Cell Adhesion Molecule Expression in Cultured Human Iris Endothelial Cells

Matthew D. Silverman,[1,2,3] *David O. Zamora,*[1,2,3] *Yuzhen Pan,*[1] *Paul V. Texeira,*[1] *Stephen R. Planck,*[1,2,4] *and James T. Rosenbaum*[1,2,4]

Purpose. To develop a method to isolate human iris microvascular endothelial cells (HIECs) for exploring their constitutive and inflammatory agent-modulated expression of intercellular adhesion molecules (ICAM)-1 and -2, vascular cell adhesion molecule (VCAM)-1, and E-selectin.

Methods. Endothelial cells from collagenase-digested irises were isolated on the basis of their expression of platelet endothelial cell adhesion molecule (PECAM)-1, using antibody-coupled magnetic beads. Cells were characterized as endothelial based on morphologic criteria, their expression of PECAM-1 and von Willebrand factor, their uptake of acetylated low-density lipoprotein, and their ability to form capillary-like networks on a synthetic basement membrane. Constitutive and inflammatory agent–modulated expression of ICAM-1 and -2, VCAM-1, and E-selectin was evaluated by the reverse transcription–polymerase chain reaction, enzyme-linked immunocellular assays (ELICAs), Western blot analysis, and functional studies of leukocyte adhesion to HIEC monolayers.

Results. HIECs constitutively expressed mRNA and protein for ICAM-1 and -2, but only low to nondetectable levels of VCAM-1 or E-selectin. When stimulated with endotoxin- or tumor necrosis factor (TNF)-α, ICAM-1, VCAM-1, and E-selectin were potently and time- and dose-dependently upregulated at both the message and protein levels. By contrast, ICAM-2 message and protein were slowly downregulated by inflammatory agents over time, but nonetheless remained present and functional. Overall, cytokine- or endotoxin-activation of HIECs resulted in enhanced adhesiveness for leukocytes.

Conclusions. ICAM-1, VCAM-1, and E-selectin have been previously implicated in mediating anterior ocular inflammation. This is a report of the selective isolation of HIECs, with a demonstration of differential expression and regulation of these adhesion molecules in them. In addition, this is the first demonstration of the regulated expression of ICAM-2 in any ocular microvascular cells. (*Invest Ophthalmol Vis Sci.* 2001; 42:2861–2866)

The molecular mechanisms responsible for the tissue-specific inflammation in anterior uveitis (AU) are incompletely understood. AU is often associated with systemic illnesses, such as ankylosing spondylitis, inflammatory bowel disease, Behçet's disease, juvenile rheumatoid arthritis, reactive arthritis, or sarcoidosis.[1,2] However, AU may also arise in an idiopathic fashion without an associated systemic disease, or it may be secondary to a localized infection, such as herpes simplex virus.[3] Regardless of its origin, the mechanism for triggering an inflammatory response specifically in the iris, while often sparing other ocular tissues, remains unknown. Recently, much emphasis has been placed on understanding how cells in the bloodstream preferentially penetrate specific tissues (e.g., in leukocyte and stem cell migration and in cancer cell metastasis).[4] In AU, transmigration of leukocytes into the anterior chamber requires that endothelial cells (ECs) and the leukocytes within the iris's microcirculation become activated and then physically approximated. These leukocytes can then leave the bloodstream and migrate through the iris stroma, often into the anterior chamber. Although leukocytes are vital cells in the pathogenesis of inflammation, the essential role of the iris microvascular ECs, the gatekeepers in AU inflammatory processes, has received little attention.

Given the limitations of investigating the molecular mechanisms of AU directly in human subjects, a variety of animal models of ocular inflammation have been developed, including endotoxin-induced uveitis (EIU), experimental autoimmune uveoretinitis (EAU), experimental melanin-induced uveitis, and experimental autoimmune encephalomyelitis.[1,2] These models attempt to mimic human disease and have provided important insights into the cause of AU. Nonetheless, species differences often make it difficult to translate the findings directly from these animal studies over to the human condition. Tissue culture systems provide another means to investigate the biology of ocular inflammation, and others have previously isolated and cultured human choroid and retinal ECs for this purpose.[5,6] Functional heterogeneity is known to exist among cultured ECs derived from the microvasculature of different, often proximally situated, tissues.[7–10] Thus, to evaluate microvascular involvement in AU in an in vitro system, we developed a methodology to selectively culture human iris endothelial cells (HIECs).

In this report we provide the initial characterization of cultured HIECs and describe the constitutive and inflammatory agent-regulated expression of several cell adhesion molecules (CAMs) in HIECs. These molecules are likely key mediators of the leukocyte infiltration observed in AU. Leukocyte adhesion assays suggest that in addition to intercellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1, both of which have been previously implicated in ocular inflammation, ICAM-2 also has the potential to play an important role in leukocyte extravasation through the iris microvascular wall in uveitis.

From the [1]Department of Ophthalmology, Casey Eye Institute, and Departments of [2]Cell and Developmental Biology and [4]Medicine, Oregon Health Sciences University, Portland.

[3]Contributed equal effort to this study.

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Corresponding author: Stephen R. Planck, Oregon Health Sciences University, Mail Code CEI-RES, 3375 SW Terwilliger Boulevard, Portland, OR 97201-4197. plancks@ohsu.edu

METHODS

Iris Endothelial Cell Cultures

All usage of human tissues and cells in this study was in accordance with institutional review board–approved protocols. Irises from anonymously donated human eyes (Lions Eye Bank, Portland, OR; donor age range 16–42; no known history of ocular or cardiovascular disease), were digested in 0.2% type II collagenase (Sigma Chemical Co., St. Louis, MO) in cell culture medium (MCDB-131; Clonetics/BioWhittaker, Walkersville, MD) for 20 to 30 minutes at 37°C, after the iris pigment epithelial layer was mechanically removed with a cotton swab. After digestion, ECs were purified from iris stromal cells, by using monoclonal anti-human platelet-endothelial cell adhesion molecule (PECAM)-1 antibody-coated magnetic beads (Dynal, Inc., Lake Success, NY), and were cultured in medium MCDB-131 supplemented with 10% fetal bovine serum (FBS; Invitrogen Corp., Carlsbad, CA), endothelial cell growth factors (EGM-MV2 BulletKit, with hydrocortisone omitted; Clonetics/BioWhittaker; complete medium), gentamicin (10 μg/ml), and amphotericin-B (250 ng/ml; Fungizone, Invitrogen Corp.). Cultures were trypsin passaged at a 1:3 split ratio and used in subsequent experiments between passages 3 and 6.

Confirmation of the EC Nature of Cultured Cells

After one or two rounds of magnetic separation, cultures were 99.5% or more pure on the basis of PECAM-1 and von Willebrand factor (vWF) expression,[7,11] and uptake of 1,1′-dioctacecyl-1,3,3,3′,3-tetramethylindocarbocyanine perchlorate acetylated-LDL (DiI-Ac-LDL).[12] In specialized culture conditions with a provisional extracellular matrix, monolayers of ECs are capable of reorganizing into capillary-like networks.[13] HIECs (75,000) were plated on polymerized synthetic basement membrane (14.1 mg/ml, 200 μl/well; Matrigel; BD Biosciences, Bedford, MA) within 24-well tissue culture plates, and were allowed to attach overnight. Cells were then refed, with some wells receiving 10 ng/ml phorbol myristate acetate (PMA; Sigma) to induce tube formation.[14]

RT-PCR Analysis of Adhesion Molecule mRNA Expression

Confluent HIEC monolayers were stimulated for up to 21 hours with either lipopolysaccharide (LPS, 10 μg/ml, from *Escherichia coli* 055:B5; List Biological Laboratories) or recombinant human tumor necrosis factor-alpha (TNFα, 10 ng/ml; R&D Systems, Minneapolis, MN). Total RNA was extracted using a commercial kit (RNApure; GenHunter Corp., Nashville, TN). Touchdown RT-PCR detection of gene expression was performed as previously described in detail.[15] Positive control total RNA was obtained from synovial samples from patients with rheumatoid arthritis. Diethyl pyrocarbonate-treated water (DEPC-H_2O; Ambion, Inc., Austin, TX) was used as a negative control. Human ICAM-1 primer sets (sense, 5′-CCGGAAGGTGTATGAACTG-3′; antisense, 5′-TCCATGGTGATCTCTCCTC-3′), ICAM-2 primer sets (sense, 5′-CCGTGGCAATGAGACTCTGCACTA-3′; antisense, 5′-ATGGTTGCTATGGCCGGAAGG-3′), and VCAM-1 primer sets (sense, 5′-CTCCGTCTCATTGACTTGC-3′; antisense, 5′GAACAGGTCATGGTCACAG-3′, all from Operon Technologies, Alameda, CA) were used to probe cDNAs reverse transcribed from the experimental, positive, and negative control RNA samples. A primer pair for cyclophilin was included in each assay as an internal control (sense, 5′-TGTTCTTCGACATTGCCGTCGAC-3′; antisense, 5′-GCATTTGCCATGGACAAGATGCCAGGA-3′; Operon). PCR reaction products were electrophoresed in 3% agarose gels in Tris-acetate buffer containing ethidium bromide, and UV-induced fluorescent bands were photographed and digitized.

Protein Analysis

CAM protein expression on intact monolayers of HIECs was evaluated using a fluorescence enzyme-linked immunocellular assay (ELICA) in a 96-well microtiter plate format, as previously described in detail.[16] Mouse monoclonal anti-human ICAM-1 antibody (Clone W-CAM-1; Neomarkers, Inc., Fremont, CA) rabbit polyclonal anti-ICAM-2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA), monoclonal anti-human VCAM-1 antibody (clone BBIG-V1-4B2; R&D Systems), and monoclonal anti-human E-selectin antibody (clone BBIG-E4-5D11; R&D Systems) were used as primary detection antibodies (all diluted to 1 μg/ml in 1% bovine serum albumin-bicarbonate-buffered saline). Affinity-purified, alkaline phosphatase-conjugated goat anti-mouse or anti-rabbit polyclonal antibodies (1:1000; Sigma) were used for secondary detection. Methylumbelliferyl phosphate (100 μg/ml; Sigma) was used as the fluorescence detection substrate. In some cases, ELICA results were confirmed by Western blot analysis in which HIEC lysates (3×10^4 cells/lane) were electrophoresed on 4% to 15% linear gradient SDS-PAGE gels, followed by transfer to nitrocellulose and protein detection with the same antibodies used in the ELICA. Bands were visualized using an nitro blue tetrazolium chloride (NBT)/5-bromo-4-chloro-3-indoyl phosphate (BCIP) detection kit (Vector Laboratories, Burlingame, CA), and images were digitized.

Leukocyte Adhesion Studies

To explore the utility of our cell culture as an in vitro model of ocular inflammation, we tested the ability of activated HIEC cultures to bind U937 monocytes (American Type Culture Collection [ATCC], Rockville, MD). HIECs were grown to confluence in 48-well plates and were then stimulated with LPS (10 μg/ml), TNFα (10 ng/ml) or vascular endothelial growth factor (VEGF; 100 ng/ml, R&D Systems) for 12 hours. In some cases, LPS-stimulated HIECs were incubated with anti-ICAM-1 mAb (20 μg/ml), anti-ICAM-2 pAb (10 μg/ml), or anti-VCAM-1 mAb (10 μg/ml, Clone 1G11.B1; Neomarkers Inc.) for the last 30 minutes before monocyte addition. All stimulations and antibody incubations were performed in complete medium at 37°C. After EC treatments, the wells were aspirated, quickly washed with medium MCDB-131 with 10% FBS, and 500 μl of the same medium containing 2.5×10^5 monocytes was added to each well and incubated for 15 minutes at 37°C. After unbound monocytes were gently washed off, the EC monolayers and adherent monocytes were briefly fixed with 0.5% glutaraldehyde, and attached monocytes were visually counted (four 1-mm^2 regions per well).

Statistical Analysis

Data presented for ELICA and monocyte adhesion assays represent mean fluorescence per well and monocytes bound per square millimeter of HIEC monolayer surface area, respectively, ± SD for the indicated number of replicates. An asterisk in the figures indicates a significant difference ($P < 0.05$) between the means of experimental and respective control groups, by ANOVA (SigmaStat, ver. 2.0 software; SPSS Science, Chicago, IL).

RESULTS

Establishment of HIEC Cultures

Initial digestion of iris tissue yielded a mixed cell population that included cells with morphologies characteristic of ECs, fibroblasts, smooth muscle cells, pigment epithelial cells, and pericytes. After a single PECAM-1–based magnetic bead separation, we were able to significantly reduce the number of contaminating cells. We have consistently been able to generate, within 2 weeks, essentially pure experiment-ready HIEC cultures. To date, we have been successful with cells from more than 30 donors. The monolayers thus generated are 99.5% pure or more, when assessed by their cobblestone morphology (Fig. 1A) and by biochemical criteria (detailed later). The average number of HIECs initially isolated from an iris pair is approximately 50,000, and these can typically be expanded in culture to approximately 3.0×10^6 cells (i.e., six to seven population doublings), without any detectable

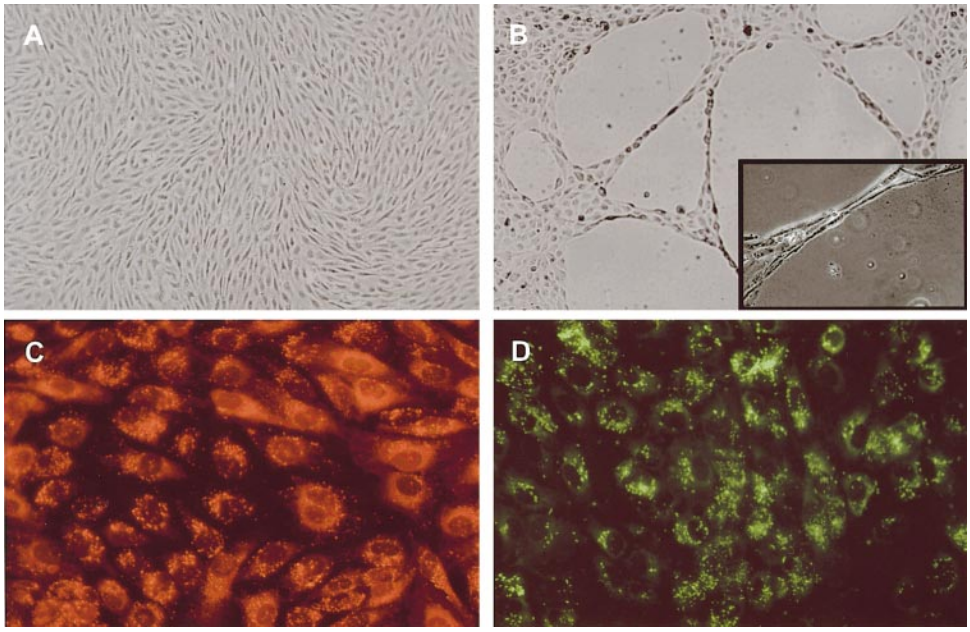

FIGURE 1. Phase-contrast and fluorescence micrographs of purified HIECs. (**A**) A confluent HIEC monolayer grown on tissue culture plastic. (**B**) HIECs forming capillary-like networks when grown atop a three-dimensional synthetic matrix and stimulated with PMA (10 ng/ml) for 24 hours. Initial monolayer reorganization was apparent as early as 4 hours after stimulation. *Inset*: higher magnification view of apparent lumen formation within capillary-like cords. (**C**) Fluorescent microscopy demonstrates DiI-Ac-LDL uptake by these cells, and (**D**) positive immunoreactivity for vWF. Original magnifications: (**A**, **B**) ×25; (**B**, *inset*) ×100; (**C**, **D**) ×100.

changes in morphology or physiology (i.e., CAM expression and stimulability). In addition, these HIEC cultures are amenable to trypsin passaging and display expected survival rates (≈70%–80%) when recovered from cryopreservation.

Physiological Characterization of HIECs

We characterized iris PECAM-1$^+$ cells as ECs by several biologic criteria. The presence of functional LDL scavenger receptors on HIECs was demonstrated by the uptake of DiI-Ac-LDL (Fig. 1C). After 4 hours' incubation with fluorescently labeled DiI-Ac-LDL, punctate staining was observed intracytoplasmically throughout the entire EC monolayer. vWF is considered to be a classic marker of ECs, because it has been found only in ECs, megakaryocytes, and platelets, the latter two of which do not pose a culture contamination problem. Immunocytochemistry of HIEC monolayers demonstrated punctate vWF expression within the cytoplasm (Fig. 1D). Although vWF immunostaining intensity varied from cell to cell, more than 99% of cells stained positively. Last, in vitro, ECs have the ability to form capillary-like networks when grown on three-dimensional matrices under specialized conditions.[13] This characteristic has been used to differentiate between cells that morphologically and biochemically appear to be ECs.[17] HIECs isolated by our methods indeed formed capillary-like structures in vitro when grown on a three-dimensional matrix (Matrigel; BD Biosciences) and stimulated with PMA. Confluent monolayers of HIECs atop this matrix quickly reorganized themselves to form tube-like structures (Fig. 1B), with reorganization apparent as early as 4 hours after addition of PMA.

CAM mRNA Expression in HIECs

By RT-PCR, cultured HIECs were tested for steady state mRNA expression of various CAMs in response to the inflammatory mediators LPS and/or TNF-α. Unstimulated HIECs constitutively expressed low but detectable levels of ICAM-1 message (Fig. 2). Stimulation with 10 μg/ml LPS caused a time-dependent upregulation of ICAM-1 mRNA—an effect obvious at 1.5 hours after exposure, maximal at approximately 3 to 5 hours, and maintained through at least 21 hours of continuous stimulation. Similar kinetics of ICAM-1 upregulation were observed when HIECs were stimulated with 10 ng/ml TNFα or with 10 ng/ml IL-1α (not shown). We also evaluated ICAM-2 mRNA expression in these cells by RT-PCR (Fig. 2). We detected significant constitutively expressed ICAM-2 mRNA in HIECs, which was not measurably altered by inflammatory agent stimulation. Only barely detectable VCAM-1 signals (Fig. 2) were seen in unstimulated HIECs. After activation with LPS or TNFα,

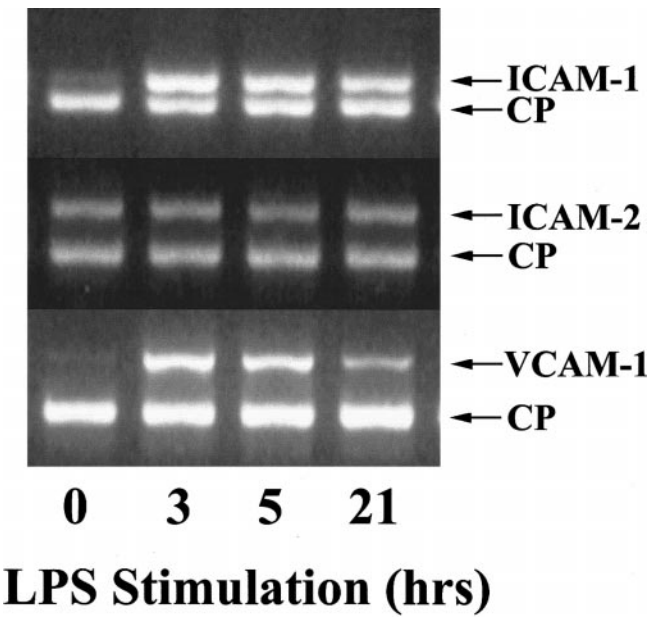

FIGURE 2. RT-PCR analysis of ICAM-1 and -2 and VCAM-1 mRNA in LPS-stimulated HIEC cultures. HIEC cultures were stimulated for up to 21 hours with 10 μg/ml LPS. Although ICAM-1 and VCAM-1 mRNA levels significantly increased with LPS-stimulation, we did not detect any change in ICAM-2 mRNA expression under the same conditions by RT-PCR. ICAM-1 and -2, VCAM-1, and cyclophilin (CP; internal control) amplicons displayed respective sizes of 319, 386, 618, and 292 bp. Shown are data from one representative experiment of three each for ICAM-1 and ICAM-2 and two for VCAM-1.

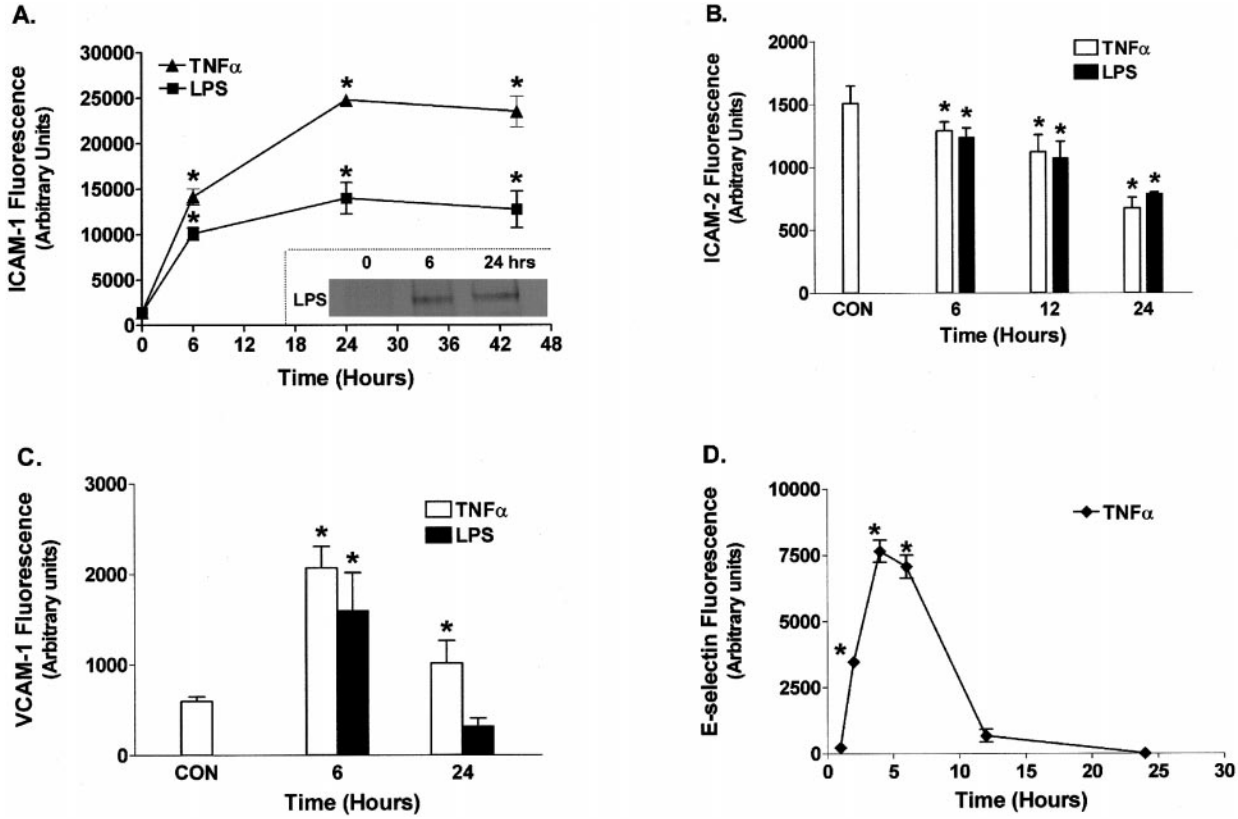

FIGURE 3. LPS and TNFα modulate adhesion molecule protein expression in HIECs. Cell surface expression of CAMs was measured in LPS-stimulated (10 μg/ml) and TNFα-stimulated (10 ng/ml) HIEC monolayers using a fluorescence ELICA. (**A**) Constitutively detectable ICAM-1 was upregulated as early as 4 hours after stimulation, with maximal protein levels attained between 8 to 12 hours, and maintained through 48 hours of exposure. *Inset*: Western blot analysis of cell lysates (≈3 × 10^4 cells/lane) from HIECs that had been stimulated with 10 μg/ml LPS for up to 24 hours confirmed upregulation of ICAM-1 protein. The band's molecular weight is approximately 95 kDa. (**B**) Constitutively expressed (CON) ICAM-2 protein in HIECs, unlike the other CAMs measured, was downregulated by LPS and TNFα stimulation (10 μg/ml and 10 ng/ml, respectively). (**C**) Cultured HIECs expressed very low levels of VCAM-1 protein in the absence of stimulation (CON). VCAM-1 expression was markedly upregulated by LPS and TNFα. Levels were maximal after 6 hours of stimulation and had begun to decline toward baseline by 24 hours. (**D**) E-selectin expression was not detectable in unstimulated HIECs, but was rapidly upregulated in response to LPS and TNFα. Levels were maximal after 4 hours stimulation and decayed to nearly to baseline by 12 hours. ELICA data are from one representative experiment of five for ICAM-1, three for ICAM-2, and two for VCAM-1 and E-selectin, each condition having been conducted in three to six wells per experiment. Data represent mean fluorescence ± SD. Western analysis data are from one representative experiment of two. $^*P < 0.05$ versus unstimulated controls, by ANOVA.

however, there was a rapid upregulation of VCAM-1 message that declined after 21 hours of stimulation.

CAM Protein Expression in HIECs

In line with the mRNA data, using an ELICA, we measured a low but detectable constitutive expression of ICAM-1 protein on HIEC cell surfaces, which was markedly elevated by both LPS and TNFα stimulation (Fig. 3A). Upregulated ICAM-1 reached maximal levels after 8 to 12 hours of LPS or TNFα stimulation and remained maximally elevated through at least 2 days of continued stimulation. ICAM-1 upregulation by LPS was confirmed by Western blot analysis of HIEC lysates (Fig. 3A, inset). We also detected significant constitutive expression of ICAM-2 protein in HIECs (Fig. 3B), which, conversely, was decreased in TNFα- and LPS-stimulated HIECs. This trend appeared as early as 6 hours after stimulation and by 24 hours had culminated in a significant 50% decrease in immunodetectable ICAM-2 on HIEC surfaces. Unlike the clear constitutive expression of ICAM-1 and -2, only very low levels of VCAM-1 and no E-selectin protein were detected on unstimulated HIECs. In response to either LPS or TNFα stimulation, however, VCAM-1 protein was markedly elevated by 6 hours (Fig. 3C). After 24 hours of stimulation, VCAM-1 in TNFα–stimulated ECs had begun declining toward baseline but was still significantly elevated compared with levels in unstimulated control cultures. After 24 hours of stimulation with LPS, VCAM-1 protein had already decayed to control levels (Fig. 3C), in good temporal concordance with the declining VCAM mRNA signal observed in these cells. In the case of E-selectin, upregulation by LPS was rapid, with maximal protein levels detected on HIEC surfaces after only 4 hours' stimulation (Fig. 3D). This was followed by a similarly rapid decline to control levels after only 12 hours.

Leukocyte Adhesion to HIECs

We next investigated whether cultured HIEC monolayers would become more adherent for leukocytes when activated by inflammatory stimuli. When HIEC monolayers were stimulated with LPS (10 μg/ml, 12 hours), subsequent monocyte adhesion increased almost 10-fold (Fig. 4). Similarly, HIEC stimulation with VEGF (100 ng/ml, 12 hours) resulted in approximately threefold increases in monocyte adhesiveness. Preincubation of LPS-stimulated HIECs with blocking antibodies against the cell adhesion molecules ICAM-1 and -2 and VCAM-1 resulted in respective 40%, 40%, and 20% decreases in monocyte binding, demonstrating the in vitro functioning of multiple adhesion molecules in these activated HIECs.

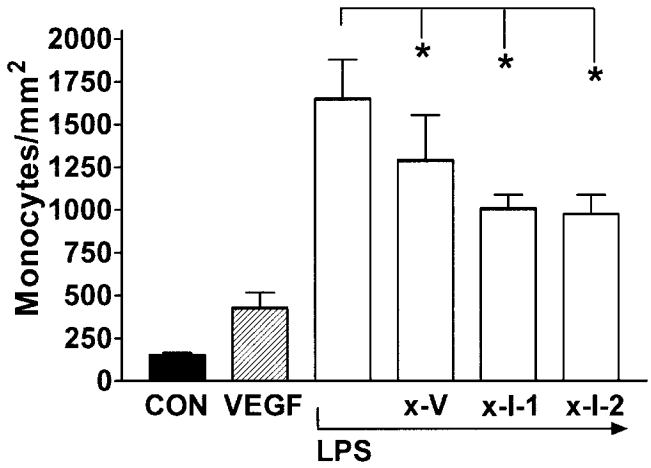

FIGURE 4. Monocyte adhesion to HIECs. Confluent monolayers of HIECs were stimulated with either VEGF (100 ng/ml) or LPS (10 μg/ml) for 12 hours, followed by the addition of U937 monocytes for 15 minutes. Unstimulated HIECs served as controls (CON). Stimulation with VEGF and LPS resulted in significant 3- and 10-fold increases in monocyte adhesion, respectively ($P < 0.01$ by ANOVA, in both cases). Anti-VCAM-1 (x-V) or anti-ICAM-1 (x-I-1) or -2 (x-I-2) neutralizing antibodies were added to some LPS-stimulated wells before monocyte incubations. All experimental variables were conducted in triplicate or quadruplicate wells per condition. Data represent mean monocytes bound per square millimeter of HIEC monolayer surface area ± SD and are presented for one of three representative experiments. *Significant inhibition of monocyte attachment ($P < 0.05$) versus LPS-stimulated HIECs.

DISCUSSION

Microvascular ECs play key regulatory roles in the initiation and progression of inflammation.[4,12] The pathologic sequelae of AU are often limited to specific tissues (i.e., iris and ciliary body), whereas neighboring tissues (e.g., retina, choroid, and sclera) are spared, supporting the likely existence of functional heterogeneity among the vasculatures of these disparate tissues. That ECs from diverse anatomic sites can differ markedly in form and function is now well appreciated. Such endothelial heterogeneity has been firmly established both by in vivo work and in studies in cultured ECs.[7–10,16] In ocular diseases, for example, cytomegalovirus preferentially infects the posterior eye, causing retinitis, whereas anterior structures are typically unaffected.[18] In addition, intraocular lymphomas also appear to selectively target the retina and not anterior eye tissues.[19] In appreciation of the likely existence of tissue-specific gene expression in the microvasculature of different ocular tissues, we endeavored to establish pure HIEC cultures to investigate some of the molecular mechanisms used in AU pathogenesis. Although other researchers have cultured and experimented with ocular ECs from human retina and choroid,[5,6] this is the first report characterizing pure microvascular ECs isolated from human irises.

We evaluated in HIECs the expression of three adhesion molecules of the immunoglobulin superfamily (ICAM-1 and -2 and VCAM-1), and one member of the selectin family (E-selectin), all of which are known to support leukocyte adhesion. Consistent with previous demonstrations in other EC types,[16] these cells display a low but measurable constitutive expression of ICAM-1 that is markedly upregulated at both the mRNA and the protein levels by stimulation with either LPS or, more potently, by TNFα. By contrast, constitutively expressed ICAM-2 protein is markedly downregulated by the same inflammatory stimuli over the same period. That we did not detect a coordinate decrease in ICAM-2 mRNA levels using a multiplex RT-PCR assay was probably due to the semiquantitative nature of this method, because in preliminary experiments using gene array hybridization assays we saw an approximate 50% decline in ICAM-2 signal strength after 24 hours of LPS stimulation.[20] Although this level of change approaches the sensitivity limits of this assay (Atlas Arrays; Clontech), it suggests that ICAM-2 mRNA declines after EC activation. By contrast to the clear constitutive expression of ICAM-1 and -2, negligible VCAM-1 and no E-selectin expression were detectable in unstimulated HIECs, at both the mRNA and protein levels. As with ICAM-1, both VCAM-1 and E-selectin are potently upregulated in iris ECs when activated by inflammatory stimuli, in line with their previously suspected roles in uveitis.[21–26] Unlike the persistent ICAM-1 elevation, VCAM-1 and E-selectin upregulation is transient.

The activated phenotype of HIECs was reflected at the functional level, wherein LPS-stimulated HIECs showed a significantly increased adhesiveness for U937 monocytes. This effect was in part blocked by the preincubation of HIECs with neutralizing antibodies against various adhesion molecules (i.e., ICAM-1 and -2 and VCAM-1). This is consistent with a demonstrated role for EC-expressed ICAM-1 in mediating ocular inflammation in both animal models and clinical investigations,[15,21–25] and suggests that these cultured HIECs can qualitatively mimic the in vivo scenario. Previously, immunohistology performed on iris biopsy specimens from patients with either acute or chronic AU has revealed upregulated expression of ICAM-1 and VCAM-1 on human iris microvascular ECs, implicating both adhesion molecules in the increased leukocyte flux through the iris in AU.[24–26] Our current data support this likelihood.

To date, this is the first report of an investigation of the role of ICAM-2 in ocular inflammation. Although ICAM-2 was appreciably downregulated in activated HIECs, as has been reported in TNFα- and IL-1β–stimulated umbilical vein ECs,[27] we observed that it nonetheless remained present in sufficient amounts to significantly mediate the binding of leukocytes to these vascular cells. Endothelial ICAM-2 is known to support the adhesion of a variety of leukocyte subsets to ECs, by acting as a ligand for the leukocyte-specific β_2 integrins, LFA-1 and Mac-1,[28,29] and appears to be essential for T-cell transendothelial migration. Of note, peptide fragments of ICAM-2 and soluble ICAM-2/Fc chimeras can bind to and rapidly enhance the affinity of these leukocyte integrins for both ICAM-1 and -2, resulting in a feed-forward enhancement of adhesion.[30] Soluble ICAM-1 is present in normal human plasma in nanogram-per-milliliter quantities, and circulating levels are significantly increased in a variety of inflammatory disorders, including uveitis.[31,32]

In addition, soluble adhesion molecules, including ICAM-1, are elevated in the aqueous humor of eyes with uveitis[32] and in the vitreous of eyes with proliferative diabetic retinopathy and proliferative vitreoretinopathy.[33] Very recently, soluble ICAM-2 has been detected in the bloodstream of normal humans and patients with leukemia, with concentrations significantly lowered after chemotherapeutic reduction of circulating leukemic cells (Gahmberg et al. personal communication, 2001). Although currently speculative, if cleavage or shedding of membrane ICAM-2 accompanies its downregulation in activated HIECs, the resultant increased local levels of soluble ICAM-2 may perpetuate inflammation in the iris by activating integrins on nearby leukocytes.[30] Taken together with prior experimental observations, our current data suggest a potentially important role for ICAM-2 in mediating leukocyte infiltration in uveitis and warrant further experimentation to this end.

Endothelial expression of various chemokines, cytokines, and cell adhesion molecules are critical determinants in the development of the inflammation in AU. Determination of the

EC expression profiles of these molecules and their relative importance in clinical uveitis is difficult because experimental analysis of patients with AU is limited to small samples of aqueous humor or iris biopsy specimens obtained from those undergoing ocular surgery. In addition, anti-inflammatory pharmacologic regimens are often under way in these patients and can complicate interpretation of findings. To circumvent these limitations while still working in a relevant human model, cultured HIECs provide a versatile means to expand our understanding of the mechanistic origins of AU. Although cell culture systems are not without inherent experimental limitations, this approach complements the existing animal models and clinical investigations in together elucidating the molecular mechanisms of uveitis.

Acknowledgments

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