Interactions between leukocytes and endothelial cells, particularly in the microvasculature, are important for the initiation and regulation of tissue inflammation. These interactions are regulated by the recognition of specific cell adhesion molecules (CAM) on both leukocytes and endothelial cells. In this study, we examined the modulation of cell surface expression of MHC antigens and the CAM intercellular adhesion molecule 1 (ICAM-1), lymphocyte function antigen 3 (LFA-3), and CD44 on human dermal microvascular endothelial cells (HDMEC) both grown in monolayers and differentiated in capillary-like structures on the basement membrane-like substrate matrigel. HDMEC grown in monolayers or differentiated on matrigel express comparable cell surface MHC class I, LFA-3, CD44, and ICAM-1. ICAM-1, but not LFA-3 or CD44, was increased in expression in a dose- and time-dependent manner by interleukin 1 (IL-1) alpha, tumor necrosis factor (TNF) alpha, lipopolysaccharide (LPS), or interferon (IFN) gamma. Comparable upregulation was observed both in cells grown in monolayers and cells differentiated on matrigel. IL-1 alpha, TNF alpha, and LPS increased ICAM-1 expression on average 100–200% whereas IFN gamma was somewhat less potent. Comparative studies with human umbilical vein endothelial cells (HUVEC) demonstrated consistently lower levels of ICAM-1 expression on HUVEC, but greater increases after cytokine stimulation. Pretreatment with dexamethasone or transforming growth factor (TGF) beta did not affect baseline expression of ICAM-1 or inhibit upregulation of ICAM-1 on HDMEC by IL-1 alpha, TNF alpha, LPS, or IFN gamma. Both IFN gamma and TNF alpha, but not IL-1 alpha increased MHC class I expression, whereas only IFN gamma induced the expression of HLA-DR on HDMEC. The effect of IL-1 alpha, TNF alpha, or IFN gamma was inhibited by antibody to the specific cytokine, but was unaffected by antibody to other cytokines. Additionally, IFN alpha or beta inhibited upregulation of HLA-DR by IFN gamma, but had no effect on the increased MHC class I or ICAM-1 expression mediated by this cytokine. These data demonstrate that the expression of CAM and MHC antigens on small vessel-derived endothelial cells is different from that observed on large-vessel HUVEC, is regulated by the presence of multiple cytokines operating via distinct pathways, and the expression and regulation of these proteins appear to be similar on cells that have been grown in monolayers to those morphologically differentiated into blood vessel-like structures.

Inflammation and tissue damage are influenced by a variety of exogenous and endogenous factors, but these processes are mediated primarily by the activity of bone marrow-derived inflammatory cells. These cells, including lymphocytes, neutrophils, or monocytes, must first interact with endothelial cells lining the microvasculature in order to gain access to organ parenchyma. The nature of this interaction is not fully defined, but the initial adherence of inflammatory cells to endothelium is controlled by adherence proteins on the surface of both leukocytes and endothelium [1–3], including MHC antigens and cell adhesion molecules such as ICAM-1, LFA-3, and CD44. The expression of these proteins on endothelial cells may be altered by a number of cytokines felt to be important in the modulation of inflammation, including interleukin 1 (IL-1), tumor necrosis factor (TNF), and interferon (IFN) gamma [4–13]. These alterations correlate in vitro with increased adherence of leukocytes to endothelium [14–19]. The expression of ICAM-1 and MHC proteins on cutaneous microvasculature has been examined on sections of human skin [9,12,13,20]. However, virtually all in vitro studies examining the expression and regulation of CAM and MHC proteins on endothelium have utilized endothelial cells derived from large vessels, usually human umbilical vein, because they are easier to isolate and culture than microvascular endothelial cells. Additionally, these cells have been utilized in monolayers. Recently, we and others have isolated and propagated small vessel endothelial cells from human dermis and demonstrated distinct differences from endothelial cells derived from large vessels [21–26]. Differences have been described in prostaglandin secretion, growth requirements in vitro,
and differences in cell surface protein expression including ICAM-1 and CD44.* In order to examine the expression and modulation of cell surface CAM and MHC proteins on cutaneous microvascular endothelial cells, we have defined the CAM on microvascular endothelial cells derived from human dermis (HDMEC). Additionally, we have evaluated the modulation of cell surface expression of MHC proteins and CAM on HDMEC in response to the proinflammatory cytokines IL-1 alpha, TNF alpha, IFN gamma, the anti-inflammatory corticosteroid dexamethasone, and the cell growth and immune modulator transforming growth factor (TGF) beta. Furthermore, in order to determine the relevance of differentiation state on expression and modulation of CAM and MHC proteins, we have studied HDMEC grown in monolayers and after induction of morphologic differentiation on matrigel.

**MATERIALS AND METHODS**

**Isolation and Culture of Human Dermal Microvascular Endothelial Cells (HDMEC) and Human Umbilical Vein Endothelial Cells (HUVEC)** HDMEC were isolated from human foreskins by a previously reported technique [21,24,25]. Briefly, HDMEC were isolated from foreskins by trypsinization and percoll gradient centrifugation. Cells were initially cultured in media consisting of medium 199 with 50% human serum, endothelial cell growth supplement 100 μg/ml (Biomedical Technologies, Stoughton, MA), heparin 50 μg/ml (Sigma Chemical, St. Louis, MO), 5 × 10^{-5} M dibutyryl cyclic AMP (Sigma), 3.3 × 10^{-5} M isobutyryl methylxanthine (Calbiochem-Behring, San Diego, CA), 2 × 10^{-9} M glutamine (Sigma), and 100 U/ml penicillin, 100 μg/ml streptomycin, and 250 μg/ml amphotericin B (Sigma). Later, cells were cultured in endothelial basal media (Clonetics Corp., San Diego, CA) with epidermal growth factor 1 ng/ml (Clonetics), hydrocortisone acetate 1 μg/ml (Sigma Chemical), dibutyryl cyclic AMP, and 20–30% human serum with identical results. Presence of greater than 99% HDMEC without contaminating fibroblasts in cultures isolated by these methods was confirmed by staining with FITC rabbit anti-human factor VIII (Atlantic Antibodies, Scarborough, ME) and by staining with acetylated low-density lipoprotein (10 μg/ml) labeled with 1,1'dioctadecyl-3,3,3',3'-tetramethyl indocarbocyanine percholate (Dil-Ac-Dil) (Biomedical Technologies). Morphologic differentiation of HDMEC was induced by plating cells on matrigel (gift of Dr. Hynda Kleinman, NIH) as described previously [21].

HUVEC were isolated from collagenase-treated umbilical vein as described previously [27]. Cells were cultured in medium 199 with 20% fetal bovine serum, endothelial cell growth supplement (100 μg/ml), heparin (50 μg/ml), 2 × 10^{-9} M glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 250 μg/ml amphotericin B.

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* Swerlick et al: Human dermal microvascular endothelial cells express a phenotype distinct from human umbilical vein endothelial cells (submitted for publication).
Monoclonal Antibodies and Biologic Response Modifiers

Purified monoclonal antibodies (MoAb) 84H10 (mouse IgG1) and TS 2/9 (mouse IgG1), which bind to intercellular adhesion molecule-1 (ICAM-1) and LFA-3, respectively, were obtained from Dr. Stephen Shaw, NIH and used at a concentration of 200 μg/ml. MoAb A1G3 (Mouse IgG1), which recognizes the protein CD44, was obtained as ascites from Dr. Bard Haynes, Duke University School of Medicine and was used at 1:40 dilution. Anti- HLA-A,B,C was obtained as tissue culture supernatants (W6/32, mouse IgG2a, Accurate Chemical, Westbury, NY) or as purified antibody (Clone B9.12.1, purified antibody [200 μg/ml], mouse IgG2a, Pel Freez biologicals, Brown Deere, WI) and was used undiluted. Anti- HLA-DR consisted of clone L234 (mouse IgG2a, Becton-Dickinson, Burlington, CA) and was used undiluted. IL-1 alpha was obtained from Genzyme Corporation (Boston, MA) and from Dr. Ira Green, NIH. TNFalpha was purchased from Amgen Corporation (Thousand Oaks, CA). IFN gamma was obtained from Amgen or Chemicon Corporation (El Segundo, CA). IFN alpha and beta were purchased from Chemicon. TGF beta was obtained from Collaborative Research (Bedford, MA). Polyclonal rabbit anti- IL-1 and polyclonal rabbit anti-TNF alpha were obtained from Endogen (Cambridge, MA). Monoclonal antibodies against IFN gamma (purified MoAb, clone A07) and IFN beta (purified MoAb, clone E54) were obtained from Olympus Corporation Immunochemicals (Lake Success, NY). FITC-conjugated goat anti-mouse IgG (Fab') and FITC-conjugated sheep anti-mouse IgG (Fab') were obtained from Tago (Burlingame, CA) and Sigma, respectively. Dexamethasone was obtained from Baxter Healthcare Corporation (Deerfield, IL).

Flow Cytometric Analysis HDMEC were grown to confluence and then stimulated with various biologic response modifiers in the presence of complete endothelial cell growth media for varying times. Control cultures had only the addition of fresh media. Cells were removed by either incubation with 1% BSA with 5 mM EDTA or by incubation with 0.05% trypsin with 0.53 mM EDTA (Biofluids, Rockville, MD), and identical results were obtained. Cells were then washed in PBS with 0.5% BSA (ICN Immunobiologicals, Costa Mesa, CA), counted, and aliquotted for staining. Ten microliters of the first antibody was added to each tube. Preliminary studies demonstrated that either isotype control antibody or PBS gave identical results, and subsequently PBS was used as a control. The cells were incubated for 30 min on ice, washed twice, and 10 μl of second step antibody diluted 1:20 in PBS was added. After another 30 min incubation, the cells were washed 3 times, and suspended in 0.5 ml PBS with 0.5% BSA. Propidium iodide was added immediately prior to flow cytometric analysis to gate out dead cells. Fluorescence was measured on a Becton Dickinson FACScan (Mountainview, CA) and expressed as either log mean channel fluorescence or percent positive cells. Results of experiments examining modulation of cell surface expression are expressed in terms of percentage increase over baseline. For example, a 100% increase in expression denotes a twofold increase in expression, whereas a 200% increase corresponds to a threefold increase.

ELISA HDMEC were plated on 96-well tissue culture plates 24-48 h prior to the assay. They were then stimulated with biologic response modifiers as described above. After stimulation, cells were fixed in 2% paraformaldehyde for 15 min at room temperature. They were then washed in HBSS and incubated with 2% BSA in HBSS for 1 h at 37°C. One hundred microliters of antibody to ICAM-1 (MoAb 84H10) or irrelevant control antibody at a concentration of 1 μg/ml in an additional hour. The cells were then washed and incubated for 1 h with peroxidase-conjugated goat anti-mouse IgG (Biorad, Richmond, CA) diluted 1:1000 in HBSS with 2% BSA. The wells were then washed and binding of antibody detected by the addition of 100 μl ortho-phenylenediamine. A stock solution was prepared by dissolving 50 mg of ortho-phenylenediamine in 5 ml methanol. A working solution was prepared by diluting 1 ml stock solution with 100 μl of 3% H₂O₂ in 100 ml distilled water. The reaction was stopped by the addition of 25 μl of 8N H₃SO₄. Plates were read on a Titertek Multiscan ELISA reader at 0.D. 492 after blanking on rows stained with irrelevant monoclonal antibody. All data points were done in quadruplicate.

RESULTS

Modulation of HDMEC Cell Surface CAM and MHC Protein Expression by IFN-Gamma Incubation of HDMEC with IFN-gamma led to a dose- and time-dependent increase in the expression of ICAM-1 (Fig 1a,b). No increase in ICAM-1 expression was noted at any dose point at 4 h. Five hundred μl of IFN gamma resulted in only an 11% increase of ICAM-1 at 24 h, and an 82% increase at 72 h. A slight effect could be seen at doses as low as 5 μl/ml and doses as high as 2000 μl/ml resulted in a 154% increase in ICAM-1 expression at 72 h.

The expression of HLA class I molecules could be increased by prolonged incubation of HDMEC with IFN gamma in a time- and dose-dependent manner. IFN gamma (2000 μl/ml) caused up to a threefold increase in HLA class I expression by 72 h (Fig 1c). The increase in class I induced by IFN gamma (500 μl/ml) was not evident after 4 h of cytokine stimulation, but was clearly apparent after 24 h of stimulation (Fig 1d). In vitro incubation of HDMEC at multiple time and dose points with IFN-gamma did not affect the expression of LFA-3 or CD44 (data not shown).

Modulation of HDMEC Cell Surface CAM and MHC Protein Expression by TNF Alpha, IL-1 alpha, and LPS HDMEC stimulated with TNF alpha demonstrated a time- and...
dose-dependent increase in the expression of ICAM-1 as determined by ELISA (Fig 2a,b) or flow cytometric analysis (data not shown). Stimulation with IL-1 alpha resulted in a similar time and dose response. Expression of ICAM-1 increased an average of 100–200% after 24 h of stimulation, although greater increases were noted in some experiments (see Fig 6). An increase in ICAM-1 was noted as early as after 2 h of incubation in some experiments, but was invariably present after 4 h of stimulation. Expression was maximal after 24 h of stimulation with either IL-1 alpha or TNF alpha at a dose of 80 μg/ml of either cytokine. Expression of ICAM-1 often fell slightly with continued stimulation at doses of 80 μg/ml or higher. Doses as low as 5 units/ml of either TNF alpha or IL-1 alpha generally elicited an increase in ICAM-1 when examined at 4 h, with increasing response noted with higher doses. In addition, incubation of HDMEC with IL-1 alpha or TNF alpha resulted in an altered morphology, with cells assuming a more spindled appearance after 24 h of exposure. LPS stimulation also resulted in an increase in ICAM-1 expression. Twenty-four hours of stimulation with doses as low as 10 ng/ml resulted in an 82% increase in ICAM-1 expression. Doses of 100 ng/ml or greater resulted in a maximal 200% increase in ICAM-1 expression. At this dose, upregulation was clearly evident by 4 h (135%) and maximal by 24 h (200%).

IL-1 alpha, TNF alpha, and LPS induced similar increases in ICAM-1 expression in large-vessel HUVEC, but increases were greater than those noted in HDMEC. Stimulation of HUVEC for 24 h with 80 μg/ml of TNF alpha or IL-1 alpha or 1 μg/ml of LPS resulted in 522%, 415%, and 517% increases, respectively. The differences in responsiveness could not be fully explained on the basis of differences in culture system. HUVEC stimulated for 24 h with IL-1 alpha (80 units/ml) or TNF alpha (80 units/ml) in HUVEC media or HDMEC media both expressed four- to fivefold increases in ICAM-1 expression whereas HDMEC stimulated in parallel only doubled ICAM-1 expression (Fig 3). However, as baseline ICAM-1 expression on HUVEC is 2–3 times less than on HDMEC, maximal expression of ICAM-1 on HDMEC and HUVEC is roughly equivalent.

IL-1 alpha and TNF alpha stimulation of HDMEC did not result in modulation of cell surface expression of LFA-3, CD44, or induction of HLA-DR (data not shown). TNF alpha induced modest increases in MHC class I expression that were evident as early as 24 h, but IL-1 alpha did not affect the cell surface expression of MHC class I (data not shown).

**Modulation of BRM Effect on HDMEC**

IFN gamma induction of HLA-DR (Fig 4a) and upregulation of HLA class I (data not shown) on HDMEC was totally abrogated by preincubation of this cytokine with a neutralizing monoclonal antibody to IFN gamma. Additionally, this antibody also completely inhibited IFN gamma-induced ICAM-1 upregulation (Fig 4b). Control antibody to an irrelevant cytokine (anti-IFN beta) did not affect IFN gamma-induced upregulation of HLA-DR (Fig 4a), ICAM-1 (Fig 4b), or class I (data not shown). In contrast, coinubcation of HDMEC with 1000 μg/ml of IFN alpha and IFN gamma inhibited the induction of class II antigens by IFN gamma (Fig 4a), but had no effect upon the upregulation of ICAM-1 (Fig 4b) or class I (data not shown).

In order to determine whether IFN gamma-induced modulation of cell-surface protein expression on HDMEC is mediated via IL-1 or TNF production, HDMEC stimulated with IFN gamma were coinubcated with neutralizing antibodies to IL-1 or TNF. Antibody against IL-1 or TNF alpha had no effect upon IFN gamma-induced expression of HLA-DR or upregulation of ICAM-1 or HLA class I antigen expression (data not shown). In contrast, specific rabbit polyclonal antibody to TNF alpha inhibited the upregulation of ICAM-1 by 40 μg/ml of TNF alpha by 50–75% when compared to preimmune rabbit sera or rabbit antiserum against an irrelevant cytokine. Similarly, polyclonal antibody to IL-1 alpha inhibited the upregulation of ICAM-1 by recombinant IL-1 alpha.

Because TGF beta has been shown to decrease neutrophil adherence to HUVEC monolayers, we examined whether TGF beta treatment of endothelial cells affected baseline expression or cytokine modulation of ICAM-1 and HLA-DR. Incubation of HDMEC with TGF beta at a concentration of 250 pm for 16 h did not consistently affect expression of ICAM-1 with minor decreases (Fig 4a,b).
Figure 5. TGF beta does not affect ICAM-1 upregulation by cytokines. HDMEC were pretreated for 24 h with 250 nM TGF beta and then stimulated with (a) IL-1 alpha or TNF alpha (40 μg/ml × 4 h), or (b) IFN gamma (500 μg/ml × 72 h).

5a) and increases (Fig 5b). Preincubation of HDMEC with TGF beta (250 pM) for 16 h prior to the addition of either IL-1 alpha or TNF alpha did not inhibit the upregulation of ICAM-1 (Fig 5a). Preincubation of HDMEC with TGF beta (250 pM) also did not affect IFN gamma-induced ICAM-1 upregulation (Fig 5b), or induction of HLA-DR. TGF beta treatment of HUVEC yielded identical results (data not shown).

Pretreatment of HDMEC for 24 h with 10^{-5} M dexamethasone also did not affect baseline ICAM-1 expression or IL-1 alpha- or LPS- (Fig 6) or TNF alpha- (data not shown) induced upregulation of ICAM-1. Additionally, neither ICAM-1, HLA class I, nor HLA-DR expression was affected by incubation of HDMEC with granulocyte-macrophage colony stimulating factor or interleukin-6 (data not shown).

Modulation of CAM Expression on Undifferentiated and Morphologically Differentiated HDMEC. HDMEC grown in monolayers or morphologically differentiated on matrigel were examined by flow cytometry for the expression of ICAM-1, LFA-3, HMC class I, and HMC HLA-DR. Mean channel fluorescence of cells stained with MoAb recognizing class I, ICAM-1, CD44, or LFA-3 was similar whether cells were examined after growing in monolayers or after morphologic differentiation on matrigel (Table I). HDMEC did not express baseline HLA-DR without IFN gamma stimulation after growth on matrigel or plastic.

Stimulation of HDMEC that had been plated on matrigel with IL-1 alpha or TNF alpha (40 μg/ml × 24 h) resulted in increases in ICAM-1 expression of 185% and 126%, respectively (Table II). Stimulation of HDMEC on matrigel with IFN gamma (500 μg/ml) for 72 h also resulted in increases in ICAM-1 of 88%. Additionally, IFN gamma stimulation of cells on matrigel increased the mean channel fluorescence of cells stained for HLA-DR, and resulted in HLA-DR expression in over 90% of cells.

DISCUSSION

Human dermal microvascular endothelial cells are strategically located to play a critical role in the induction and modulation of cutaneous inflammation. Effector leukocytes must first interact with endothelial cells lining microvessels before they can gain access to extravascular spaces. The interaction of leukocytes and endothelial cells is mediated by a number of proteins on each cell type [1,3,14-19], and modulation of the expression of these proteins may alter the interactions of leukocytes with endothelium and ultimately impact upon the intensity of inflammatory responses.

The expression and regulation of these immunologically relevant cell-surface proteins on endothelial cells has been examined in vivo and in vitro. Histologic studies have demonstrated the expression of ICAM-1 and MHC proteins on endothelial cells in normal and inflamed skin, and in sections of skin from organ culture. Direct examination of cytokine effect on expression of CAM and MHC proteins on cultured human endothelial cells have been done almost exclusively on large-vascular human umbilical vein endothelial cells [1-11] because of the difficulty in isolating sufficient quantities of microvascular endothelium. These studies have shown that the cell adhesion molecules ICAM-1 and ELAM-1 (endothelial-leukocyte adhesion molecule-1) are upregulated by the cytokines IL-1, TNF, and IFN gamma [2,5,8,9]. Additionally, TNF and IFN gamma were capable of altering cell-surface expression of MHC proteins [5-13].

Because leukocytes that mediate cutaneous inflammation interact with endothelium in microvessels in the skin, the ideal cell to study is the cutaneous microvascular endothelial cell. Large-vascular endothelial cells demonstrate distinct differences from small vessel endothelial cells from skin. These include differences in prostanoid production [26], in vitro growth requirements [21,24,25], and cell-
Table II. HDMEC Differentiated on Matrigel are Responsive to IL-1 Alpha, TNF Alpha, and IFN Gamma

<table>
<thead>
<tr>
<th>Control</th>
<th>IL-140 μg/ml</th>
<th>TNF 40 μg/ml</th>
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<tr>
<td>92 ± 12</td>
<td>262 ± 12</td>
<td>208 ± 12</td>
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*HDMEC were cultured in the presence or absence of cytokine on matrigel-coated plastic. Cells were then removed and stained by flow cytometric analysis as described in Materials and Methods. Results are expressed as log mean channel fluorescence ± SD.

The expression of ICAM-1 on HDMEC differed distinctly from that seen on large-vessel human umbilical vein endothelial cells (HUVEC) in two ways. As we have previously reported, direct comparison using flow cytometric analysis demonstrated that the baseline expression ICAM-1 was consistently higher on HDMEC than HUVEC [23]. The differences in baseline expression are not due to differences in culture conditions as prolonged growth of HUVEC in HDMEC media does not increase expression of ICAM-1 on HUVEC to the levels observed on HDMEC.* Additionally, the magnitude of the upregulation of ICAM-1 on HDMEC that was induced by IL-1 alpha, TNF alpha, or LPS was on average less than that induced on HUVEC. ICAM-1 on HDMEC increased an average of 100–200% after cytokine stimulation, whereas it increased on HUVEC an average of 400–500% after similar stimulation. Both IL-1 alpha and TNF alpha rapidly induced upregulation of ICAM-1 on HDMEC. Doses of TNF alpha or IL-1 alpha as low as 5 μg/ml caused increases in ICAM-1, with maximal effect noted at around 80 μg/ml. These data show that the time and dose responses of HDMEC are similar to HUVEC, although the percent increase in ICAM-1 expression is less.

IFN gamma also upregulated the expression of ICAM-1 on HDMEC, but its onset of action was much slower than either IL-1 alpha or TNF alpha. No evidence of increased expression of ICAM-1 was observed after 4 h of stimulation with 500 μg/ml of IFN gamma and only minimal increases were noted after 24 h. This is in contrast to the induction of ICAM-1 by IFN gamma on fibroblasts or keratinocytes after only 6–10 h of stimulation [9,28]. Previous studies on large-vessel endothelial cells have demonstrated small increases that have been seen as early as after 24 h of IFN gamma stimulation [2], but maximal stimulation is evident only after longer incubations. IFN gamma was not as potent as either IL-1 alpha or TNF alpha in inducing ICAM-1 upregulation on HDMEC. Seventy-two hours of stimulation with 500 μg/ml of IFN gamma generally resulted in a doubling of ICAM-1 expression, although occasionally greater increases were noted.

The effect of IL-1 alpha and TNF alpha on ICAM-1 expression is very rapid with increased expression often evident after 2 h of incubation and is similar to observations in HUVEC. This correlates well with increases in T-cell adherence to endothelial cells in vitro where increased T-cell adherence is noted after 4 h of cytokine stimulation [14,15]. However, IFN gamma stimulation of endothelium also results in increased T-cell adherence as early as after 4 h of stimulation [16,19]. Because upregulation of ICAM-1 does not occur this rapidly, increased adherence of T cells must be via an ICAM-1–independent mechanism.

Both TNF alpha and IFN gamma induced upregulation of HLA class I molecules on the surface of HDMEC. The kinetics of class I upregulation were distinctly different from ICAM-1 upregulation with either cytokine. Whereas TNF alpha induced ICAM-1 upregulation after 2–4 h of stimulation, and IFN gamma required more than 24 h to induce ICAM-1 increases, increases in HLA class I expression were evident after 24 h of stimulation with either cytokine. IFN gamma was more potent in inducing class I upregulation than TNF and, in addition, IFN-gamma was a potent inducer of HLA class II on HDMEC. Like the upregulation of ICAM-1 induced by IFN-gamma, the induction of class II on HDMEC required greater than 24 h to become evident and showed continued increases when examined at 72 h.

The upregulation of cell surface epitopes by IL-1 alpha, TNF alpha, and IFN gamma were all inhibited by polyclonal antisera to specific cytokines. Specific antibody to IFN-gamma could completely abrogate the effects of IFN-gamma on class I and class II expression, as well as the upregulation of ICAM-1. Coincubation of IFN-gamma with either interferon alpha or interferon beta virtually eliminated the induction of class II on the surface of HDMEC as has been reported with HUVEC [6,11], but interestingly, had no effect on the upregulation of either ICAM-1 or class I. This suggests that the effect of IFN gamma on HDMEC is mediated through multiple distinct pathways that can be independently regulated. The relatively long lag time in the introduction of ICAM-1 by IFN-gamma is suggestive of the induction of a second messenger that is responsible for ICAM-1 upregulation. It is possible that this messenger may be IL-1 alpha or TNF alpha produced by the endothelial cells, but this is unlikely because specific antibody to these cytokines in the presence of IFN gamma had no effect on ICAM-1. Furthermore, similar experiments on large-vessel endothelial cells have found that IFN gamma stimulation does not result in increase in IL-1 mRNA [5].

TGF beta has been shown to inhibit the increased adherence of neutrophils to TNF-alpha treated HUVEC [29]. It has been suggested that this effect may be due to inhibition of TNF alpha-mediated ICAM-1 upregulation. We have examined this on both HUVEC and HDMEC and found that TGF beta did not reduce baseline ICAM-1 expression, and had no effect upon either IL-1 alpha, TNF alpha, or IFN-gamma upregulation of ICAM-1. Additionally, TGF beta did not affect upregulation of HLA class I or induction of HLA-DR by IFN gamma. The upregulation of ICAM-1 by IL-1 alpha or TNF alpha was also unaffected by pre-treatment of HDMEC with dexamethasone. Although decrease in ICAM-1 expression has been reported after corticosteroid treatment in certain cultured cell lines [30], this does not appear to be relevant to its expression on endothelial cells.

We also examined the expression of these cell surface epitopes on cells that have undergone morphologic differentiation on the basement membrane substrate matrigel. Endothelial cells that have formed capillary-like tubes demonstrated no significant alteration in the expression of cell surface epitopes when examined by flow cytometric analysis. Additionally, these cells retained their responsiveness to the cytokines IL-1 alpha, TNF alpha, and IFN gamma.
This indicates that endothelial cells grown in monolayers are appropriate models for the study of the induction and regulation of cell adhesion molecules, and that the use of morphologically differentiated cells for these purposes confers no particular advantages.

In summary, IL-1 alpha, TNF alpha, and IFN gamma modulate the expression of ICAM-1 and MHC proteins on HDMEC. Although the expression and regulation of these proteins appears to be similar to what has been described on large-vein endothelial cells, HDMEC are distinctly different in regards to baseline expression of ICAM-1, and the magnitude of their response to IL-1 alpha, TNF alpha, and IFN gamma. Pretreatment of HDMEC with TGF-beta or dexamethasone, or morphologic differentiation on matrigel did not affect baseline expression or cytokine upregulation of ICAM-1 or MHC proteins. IFN beta or alpha inhibited IFN gamma-induced HLA-DR expression on HDMEC, but did not affect IFN gamma upregulation of ICAM-1 or class I. These data demonstrate the complexity of cytokine-modulated regulation of HDMEC cell surface proteins and provide further evidence demonstrating distinct differences between large- and small-vein endothelium.

REFERENCES