supernatants to induce directional migration of monocytes as described (16).

Northern Analysis. Mesothelial cells were detached from the culture surface by brief exposure to trypsin/EDTA and washed repeatedly in cold PBS (2×10^7 mesothelial cells in total for each experimental condition and at any time point). After centrifugation at 800 *g* for 10 min, total RNA was extracted from the pellet by the guanidine isothiocyanate method (17), with minor modifications (16, 18).

Total RNA (10–15 μg) was analyzed by electrophoresis through 1% agarose-formaldehyde gels, followed by Northern blot transfer to Gene Screen Plus sheets (New England Nuclear, Boston, MA). The IL-8 and MCP-1 probes (16, 18) were labeled to high specific activity (10^8 cpm/μg) by nick translation and α-[^{32}P]dCTP (3,000 Ci/mmol; Amersham). Membranes were pretreated and hybridized in 50% formamide with 10% dextran sulfate, washed twice with 2× SSC at 60°C for 30 min, and washed again twice with 0.1× SSC at room temperature for 30 min. The membranes were exposed for 24 h at −80°C with intensifying screens. The quality and quantity of RNA, blotted onto filters, were checked by visualization under UV light.

Reverse Transcriptase (RT)-PCR. 1 μg total RNA was reverse transcribed and then amplified by PCR. RNA was mixed with 2.5 mM random hexamers in 5 mM $MgCl_2$, 25 mM KCl, 10 mM Tris-HCl, pH 8.3, 1 mM each dNTP, and 2.5 U/ul Moloney murine leukemia virus RT (Perkin Elmer Cetus, Norwalk, CT) in a final volume of 20 μl. Reverse transcription was carried out at 42°C for 15 min. This reaction was then amplified by adding in a final volume of 100 μl 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 U Taq DNA polymerase (Perkin Elmer Cetus), and 0.15 M each specific primers. Primers were as described (19): oligomer 370-y is 5′-GGAACCTTGCAGCTTACAGTGACAG-AGCTCCC-3′, and oligomer VC is 16 5′-CAAGTCTACATAT-CACCCAAG-3′. Samples were amplified in a thermal cycler (Perkin Elmer Cetus) using 30 cycles at 95°C (1′), 55°C (2′), and 72°C (3′). 10 μl of each RT-PCR was then subjected to electrophoresis through a 2% agarose gel, which was stained, photographed, and blotted onto Gene Screen Plus membranes. These were then hybridized to the vascular cell adhesion molecule 1 (VCAM-1) probe as described according to standard procedures for Southern blotting (20).

Statistical Analysis. In each functional assay, three to six replicates per experimental group were used. Results, presented as mean ± SD, are representative of at least three experiments performed, unless otherwise specified.

Results

Characterization of Mesothelial Cells. Mesothelial cell cultures were characterized in terms of morphology, growth properties, DNA content, karyotype, tumorigenicity, and mAb-defined structures, in an effort to define their cell lineage and possible contaminants (Table 1, and data not shown). Mesothelial cultures maintained for 2–7 d in the absence of growth factors had flat, cobblestone morphology, homogeneous appearance, and no three-dimensional outgrowth. Cells had large nuclei with typical multiple nucleoli. Unlike endothelial cells, mesothelial cells did not express factor VIII antigen, CD31, and caderin 5 (mAb 7B4) (Table 1).

Table 1. *Characterization of Mesothelial Cell Cultures*

Marker	Mesothelial cells	Monocytes
CD2	−	−
CD13	+	+
CD14	−	+
CD16	−	−*
CD20	−	−
CD31	−	+
CD45	−	+
Class II MHC	±‡	+
Nonspecific esterase	−§	+
CEA	−	−
MOV18	−	−
Cytokeratins	+	−
Vimentin	+	NT‖
Factor VIII	−	−
Caderin 5	−	−

CD2, CD13, CD14, CD16, CD20, CD31, and HLA-DR were studied by flow cytometry, whereas immunocytochemistry was used for cytokeratins, vimentin, CEA, MOV18, factor VIII, caderin 5, and CD45.
* Mature macrophages are CD16$^+$.
‡ Mesothelial cells were weakly (6–20%) HLA-DR$^+$, but culture with IFN-γ for 24 h resulted in >80% HLA-DR$^+$ cells.
§ Occasionally, few esterase-positive cells at the beginning of the culture.
‖ Non tested.

Moreover (see below), they expressed a distinct set of adhesion molecules. Unlike most ovarian carcinomas (most cultures were derived from ovarian ascites), mesothelial cells were MOV18$^-$ and CEA$^-$. Moreover, flow cytometric analysis showed that mesothelial cell cultures had a diploid DNA content, whereas a substantial proportion of ovarian carcinomas are aneuploid. Karyotype analysis of mesothelial cells revealed no abnormality, and these cells did not form colonies in soft agar and did not cause tumors in nude mice. In contrast to leukocyte populations, mesothelial cells did not express CD2, CD14, CD16, CD20, CD31, and CD45 (Table 1). Monocytes are shown in Table 1, as mononuclear phagocytes were a major contaminant at the initiation of the cultures. Mesothelial cells express CD13, a differentiation structure of the myelomonocytic pathway also present on fibroblasts. Only occasional cells at the initiation of mesothelial cultures stained for nonspecific esterase. Mesothelial cells were weakly HLA-DR$^+$ (<5–20%), and exposure to IFN-γ caused strong expression of class II MHC (>80% positive cells). Typically mesothelial cells expressed high and low molecular weight cytokeratins (7, 8, 18, and 19) as well as vimentin. Thus, the cultures used for the present study conform to criteria used to identify mesothelial cells (e.g., references 21–24).

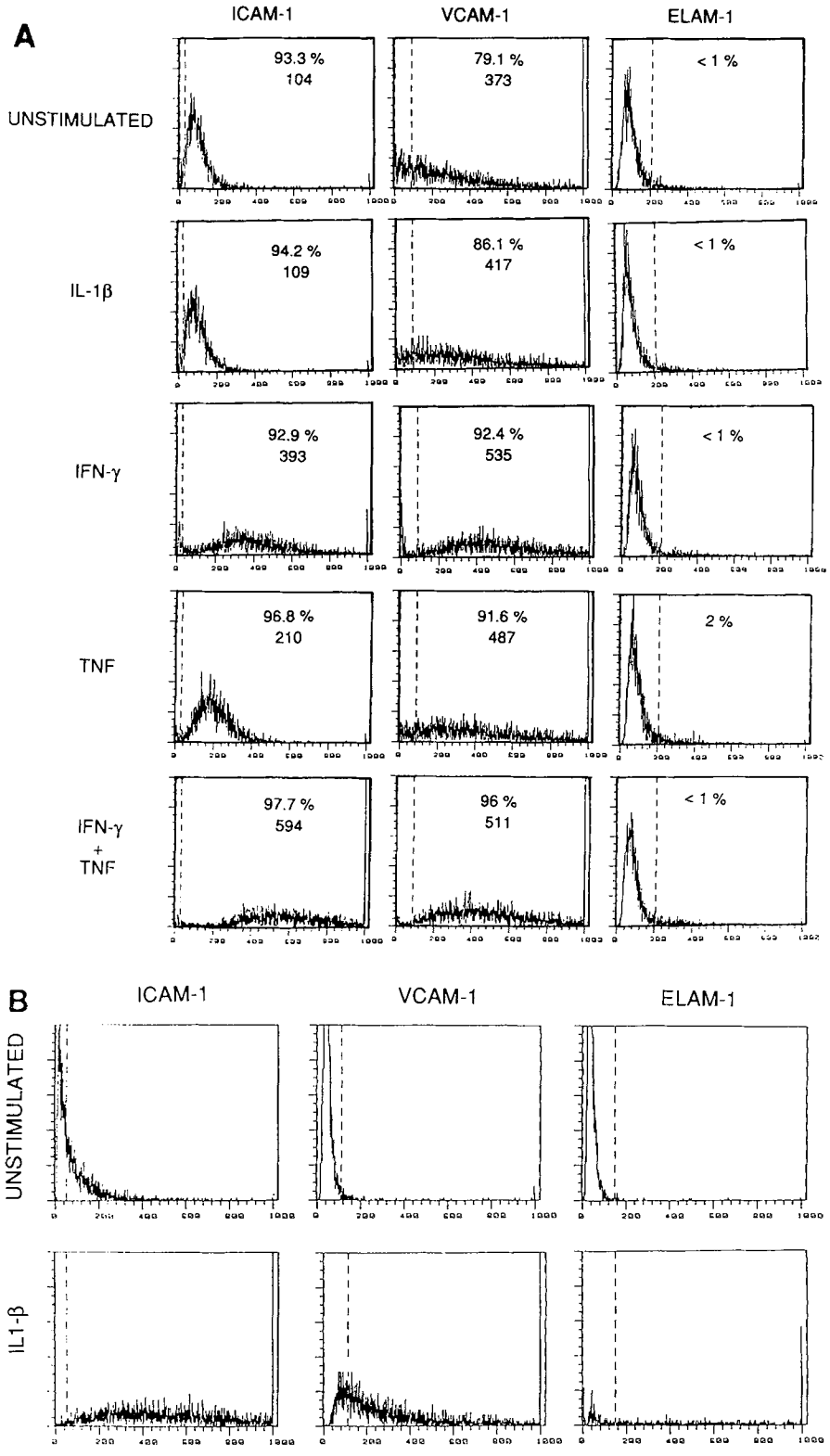

Figure 1. Expression of adhesion molecules on mesothelial cells. Peritoneal mesothelial cells were exposed to cytokines for 24 h (10 ng/ml IL-1; 500 U/ml TNF; 500 U/ml IFN-γ), and expression of adhesion molecules was assessed by FACS® (*A*) after staining with appropriate mAb or an irrelevant control mAb. Numbers indicate the percent positive cells and the mean channel of fluorescence on a linear scale. EC exposed to IL-1 (20 ng/ml) served as reference population (*B*). EC were cultured with IL-1 for 24 h except for E-selectin (ELAM-1), which was determined at 4 h since expression subsequently declined.

In particular, morphology, ploidy, and mAb-defined structures distinguish mesothelial cells from vascular endothelium, mononuclear phagocytes, and carcinoma cells, and exclude appreciable contamination of cultures by these cell types.

Expression of Adhesion Molecules. The expression of adhesion molecules was studied in mesothelial cells and, by way of comparison, in endothelial cells. Representative experiments are shown in Figs. 1 and 2, and results obtained are summa-

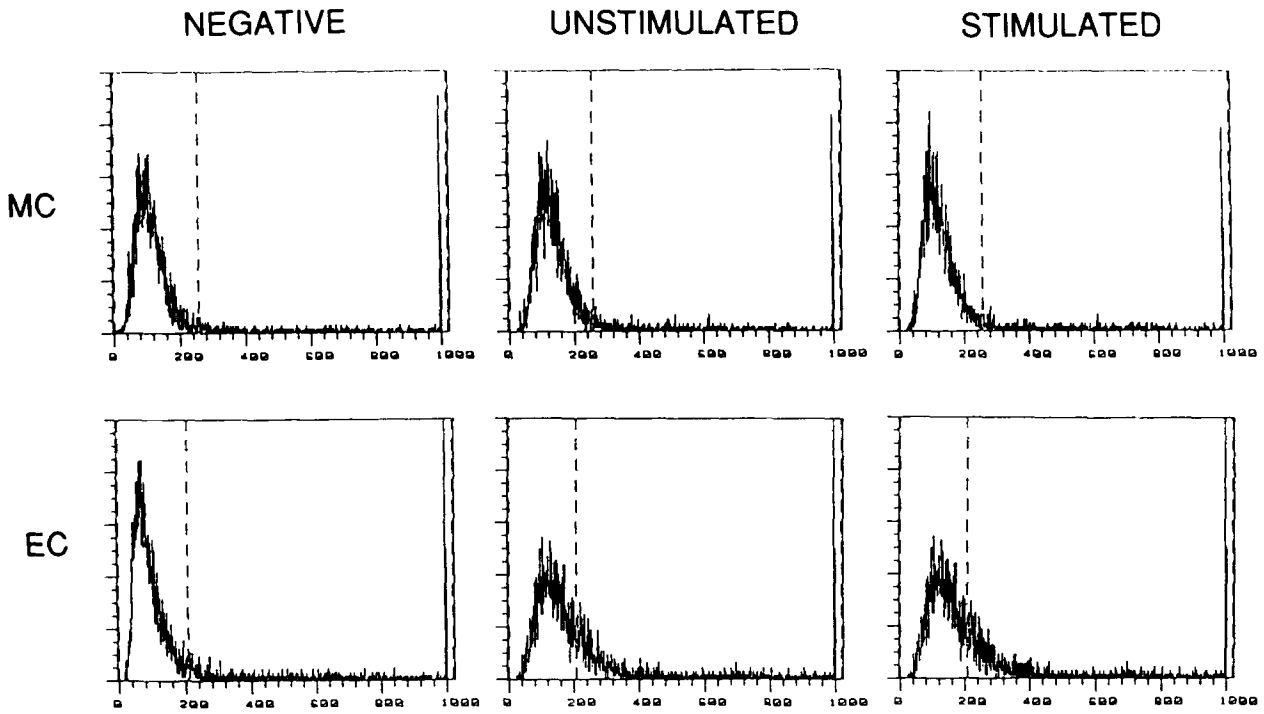

Figure 2. Expression of ICAM-2 in EC and mesothelial cells. Cells were exposed to medium or TNF (500 U/ml) and IFN-γ (500 U/ml) for 24 h before FACS® analysis. Results are shown on a linear axis.

rized in Table 2. In the absence of deliberate stimulation, mesothelial cells expressed appreciable levels of intercellular adhesion molecule 1 (ICAM-1) and VCAM-1, whereas ICAM-2 and E-selectin (previously designated ELAM-1) were undetectable. Unstimulated mesothelial cells were routinely maintained for 24–72 h in the absence of PHA-conditioned medium before being used. The expression of VCAM-1 may have resulted from exposure to cytokines present in PHA-conditioned medium. To investigate this possibility, in three experiments mesothelial cells were cultured in medium without added growth factors for 7 d. Also under these conditions, appreciable expression of VCAM-1 was detectable with 10, 90, and 85% positive cells in the three assays performed (not shown). More prolonged culture in the absence of growth supplements resulted in gross damage of mesothelial cells.

Table 2. *Expression of Adhesion Molecules on Mesothelial Cells*

Adhesion molecule	Endothelial cells		Mesothelial cells	
	Unstimulated	Stimulated	Unstimulated	Stimulated
ICAM-1	+	+ + +	+	+ + +
ICAM-2	+	+	−	−
E-selectin	−	+ + +	−	−
VCAM-1	±	+ + +	+ +	+ + +

Results presented summarize 10 experiments performed, except for ICAM-2, which was investigated twice. Cells were exposed to TNF (500 ng/ml) for 4–48 h. Stimulated expression of adhesion molecules refers to the 24-h time point, except for E-selectin, which peaked at 4–6 h in endothelial cells. E-selectin was undetectable in mesothelial cells throughout the 48-h observation period.

Cytokines regulate the expression of adhesion molecules in vascular endothelial cells (for review, see references 8 and 9). Therefore, mesothelial cells were exposed for 4–48 h to IL-1, TNF, or IFN-γ/TNF. IFN-γ/TNF augmented the expression of ICAM-1 and VCAM-1 in mesothelial cells in all 10 experiments performed. In contrast, IL-1 had inconsistent effects: in part of the experiments (2), IL-1 had little affect on adhesion molecule expression (e.g., Fig. 1), whereas in the others, it induced augmented expression and leukocyte binding (e.g., see Fig. 6). When optimal concentrations of TNF (500 U/ml) and IFN-γ (500 U/ml) were combined, higher levels of adhesion molecule expression were observed. For instance, in the experiment shown in Fig. 1, ICAM-1 fluorescence units at 24 h were 104 for control cells and 393, 210, and 594 for IFN-γ-, TNF-, and IFN-γ/TNF–stimulated cells, respectively. Even when the IFN-γ/TNF combination was not dramatically better than the individual cytokines, as for VCAM-1 at the optimal 24-h time point (Fig. 1), it caused a faster appearance of the adhesion molecule compared with the two individual cytokines (Fig. 3). Resting or cytokine-stimulated mesothelial cells did not express ICAM-2 and E-selectin (e.g., Figs. 1 and 2). Under the same conditions, in endothelial cells IL-1 and TNF induced expression of ELAM-1 with a peak at 4–6 h (Fig. 1 *B*), and

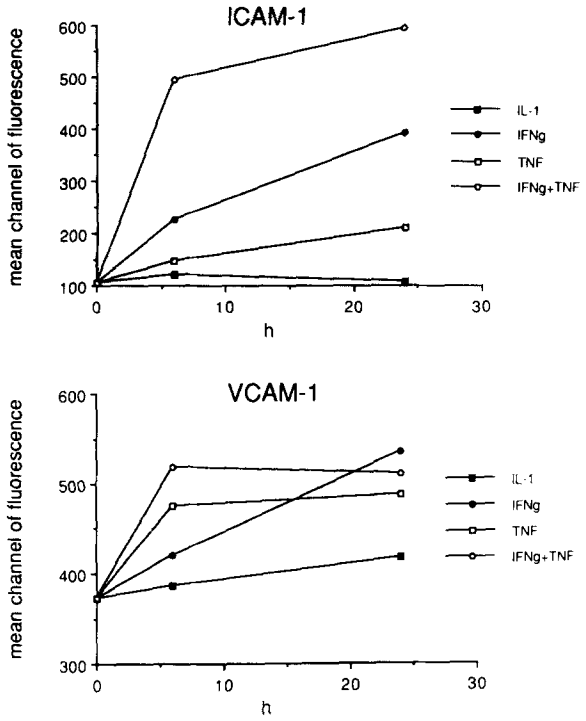

Figure 3. Time course of expression of adhesion molecules on mesothelial cells in culture. Mesothelial cells were exposed to cytokines for different times before analyzing adhesion molecule expression by FACS®. Results are mean channel of fluorescence.

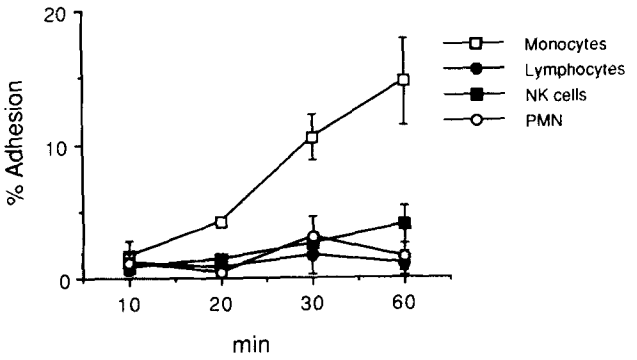

Figure 5. Binding of different leukocyte populations to unstimulated mesothelial cells. ^{51}Cr-labeled leukocytes were cultured for different times on monolayers of mesothelial cells previously maintained for 48 h in the absence of growth supplements. After washing, the percentage of cells remaining adherent to mesothelial cells was determined.

these cells had appreciable levels of constitutive ICAM-2 (Fig. 2).

In endothelial cells, splicing results in two mRNA species encoding VCAM-1 molecules with six or seven Ig domains (19, 25). To identify the form(s) of VCAM-1 expressed in mesothelial cells, we used RT-PCR to amplify fragments of different size. As shown by the ethidium bromide–stained gel and the Southern blot shown in Fig. 4, mesothelial cells expressed a predominant 631-bp fragment and a 355-bp band; stimulation with TNF/IFN-γ augmented both bands.

Monocyte Binding. Having found that mesothelial cells have the capacity to express adhesion molecules, we studied the binding of leukocytes to confluent cultures. It should be emphasized that fully confluent cultures could only be obtained with early passage (greater than or equal to three) mesothelial cells, whereas cell preparations undergoing senescence grew more slowly and remained sparse. As shown in Fig. 5, among leukocyte populations, monocytes showed the highest levels of spontaneous binding to unstimulated mesothelial cells, whereas PMN, NK cells, and lymphocytes had lower adhesion. Exposure of mesothelial cells to cytokines augmented monocyte binding considerably (Fig. 6). As expected on the basis of phenotype analysis (see above), maximal augmentation of monocyte adhesion was observed when mesothelial

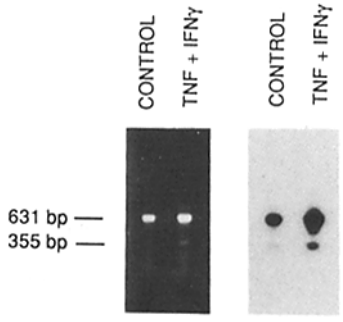

Figure 4. RT-PCR analysis of VCAM-1 transcripts in mesothelial cells. Mesothelial cells were exposed to TNF (500 U/ml) and IFN-γ (500 U/ml) for 4 h. IL-1-stimulated EC served as positive control. (*Left*) Ethidium bromide gel; (*right*) the same after Southern transfer and hybridization with a VCAM-1 probe.

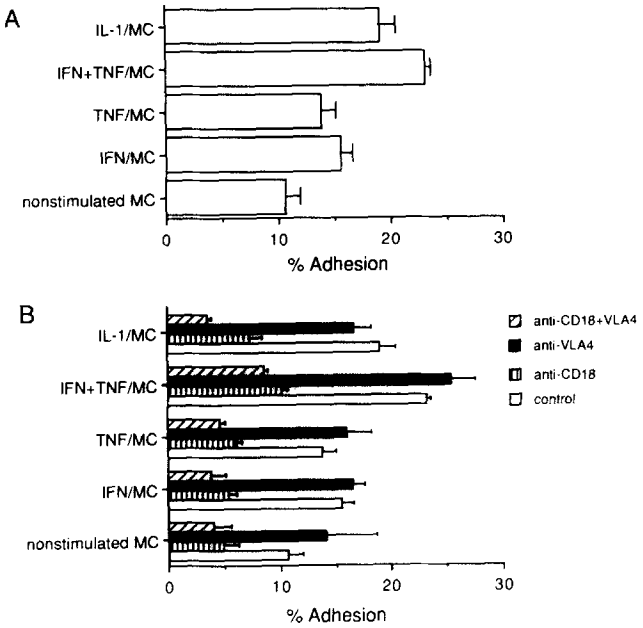

Figure 6. Binding of unstimulated monocytes to cytokine-activated mesothelial cells. (*A*) Mesothelial cells were cultured for 24 h with IL-1 (10 ng/ml), TNF (500 U/ml), IFN-γ (500 U/ml), or TNF/IFN-γ. (*B*) The influence of different adhesion pathways was studied by adding appropriate mAb or control irrelevant mAb.

cells were exposed to a combination of IFN-γ and TNF, with a 110% increase in binding in the experiment shown in Fig. 6.

As illustrated in Fig. 6 *B*, where one experiment, representative of three performed, is presented, anti-CD18 mAb, directed against the ICAM-1 and -2 counter-receptors (26), caused a significant inhibition of monocyte binding to resting (67% blocking) and stimulated (41% inhibition) mesothelial cells. A mAb directed against the α4 β1 integrin very late antigen 4 (VLA-4), the VCAM-1 counter-receptor (27), had little or no effect alone, but in all three experiments it augmented the inhibitory activity of anti-CD18, with 78 vs. 67% inhibition for "resting" mesothelium and 65 vs. 41% inhibition for IFN-γ/TNF-stimulated mesothelial cells. When the role of different β2 integrins was studied, anti-LFA1 mAb inhibited monocyte binding to mesothelium, whereas anti-Mac-1 and anti-p150,95 had little or no effect (only two experiments performed; data not shown).

Exposure to bacterial products or cytokines modulates the adhesive properties of leukocytes, including monocytes, to vascular endothelium (e.g., 11–13,28). In our previous studies, we used LPS and IFN-γ as prototypic stimuli for the interaction of mononuclear phagocytes with endothelium (11–13). Monocyte activation by IFN-γ/LPS resulted in augmented binding to resting (not shown) and stimulated (Fig. 7) mesothelial cells, with a 40.7% of increase in a series of five experiments.

Expression of Chemotactic Cytokines. Recruitment of leukocytes involves the regulated expression of adhesion molecules and the response of circulating cells to tissue-derived chemotactic signals. It was therefore important to investigate whether mesothelial cells were able to express two members (IL-8 and MCP-1) of a recently identified chemotactic cytokine superfamily (for review, see reference 29). As shown in Fig. 8, exposure to IFN-γ/TNF or IL-1 (data not shown) induced a substantial increase in IL-8 and MCP-1 mRNA expression over variable, but appreciable baseline expression. It should be noted that the PHA-conditioned medium used for culturing mesothelial cells augmented IL-8 expression (data not shown). Immunoreactive IL-8 as well as MCP-1 biological activity were detected in the supernatant of stimulated mesothelial cells (Table 3).

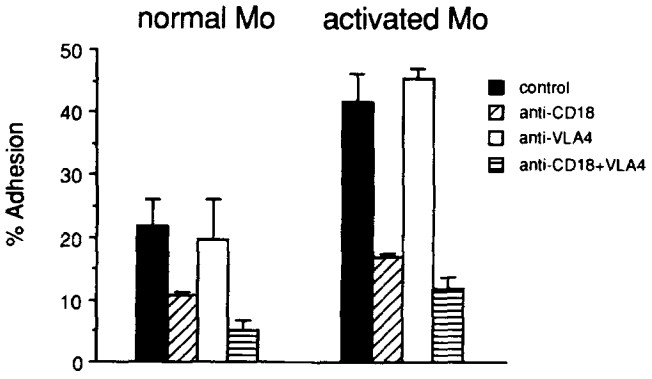

Figure 7. Binding of resting and activated human monocytes to mesothelial cells. Monocytes were activated by culture for 20 h with IFN-γ (500 U/ml) and LPS (100 ng/ml). After washing, binding was determined using cytokine (TNF/IFN-γ) activated mesothelium as substratum.

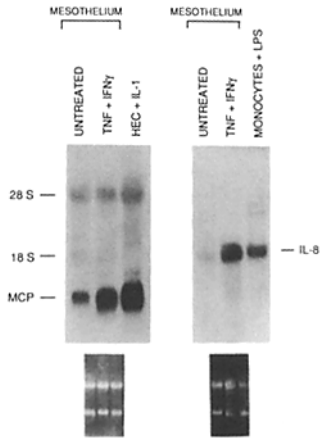

Figure 8. Expression of IL-8 and MCP-1 in cytokine-stimulated mesothelial cells. Mesothelial cells were exposed for 4 h to TNF (500 U/ml) and IFN-γ (500 U/ml), the RNA was extracted, and cytokine gene expression was studied by Northern analysis.

Discussion

The results presented here demonstrate that human mesothelial cells in culture can express a defined set of adhesion molecules as well as chemoattractant cytokines, two fundamental mechanisms of regulation of leukocyte traffic (8, 9). Mesothelial cells expressed, in the absence of deliberate stimulation, ICAM-1 and VCAM-1, and the levels of adhesion molecules were augmented by TNF, IFN-γ, TNF/IFN-γ, and IL-1, though the response to the latter stimulus was variable. VCAM-1 transcripts in endothelial cells undergo differential splicing to yield two mRNA populations encoding a six- and seven-Ig domain version of the molecule (19, 25). The predominant version of VCAM-1 expressed on activated endothelium is a seven-Ig domain molecule. There is evidence based on gene transfer that the six-domain VCAM-1 also interacts with the leukocyte counter-receptor VLA-4. By PCR

Table 3. *Production of Cytokines by Mesothelial Cells*

Stimulus	IL-6	IL-8	MCP-1 (migrated monocytes)
	U/ml	*ng/ml*	
-	1,250	120	98 ± 12
IL-1 (10 ng/ml)	22,458 ± 2,812	NT	172 ± 16
TNF (500 U/ml)	17,892 ± 456	580	NT
IFN-γ (500 U/ml)	NT	160	NT
TNF + IFN-γ	NT	600	NT

Cells were maintained for 48 h without PHA-conditioned medium, exposed to stimuli for 20 h, washed, and cultured for further 24 h. Results are mean ± SD of three replicates, except for IL-8, which was tested once. NT, not tested.

analysis we established that mesothelial cells express both the seven- and six-Ig domain transcripts, with predominance of the longer species. Thus, the patterns of alternative splicing and relative abundance of VCAM-1 mRNA transcripts are similar in mesothelium and endothelium, with no evidence of tissue-specific regulation.

It should be noted that mesothelial cells used for the present study were grown in the presence of conditioned medium of PHA-stimulated mononuclear cells, a classical source of cytokines. Although the cells were routinely rested for 24–72 h (in three experiments, 7 d) before experiments, it is possible that the relatively high baseline expression of VCAM-1, compared with endothelial cells, may indeed result from previous exposure to mediators present in the growth medium.

Unlike endothelial cells (10, 30), mesothelial cells did not express ICAM-2 as assessed by flow cytometry. Moreover, stimuli active on endothelial cells (TNF and IL-1) tested repeatedly at different times failed to induce E-selectin in mesothelial cells. Thus, the repertoire of adhesion molecules that can be expressed in mesothelial cells, which includes ICAM-1 and VCAM-1, but not ICAM-2 and E-selectin, overlaps with, but is clearly distinct from, that available in the endothelial armamentarium.

E-selectin acts in leukocyte-endothelium interactions as a lectin counter-receptor for carbohydrate-containing molecules (for review, see references 8 and 9). This strong sugar-lectin interaction is particularly important for the rolling and initial capture of circulating leukocytes under conditions of shear stress in blood vessels (31, 32). This type of interaction may be dispensable in the less stressful conditions found in the peritoneal cavity.

Monocyte-endothelial cell interactions can be modulated both at the level of vessel wall elements and circulating white cells. As discussed above, at the level of the endothelium, inflammatory signals induce adhesion structures de novo or in augmented quantities. Conversely, chemotactic agonists and cytokines augment the adhesive properties of mononuclear phagocytes (12, 13, 33–35). A similar regulated interaction of monocytes with mesothelial cells was observed in the present study. Monocytes activated by in vitro exposure to IFN-γ and LPS (serving as prototypic activation signals previously utilized in studies on endothelium; references 12 and 13) bound with increased efficiency to mesothelial cells. The main $\beta 2$ integrin involved was LFA-1, with little or no contribution of Mac-1 and p150,95. mAb directed against VLA-4, the VCAM-1 counter-receptor, had no consistent effect on monocyte binding to mesothelium, but, when the CD18 pathway was blocked, a role for VLA-4-dependent adhesion was detected. These results, parallel to those obtained in similar studies with EC (11–13, 28, 36), establish the functional significance of adhesion molecules expressed on mesothelial cells.

In the present study, the functional relevance of adhesion molecules expressed on mesothelial cells was investigated by focusing on mononuclear phagocytes, a major peritoneal cell population. However, studies on EC have revealed that disseminating pathogens, most notably tumor cells, use the same adhesion pathways for interacting with endothelial cells in vitro and in vivo (e.g., 37–39). The peritoneal cavity can represent a route for cancer cell dissemination and a site of implantation from primary sites located in peritoneal organs, including the gastrointestinal tract and the ovary. In analogy with endothelium, the expression of adhesion structures on mesothelial cells is probably involved in the peritoneal dissemination and seeding of cancer cells.

Serous cavities, and most notably the peritoneum, can be involved by infectious, inflammatory, and immunological processes. It has long been known that local application of inflammatory or immunological stimuli can cause recruitment of leukocytes in serous cavities. Production by mesothelial cells of the chemotactic cytokines IL-8 and MCP-1, also expressed by activated EC (16, 18, 40–43), can be an important determinant of leukocyte recruitment. On the other hand, intraperitoneal challenge with antigen in a peritoneum containing an inflammatory exudate results in disappearance of macrophages from the fluid (MDR 2–7). Lymphokine supernatants can substitute for antigen in eliciting the MDR (4, 7). The results presented here suggest that the inducible expression on the mesothelium of adhesion molecules recognized by macrophage counter-receptors underlies the MDR. These observations also suggest that IFN-γ and TNF may be central cytokine mediators of the MDR. By and large, the results reported here suggest that the mesothelial lining plays an important, active role in regulating the traffic of leukocytes, monocytes in particular, between the circulation, the peritoneal fluid, and the walls of the peritoneum.

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