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Original Article

Effect of Ultraviolet Light on the Expression of Adhesion Molecules and T Lymphocyte Adhesion to Human Dermal Microvascular Endothelial Cells

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In order to determine the effect of ultraviolet radiation (UVR) on the cell adhesion molecules expressed in human dermal microvascular endothelial cells (HDMEC), the cells were exposed to varying UVR doses and the cell surface was examined for expression of intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin. The effect of UVB irradiation on the binding of T lymphocytes to HDMEC was also examined.

UVA irradiation did not affect the surface expression of ICAM-1, VCAM-1, or E-selectin on the HDMEC. However, following UVB exposure, ELISA demonstrated a significant increase in the baseline ICAM-1 cell surface expression on the HDMEC. However, no induction of either E-selectin or VCAM-1 was noted. UVB also significantly augmented ICAM-1 induction by IL-1α and TNF-α. VCAM-1 was induced by stimulating HDMEC with IL-1α following a UVB irradiation dose of 100 mJ/cm^2. Flow cytometric analysis of the HDMEC stimulated with IL-1α for 24h demonstrated that 12% of the cells expressed VCAM-1 but either IL-1α or UVB irradiation alone failed to induce VCAM-1 expression. Enhancement of T cell-HDMEC binding by IL-1α or TNF-α treatment was not significantly affected after UVB irradiation. This study demonstrated that UVB irradiation can alter ICAM-1 and VCAM-1 expression on the HDMEC surface and that augmentation of ICAM-1 expression and the IL-1α -dependent induction of VCAM-1 following UVB exposure might be important steps in the pathogenesis of sunburn.

Key Words: HDMEC, ultraviolet radiation, adhesion molecules, intercellular adhesion molecule-1, E-selectin, vascular cell adhesion molecule-1

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INTRODUCTION

Vascular endothelial cells line the innermost layer of the blood vessels and are therefore essential cells in leukocyte migration, homing, and the inflammatory process. In order for a circulating leukocyte to migrate from inside a blood vessel to an inflammation site, it needs to adhere to the endothelial cells. The adherence is altered by exposing leukocytes or endothelial cells to biological response modifiers (BRM)[1] and exposing endothelial cells to either interleukin-1 (IL-1), tumor necrosis factor (TNF), or lipopolysaccharide (LPS) either induces or stimulates the adherence of neutrophils, mononuclear cells, lymphocytes, basophils, or eosinophils.[2-5]

This process is mediated by the cell adhesion molecules[6] and recent advances in this area have led to the identification of at least 3 adhesion molecules, i.e., the intercellular adhesion molecule-1 (ICAM-1), the vascular cell adhesion molecule-1 (VCAM-1), and E-selectin.[7-11] Surface expression of these adhesion molecules is modulated by stimulation with BRM[8,12,13] and this modulation plays an important role in regulating the inflammatory and immunologic reactions, and tumor metastasis.[8] Research in this area has mostly been carried out using human umbilical vein endothelial cells (HUVECs) despite the fact that most pathophysiological phenomena take place at the microvascular levels. However, not many studies using human dermal microvascular endothelial cells (HDMEC) have been reported due to difficulties in separating and culturing the cells. It is known that microvessels constitute the

main part of the human vasculature and that the nutritious growth condition, immunologic phenotype and regulation of cell surface antigens, secretion of chemical mediators, and even regulation of tumor cell adhesion by BRM differ between the microvascular and the large vessel endothelial cells.[14-19] Moreover, tissue specific stimulation and reactions, which are important in the local pathophysiologic process, are different among the endothelial cells originating from different organs.[20,21] Therefore, it is most ideal to use the HDMEC to investigate cell adhesion in a cutaneous inflammation.

Ultraviolet radiation (UVR) is known to induce cell membrane changes, alter the cell surface antigens, interfere with the cell-to-cell interaction and antigen introduction, and also to regulate cytokine secretion.[22] UVB irradiation induces ICAM-1 expression and functional changes in the peripheral blood mononuclear cells and keratinocytes by causing intramembranous changes.[23-26] The effect of UVR on epidermal cells has been widely investigated but its effect on microvascular endothelial cells, which is the key player in dermal inflammatory and immunologic reactions, is not well known. Therefore, the enzyme-linked immunosorbent assay (ELISA), immunofluorescence flow cytometry, and T lymphocyte adherence assay were employed to assess the effect of UVR on the expression of adhesion molecules on the HDMEC.

MATERIALS AND METHODS

Isolation and culture of HDMEC

The HDMEC were isolated from human neonatal foreskins by trypsinization and percoll gradient centrifugation as previously described.[27] The cells were cultured in endothelial basal media (Clonetics Corp., San Diago, CA, USA) with epidermal growth factor 5 ng/ml (Clonetics), hydrocortisone acetate 1 μg/ml (Sigma Chemical Co., St. Louis, MD, USA), dibutyryl cyclic AMP 5×10^{-5}M (Sigma), penicillin 100 μU/ml, streptomycin 100 μg/ml (Sigma), and 30% human serum (Irvine Scientific, Santa Ana, CA, USA). The resulting cell cultures were 100% pure, as assessed by the morphologic and immunohistochemical criteria. Experiments were conducted with the endothelial cells at passages 2 - 6.

Antibodies

Monoclonal antibodies (Mab) 4B9 recognizing VCAM-1 was obtained as a gift of Dr. J. Harlan (University of Washington, Seattle, WA, USA). MAb 3B7 recognizing E-selectin was a generous gift of Dr. Walter Newman (Otsuka Pharmaceuticals, Rockville, MD, USA). MAb CD54 (anti-ICAM-1) was purchased from Amac Inc. (Westbrook, ME, USA). MAb W6/32 (anti-HLA class I), CD8 (Leu 2), CD14 (Leu M3), CD19 (Leu12), CD 11c (Leu-M5), and anti-HLA-DR were purchased from Becton-Dickinson (Mountain View, CA, USA) MAb Mo-1 (Mac-1) was purchased from Coulter Immunology (Hialeah, FL, USA) and other anti-HLA-DR were purchased from Serotec (Oxford, England).

UVR treatment

HDMEC were exposed in vitro to UVR either before or after BRM stimulation. Sellas UVA (Sellmeier Co., Gevelaberg, Germany) lamps were used as the UVA radiation source. The energy emission spectrum from these lamps ranged between 320 and 400 nm with peak wavelength of 365 nm. At a 20 cm lamp to target distance, the energy flux from these lamps was 50 mw/cm^2. To eliminate the UVB wavelengths, UVA radiation was filtered through a Mylar sheet. The output of the filtered light source was measured using an IL442 UV spectro-radiometer system (International Light, Inc., Newburyport, MA, USA) using a SEE 015 detector, which provides a measure of the irradiance for the integrated waveband between 320 - 400 nm. FS 72 T 12-UVB-HO lamps were used as the UVB light source. The energy emission spectrum from these lamps ranged between 290 and 325 nm with a peak wavelength of 301 and 312 nm. The UVB output was monitored by means of an IL 442A (Ultralight Enterprises Inc., Lawrenceville, CA, USA) radiometer and SEE 015 detector and was approximately 0.6 mW/cm^{2}at a 30 cm tube-to- target distance. The medium was removed from the cultures and replaced with a thin film of phos-

phate buffered saline without phenol red and then exposed to various UVR doses. In order to obtain a homogenously irradiated cell population, the culture dishes were gently shaken every 15 seconds during UVR exposure. The effect of UVR on CAM was investigated using two different protocols. The cells were irradiated, refilled with medium, and then analyzed for CAM expression after 24, 48, and 72 hours of incubation in order to examine the effect of UVR on CAM induction. In the second protocol, the same procedure was followed, but BRM was added to the refilled media after the UVR exposure to examine the effect of UVR on the CAM expression regulated by BRM.

ELISA measurement of UVR effects on the expression of adhesion molecules on HDMEC

The HDMEC were plated in 96 well plates and allowed to grow to confluence over 24 h. The cells were then stimulated with either the cell culture media alone or with IL-1 α (1-100 U/ml, Genzyme Corp, Boston, MA, USA), TNF-α (1 - 100 U/ml, Amgen Biologicals, Thousand Oaks, CA, USA) for 1 - 72 h at 37 ℃. A total of 100 μl of the monoclonal antibodies CD54, 4B9, or 3B7 (1 μg/ml) were added to each well and the plates were incubated at 37℃ for 1 h. After washing, 100 μl of peroxidase-conjugated goat anti-mouse IgG (Sigma), diluted 1 : 500 with Hank's balanced salt solution (HBSS) with divalent cations (Irvine) and 5% neonatal calf serum, was added to each well and the plates were incubated for 1 h. The plates were again washed and the level of antibody binding was quantified colorimetrically by adding tetramethylbenzidene (TMB, 1 mg/ml, Sigma). One ml of a 100 mg/ml TMB stock solution in acetone was added to 100 ml of distilled water. Ten microliters of 30% H_2O_2 was added immediately prior to use. The chromogenic reaction was stopped with 25 μl 8N H_2SO_4 and the plates were read spectrophotometrically at 450 nm on an ELISA reader (Dynatech Laboratories Inc., Alexandria, VA, USA).

Flow cytometric analysis

The HDMEC were grown to confluenceand subsequently removed by incubating with trypsin/EDTA (Biofluids, Rockville, MD, USA) and washing in PBS with 0.5% BSA. The cells were counted, and aliquotted for staining. The primary antibodies at the appropriate dilution were added to each tube. The cells were incubated for 30 min on ice, washed, and secondary antibodies diluted 1/20 in PBS with 0.5% BSA was added. After another 30 min incubation, the cells were washed, and resuspended in 0.5 ml PBS with 0.5% BSA. Propidium iodide was added immediately before flow cytometric analysis to identify the dead cells. The fluorescence level was examined on a Becton Dickinson FACStar flow cytometer and the values are expressed as the log mean channel fluorescence.

Separation of peripheral blood mononuclear cells and T lymphocytes

The pheripheral blood mononuclear cells (PBMC) from healthy donors were separated using Ficoll-Hypaque (LSM, Organon Technik Corp., Durham, NC, USA) density gradient centrifugation. The adherent mononuclear cells were separated from the nonadherent ones by adherence to plastic at 37℃ for 1 h. To dispose of all cells other than the T lymphocytes, selective killing with low tox baby rabbit complement (Cedarlane Lab., Westburg, NY, USA) and mAb CD19, CD11C, anti-Mo-1, anti- HLA-DR (Becton-Dickinson), and anti-HLA-DR (Serotec) was performed. A cocktail of complement and mAb was added to the cells and incubated at room temperature for 30 min. The pellet was resuspended in complete T cell media consisting of RPMI 1640 (Gibco), l-glutamine 2 mM, 2-mercaptoethanol 50 mM, penicillin 100U/ml, streptomycin 100 μg/ml, amphotericin B 250 μg/ml.

T lymphocyte-HDMEC adherence assay

The HDMEC were plated in gelatin-coated 96-well flat-bottomed culture plates. They were preincubated with either the cell culture media alone or with different BRM concentrations and times. The T lymphocytes were labeled with ^{51}Cr (ICN Biomedicals, Costa Mesa, CA, USA) by incubating 100 μCi per 10^6 cells for 2h at 37℃. They were washed, suspended to 4×10^6/ml in RPMI with 10% FBS and 100 μl of the cell suspension was

added to each well containing the HDMEC and incubated for 4 h. After incubation at 37℃, the plates were washed twice, 100 μl of 1% triton-X (Sigma) was added to each well, and the contents were harvested with a cotton swab and counted using a gamma counter. The percentage binding was calculated using the following equation.

$$\% \text{ T lymphocyte binding} = \frac{\text{adherent counts} - \text{background counts}}{\text{counts added per well} - \text{background counts}} \times 100$$

In some experiments, the HDMEC monolayers were preincubated with 100 μl of mAb for 45 min. One hundred μl of 10 μl/ml suspension of the purified antibodies were used. After preincubating with antibodies, an adherence assay was performed in the continuous presence of antibodies using the method as described above.

Statistical analysis

Statistical analysis of the data was done using a Mann Whitney test and a Krulkal-Wallis one-way analysis of variance in SigmaStat v 2.0 program. Statistical significance was determined at a level of $p < 0.05$.

RESULTS

Effect of UVA irradiation on the expression of HDMEC adhesion molecules

In order to assess the effect of UVA irradiation on VCAM-1, ICAM-1, and E-selectin expression in the HDMEC, their expression level in the cells either stimulated or not stimulated with IL-1 α or TNF-α following varying UVA doses. UVA irradiation did not affect the low constitutive ICAM-1 expression and failed to induce VCAM-1 or E-selectin expression in the non-stimulated HDMEC at 24 h following UVA exposure (Fig. 1). Although the data presented in Fig. 1 employed 10 J/cm^2 of UVA and was measured 24h later, similar results were obtained with a wide range of UVA doses (1 - 10 J/cm^2) and incubation times (4, 16, 24, 48, and 72 h) following UVA exposure (data not shown).

ICAM-1 and E-selectin expression in the HDMEC was higher 24h after treatment with IL-1 α and TNF-α, but only TNF-α induced VCAM-1 expression in the HDMEC, as has been previously reported.[8] UVA irradiation did not affect the cytokine-induced ICAM-1, VCAM-1 or E-selectin expression in the HDMEC that were exposed to 10 J/cm^2 of UVA then stimulated for 24 h with IL-1 α or TNF-α (Table 1).

Effect of UVB irradiation on the expression of HDMEC adhesion molecules

Following UVB exposure, ELISA analysis demonstrated a significant increase in the baseline ICAM-1 expression in the HDMEC, but no induction of either E-selectin or VCAM-1 was observed (data not shown). UVB significantly augmented ICAM-1 induction by IL-1 α or TNF-α at 24 h with 100 mJ/cm^2 of UVB (Fig. 2). Stimulating the HDMEC with IL-1 α did not induce significant VCAM-1 expression at multiple time

Table 1. Effect of UVA Irradiation on ICAM-1, VCAM-1, and E-selectin Expression on the HDMEC Stimulated with IL-1 and TNF-α

	Optical density at 450 nm					
	Unstimulated		IL-1 α treated		TNF-α treated	
	UVA 0 J/cm^2	UVA 10 J/cm^2	UVA 0 J/cm^2	UVA 10 J/cm^2	UVA 0 J/cm^2	UVA 10 J/cm^2
ICAM-1	0.243 ± 0.051	0.249 ± 0.019	0.386 ± 0.084*	0.372 ± 0.032*	0.386 ± 0.017*	0.370 ± 0.018*
VCAM-1	0.021 ± 0.012	0.015 ± 0.014	0.026 ± 0.002	0.007 ± 0.006	0.116 ± 0.022	0.109 ± 0.029
E-selectin	0.016 ± 0.009	0.018 ± 0.015	0.120 ± 0.022	0.117 ± 0.014	0.118 ± 0.039	0.119 ± 0.039

* Statistically significant difference between unstimulated and cytokine treated groups is marked by an asterisk.

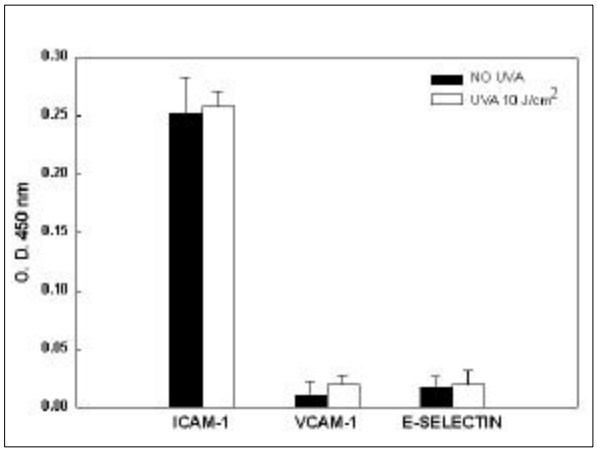

Fig. 1. Effect of UVA irradiation on ICAM-1, VCAM-1, and E-selectin expression in the HDMEC. The confluent HDMEC were irradiated with 10 J/cm^2 of UVA and analyzed by ELISA at 450 mn after 24 hours.

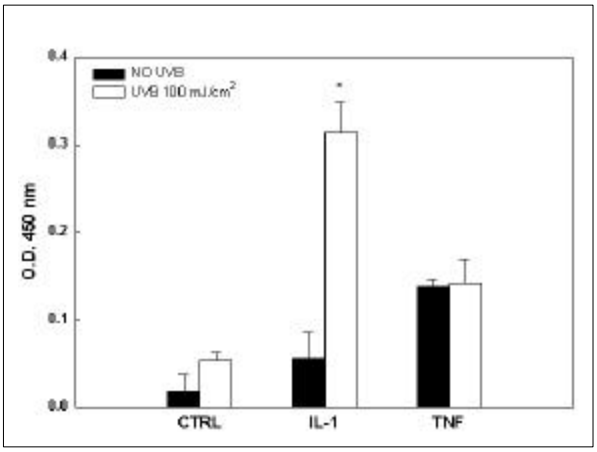

Fig. 3. UVB irradiation induces VCAM-1 by IL-1α on HDMEC. IL-1α (100 U/ml) and TNF-α (100 U/ml) were added after UVB irradiation of 100 mJ/cm^2, cultured for 48 hours, and analyzed by ELISA at 450 nm. Any statistically significant difference between the before and after the UVB irradiation samples is marked by an asterisk (*). CTRL: control.

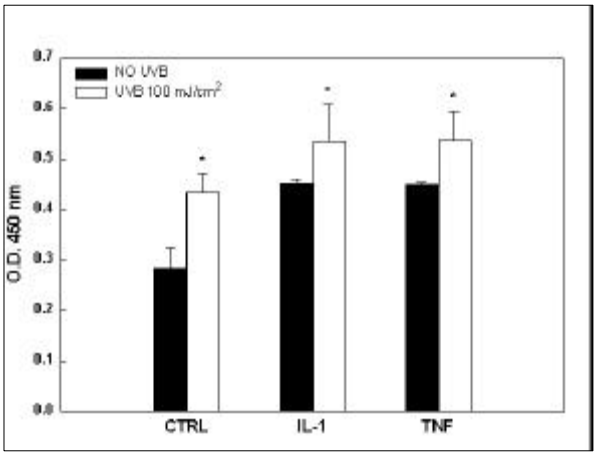

Fig. 2. UVB irradiation augments ICAM-1 induction by IL-1α and TNF-α. IL-1α (100 U/ml) and TNF-α (100 U/ml) were added after UVB irradiation of 100 mJ/cm^2, cultured for 48 hours, and analyzed by ELISA at 450 nm. Any statistically significant difference between the before and after the UVB irradiation samples is marked by an asterisk (*). CTRL: control.

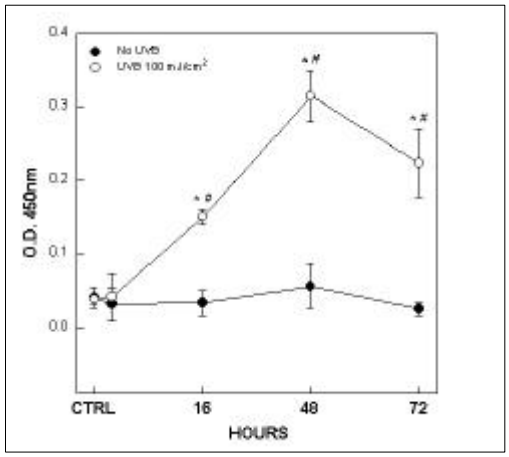

Fig. 4. Time course of VCAM-1 expression on IL-1α stimulated HDMEC with and without UVB irradiation. IL-1α 100 U/ml was added after UVB irradiation of 100 mJ/cm^2, cultured for 1, 16, 48, and 72 hours, and analyzed by ELISA at 450 nm. Any statistically significant difference between the control and each time point is marked by an asterisk (*), and between the before and after the UVB irradiation samples is marked by a sharp (#). CTRL: control.

points, as previously described. Interestingly, VCAM-1 was induced by stimulating HDMEC with IL-1α following UVB irradiation of 100 mJ/cm^2 (Fig. 3). VCAM-1 expression in HDMEC was generally detectable after 4 h of stimulation with IL-1α and 100 mJ/cm^2 of UVB irradiation. However, significant expression was observed at 16 h after stimulation with a peak at 48 h, which persisted until 72h after stimulation (Fig. 4). Flow cytometric analysis of the HDMEC stimulated with IL-1α for 24 h demonstrated that 12.0% of the cells expressed VCAM-1, but IL-1α alone without UVB irradiation or UVB irradiation alone without IL-1α treatment failed to induce VCAM-1 expression. VCAM-1 expression induced by TNF-α was unaffected by UVB irradiation.

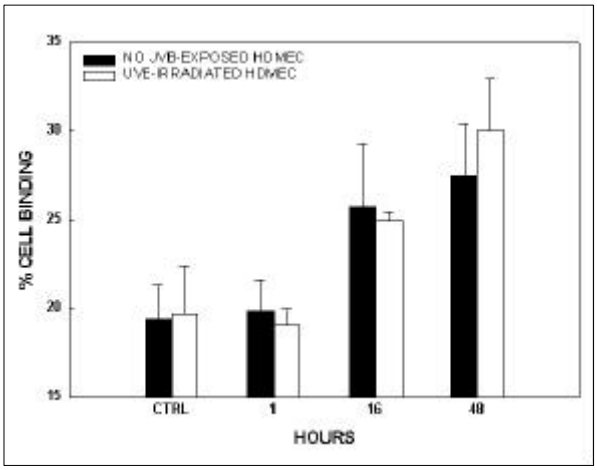

Fig. 5. UVB effect on T cell binding to IL-1α treated HDMEC. IL-1α, 100 U/ml, was added after UVB irradiation of 100 mJ/cm^2, cultured for 1, 16, and 48 hours, and 51Cr labeled T cells were added. CTRL: control.

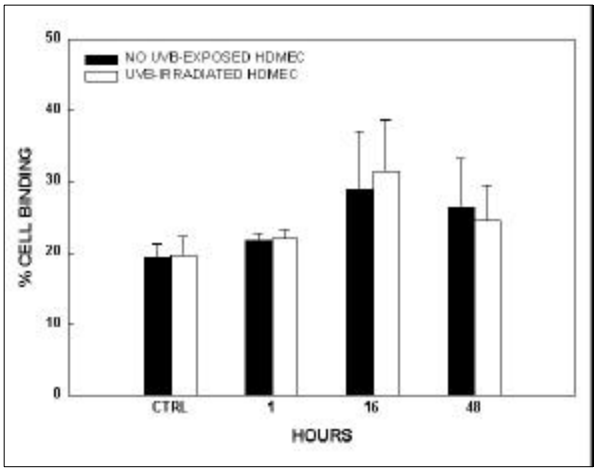

Fig. 6. UVB effect on T cell binding to the TNF-α treated HDMEC. TNF-α, 100 U/ml, was added after UVB irradiation of 100mJ/cm^2, cultured for 1, 16, and 48 hours, and 51Cr labeled T cells were added. CTRL: control.

Although the E-selectin expression fell after 24 h of IL-1α or TNF-α stimulation, it was still detectable. However, UVB irradiation with IL-1α and TNF-α treatment on the HDMEC did not significantly affect E-selectin expression (data not shown).

Effect of UVB on T lymphocyte-HDMEC adherence

The binding of T lymphocytes increased in a time dependent manner by stimulating the HDMEC with IL-1α (100 U/ml) or TNF-α (100 U/ml) (data not shown), but UVB irradiation did not affect T lymphocyte binding to the non-stimulated or cytokine-treated HDMEC (Fig. 5 and 6). In order to determine whether the cell surface ICAM-1 and VCAM-1 expression induced by UVB irradiation with cytokine treatment is correlated with T lymphocyte binding to HDMEC, the effect of the mAb directed against ICAM-1, VCAM-1 and E-selectin on the binding of T lymphocyte to the HDMEC monolayers that were not stimulated or stimulated with IL-1α or TNF-α was examined. Although the increase in T lymphocyte binding to the IL-1α stimulated HDMEC was inhibited significantly by antibodies recognizing ICAM-1 (CD54), antibodies recognizing VCAM-1 (4B9) did not block the increased binding of T lymphocytes to the IL-1α-stimulated HDMEC. The increased binding of the T lymphocytes to the TNF-α-stimulated HDMEC was only partially inhibited by mAb 4B9 but this inhibition was not statistically significant. The inhibitory effects of mAbs CD54 and 4B9 were not significantly different between the UVB-irradiated and -non-irradiated groups (Table 2).

DISCUSSION

Vascular endothelial cells play essential roles in various biologic phenomena such as the migration of leukocytes, inflammation, wound healing and angiogenesis.[6,21,27-30] Until recently, these studies were done using endothelial cells separated from the human umbilical vein.[31] However, it has recently been revealed that most of the pathophysiologic phenomena take place in microvasculature, which constitute the main vascular system in humans. In addition, endothelial cells that originated from certain tissues exhibit many differences from large vessel endothelial cells. In contrast to the large vessel endothelial cells, microvascular endothelial cells have stricter *in vitro* culture requirements,[32] differentiate rapidly into capillary-like morphology *in vitro*,[33] differ from the large vessel-derived endothelial cells in the type of prostaglandin they produce[15] and also the type of adhesion molecules they express, and their

Table 2. Effect of Anti-ICAM-1, Anti-VCAM-1, and Anti-E-selectin Monoclonal Antibodies on T Cell Binding to IL-1 and TNF--treated HDMEC with or without UVB Irradiation

	Percent adherence					
	Unstimulated		IL-1 α treated		TNF- α treated	
	UVB 0 J/cm^2	UVB 100 mJ/cm^2	UVB 0 J/cm^2	UVB 100 mJ/cm^2	UVB 0 J/cm^2	UVB 100 mJ/cm^2
None	12.5 ± 1.4	13.9 ± 2.0	23.0 ± 1.8	22.6 ± 3.1	24.3 ± 5.6	22.6 ± 3.1
CD54	11.1 ± 1.4	13.3 ± 3.4	17.0 ± 2.1*	17.8 ± 3.8*	18.7 ± 2.8	19.9 ± 5.0
4B9	13.0 ± 2.7	14.6 ± 3.9	22.3 ± 2.3	22.3 ± 2.3	20.6 ± 3.4	21.7 ± 1.4
3B7	14.8 ± 0.4	15.7 ± 3.3	25.1 ± 7.4	25.1 ± 7.4	22.4 ± 1.3	22.5 ± 3.1

* Statistically significant difference between no antibody and antibody treated groups is marked by an asterisk.
CD54 (anti-ICAM-1 mAb), 4B9 (anti-VCAM-1 mAb), 3B7 (anti-E-selectin mAb).

degree of expression.[16,19] In addition, the two types of endothelial cells differ in the regulation of the cell adhesion molecules[17] and tumor cell adhesion in response to BRM.[18,34] Leukocyte "homing" occurs at the microvasculature level and this is mediated by tissue-specific cell adhesion molecules.[30] All these events attest to the fact that it is ideal to use human dermal microvascular endothelial cells isolated from the human dermis in the study of cutaneous inflammation.

UVR can be used to effectively treat inflammatory skin diseases such as psoriasis or eczemas but they are also implicated as a cause of skin cancer, sunburn, and aging. In addition to its effect on pigment cells, which is the most well known effect of UVR, it is also known to affect the cell membranes, to change the cell surface antigens, to block the cell-to-cell interactions, to interfere with the antigen presenting function and to regulate cytokine secretion.[22] It is also known to regulate the T cell-related cell mediated immunity (CMI) in the graft versus host reaction or contact sensitivities.[22] Large UVR doses can induce systemic alterations in the antigen presenting function[35] and small or large doses promote the production of inhibitor cells that suppress the contact hypersensitivity that is related to the development of UVR induced skin cancer.[36,37] It is known that the main effect of UVR on the immune functions is UVB (280 - 321 nm)[38] but UVA[39] or psoralen ultraviolet A(PUVA)[40] are also responsible for the changes. UVB elicits change in the cell membranes and therefore leads to the expression and/or functional changes in the peripheral mononuclear cells or keratinocytes.[23-26]

The cell adhesion molecules are the cell surface receptors that mediate the cell-cell or cell-matrix interactions that play a major role in inflammatory reactions, wound healing, coagulation, metastasis of cancer cells, and growth and differentiation. ICAM-1 is an inducible surface glycoprotein with a molecular weight of 90-114 kDa and is expressed on the surface of fibroblasts, keratinocytes, and vascular endothelial cells.[7,41,42] It belongs structurally to the immunoglobulin gene superfamily and functions as a ligand to the leukocyte-expressed lymphocyte function associated antigen-1 (LFA-1). Their reaction is essential to regulating the immune system in inflammatory conditions where the association between the T cells and keratinocyte or endothelial cell are involved. In normal conditions, ICAM-1 is only scarcely expressed on the endothelial cell surface but the expression is boosted by IL-1 α and TNF- α stimulation.[29] Previous studies have shown that ICAM-1 expression in keratinocytes is suppressed by UVR and this phenomenon was used to explain the result of in vivo experiments in which ICAM-1 expression was lost after UVR treatment for atopic dermatitis or psoriasis.[23,25] However, later studies showed that the UVR-induced suppression of ICAM-1 expression is only temporary. The expression significantly increases after some time and this delayed induction of ICAM-1 was postulated to be TNF- α -mediated.[43-45] Furthermore, ICAM-1 expression is cell-specific and endothelial cells from different tissues show varied responses to BRMs, e.g., after stimulation with PMA for 24 hours, ICAM-1 expression was

higher in the HUVEC whereas it was lower in HDMEC.[17,19] These experiments, which were intended to evaluate the influence of UVA and UVB on the cell adhesion molecules in the HDMEC, showed that UVA, even in a high dose of 10 J/cm^2, did not affect ICAM-1 expression. ICAM-1 expression in the HDMEC did not show any significant changes after the combined stimulation of UVB and IL-1α or TNF-α, which further supports the fact that there is a difference between the cell types in terms of the ICAM-1 expression level after UV irradiation.

E-selectin is an inducible cell adhesion molecule that acts as a ligand for neutrophils. It was first characterized in the HUVEC[46] and belongs to the selectins or LEC-CAMs family with gp90^{mel}, which is a homing receptor expressed on T and B cells against the lymph node high endothelial cells, and GMP-140, which is a membrane protein rapidly expressed on activated platelets and endothelial cells.[47] They have a common molecular structure constituting of a lectin region, an epidermal growth factor region and a repetitive complement receptor region.48 E-selectin was not detected on the non-stimulated endothelial cells but could be induced on the HUVEC after stimulation with IL-1, TNF, LPS and IFN-γ.[13,46] E-selectin expression on the HUVEC after BRM stimulation reached a maximum after 4 hours and became normalized after 24 hours, which is more rapid than those of ICAM-1 or VCAM-1. E-selectin is known to mediate the adhesion of neutrophils to the cytokine-activated endothelial cells.[12,46] However, later reports showed that E-selectin is also involved in T cell adhesion to HUVEC[9] and was also found to be a ligand on endothelial cells for memory T lymphocytes that play an important role in CMI.[10] This study did not show any significant effect of UVR on E-selectin expression in the HDMEC.

VCAM-1, a ligand for $\alpha_4\beta_1$ (VLA-4) integrin, is a BRM-induced glycoprotein on the vascular endothelial surfaces that belong to the immunoglobulin gene superfamily with ICAM-1.[8] VCAM-1 is induced by the same BRMs as ICAM-1 and promotes the adhesion of lymphocytes to activated endothelial cells in inflammatory locations.[8,49] A previous study showed that VCAM-1 is induced by TNF-α, LPS, IL-1α and IL-4 on the HUVEC and by only TNF-α and LPS on the HDMEC. It concluded that VCAM-1 expression is tissue-specific and TNF-α was the strongest inducing factor.[11] This study revealed that UVB, at a high dose of 100 mM/cm^2, stimulates VCAM-1 expression after being incubated with IL-1α, which does not alter the VCAM-1 expression when used alone. The fact that IL-1α could stimulate VCAM-1 expression with the aid of high dose UVB has not been reported before. From this in vitro study, it can be presumed that a combination of the two stimuli could elicit identical phenomena *in vivo* and this result could be used to explain the pathogenesis of UVR-induced inflammatory diseases such as sunburn since ICAM-1, VCAM-1, and E-selectin are all upregulated after UVR exposure in the skin.[50] However, these results cannot explain why UVB induced only IL-1α to stimulate VCAM-1 expression but there are two hypotheses. Firstly, IL-1α alone is not sufficient to activate the signal needed to stimulate VCAM-1 expression but UVB releases an IL-1α-specific factor that helps to express VCAM-1. Secondly, IL-1α-specific BRM might be regulated by UVB, which acts to stimulate VCAM-1 expression. A T lymphocyte-HDMEC adhesion assay was conducted in order to observe the role of VCAM-1 expression in actual inflammatory reactions. The results showed that increased adherence by identical cytokines did not show any change after UVB irradiation and the addition of anti-VCAM-1 antibodies did not suppress the increased adherence. Therefore, VCAM-1 expression after UVB irradiation in these experiments might not be strong enough to actually influence the adhesion of T lymphocytes to the HDMEC and the necessity of a specific local regulatory factor for T lymphocyte activation is suggested. Based on these results, UVB induced IL-1α stimulation of VCAM-1 in the HDMEC might be an indirect factor in the inflammatory reaction.

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