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Research Article

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Cell Membrane Is a Major Locus for Ultraviolet B–induced Alterations in Accessory Cells

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Abstract

In vitro ultraviolet B (UVB) irradiation of human blood monocytes inhibits their accessory cell function for antigen- and mitogen-induced T cell responses. These studies were designed to characterize the nature of the UVB-induced defect in human monocyte accessory cell function. Irradiated monocytes were deficient in their ability to serve as accessory cells for OKT3-induced T cell activation. In vitro exposure of monocytes to 100 J/m² UVB completely inhibited the T cell proliferative response (51502 cpm, non-UVB-irradiated; 302 cpm, UVB-irradiated). Analysis of the accessory signals altered by UVB indicated that irradiated monocytes were incapable of binding to OKT3 molecules attached to the CD3 antigen on T cells. Provision of an alternative mechanism for binding of OKT3 molecules by attaching anti-mouse IgG to the bottom of microtiter wells completely restored accessory cell function. Further characterization of the defect demonstrated that UVB radiation did not deplete p72 Fc receptors from the surface of irradiated monocytes. However, UVB exposure did produce a dose-dependent decrease in monocyte membrane expression of ICAM-1. It is proposed that UVB radiation leads to changes within the cell membrane that inhibit the ability of monocytes to express selected molecules necessary for binding of T cells. (*J. Clin. Invest.* 1990. 85:1529–1536.) photoimmunology • antigen presentation • ultraviolet B radiation • ICAM-1 • monocytes

Introduction

Ultraviolet B radiation (UVB)[1] (290–320 nm) is a potent modulator of specific cell-mediated immune responses (1–3). UVB-irradiated mice are deficient in their ability to initiate immune responses to UVB-induced skin cancer (4), to contact sensitizing agents (5–7) and to certain microorganisms (8, 9). These observations have been ascribed both to an inhibition in the function of antigen presenting cells responsible for activation of helper T lymphocytes (5, 10, 11) and to the preservation of the antigen presenting function of cells (12) required for the activation of suppressor T cell circuits (13, 14). This translates into excessive numbers of suppressor T lymphocytes and deficient numbers of helper T lymphocytes in UVB-irradiated animals.

In humans, relatively modest doses of UVB delivered in vitro can inhibit the function of peripheral blood monocytes (MN) and epidermal Langerhans cells in accessory cell dependent assays of helper T cell activation (15–17). The situation is somewhat more complex when humans receive this form of nonionizing radiation in vivo in that UVB exposure first inhibits antigen presentation by epidermal cells, but later augments that function (16). However, the augmented antigen presenting capacity that occurs later appears to result from immigration into the epidermis of $CD1^-DR^+$ antigen presenting cells that are responsible for the preferential activation of CD4+, 2H4+ T cells (17). One function of CD4+, 2H4+ T cells is to induce suppressor T cell formation.

The repeated observation that a decrease in the viability of UVB-irradiated cells is not a necessary precondition for the loss of antigen-presenting function has led to an examination of the accessory signals that are inhibited by UVB (18–22). There is some evidence to indicate that altered IL-1 production contributes to this effect (21, 22). However, the addition of exogenous IL-1 (15, 22) only partially reconstitutes the response when added to cultures containing UVB-irradiated antigen presenting cells. Other studies have implicated deficient antigen processing as an accessory signal altered by UVB (15, 18, 20, 21). This, too, does not appear to be the sole accessory cell deficit imposed by UVB since denatured antigen also fails to completely restore the T cell proliferative response (23). Furthermore, a UVB-induced impairment in antigen processing is unlikely to be the only accessory deficit, because essentially identical doses of UVB inhibit monocyte accessory activity for mitogens, which are stimuli that are not processed (15).

This study was designed to further investigate the accessory cell defects that accompany UVB radiation exposure. We were particularly interested in effects on the cell membrane since this aspect of accessory function has not been investigated previously after UVB radiation. For this purpose, an accessory dependent system was employed in which OKT3 served as the mitogenic stimulus. In this system, MN have been shown to bind, via their surface membrane associated Fc receptors, to the Fc portion of OKT3 antibodies that have attached to the CD3 complex on T cells (24, 25). They also secrete the soluble cytokine IL-1 (25–27). Neither major histocompatibility complex restriction nor a stimulus processing step is required for activation of T cells by OKT3. Our studies demonstrate that UVB has a major effect on cell membrane expression of the

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1. *Abbreviations used in this paper:* aMIgG, anti-mouse IgG; FITC-OKT3, FITC-conjugated OKT3 MAb; ICAM-1, intercellular adhesion molecule 1; MN (noUV), unirradiated human peripheral blood monocytes; MN(UV), human peripheral blood monocytes, irradiated in vitro with 100 J/m² UVB; UVB, ultraviolet B radiation.

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adhesion molecule ICAM-1, an additional signal provided by monocytes for T cell activation by OKT3.

Methods

Antibodies and cytokines. Both unconjugated and FITC-conjugated purified OKT3, and unconjugated OKT4 and OKT8 MAb, which identify the CD3, CD4, and CD8 differentiation antigens, respectively, and purified OKIa, which is a MAb to a framework determinant of the HLA-DR antigen, were purchased from Ortho Diagnostic Systems, Inc. (Raritan, NJ). The MAb FcR32, which binds to the high affinity p72 Fc receptor on human MN, was generously provided by Dr. P. M. Guyre, Department of Microbiology and Physiology, Dartmouth Medical School (Hanover, NH). Its properties have previously been described in detail (28). RR 1/1 MAb, which reacts with ICAM-1, was kindly provided by Dr. Robert Rothlein, Boehringer Ingelheim Pharmaceuticals, Inc. (Indianapolis, IN) (29). Affinity purified goat anti-mouse IgG (aMIgG,) was obtained from Cooper Biomedical, Inc., Malvern, PA. FITC-conjugated goat anti-mouse IgG (Fab_2) was purchased from Tago, Inc. (Burlingame, CA) or Chemicon International (El Segundo, CA). Human recombinant IL-1 alpha and IL-1 beta were the gift of Dr. P. Lomedico, Hoffmann-La Roche (Nutley, NJ). The bioactivity of these cytokines was verified before use by adding them to cultures of mouse thymocytes in the mouse-thymocyte proliferation assay for IL-1. Recombinant human interferon gamma was purchased from Amgen Biologicals (Thousand Oaks, CA).

Purification of T lymphocytes. Peripheral blood mononuclear cells were obtained from the heparinized blood of healthy human volunteers by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density sedimentation. T-lymphocytes were depleted of accessory cells by a rigorous four-step purification procedure as described previously (30). Briefly, peripheral blood mononuclear cells were allowed to adhere to plastic tissue culture dishes (Falcon Labware, Oxnard, CA) for 1 h. Plastic nonadherent cells were then placed on nylon-wool columns (Fenwall Laboratories, Deerfield, IL) for 45 min. Nylon-wool nonadherent cells were subsequently exposed to the lysosomotropic agent L-leucine-methyl-ester (Sigma Chemical Co., St. Louis, MO) for 40 min according to the method described by Thiele et al. (31). Finally, these T cells were treated with a 1:50 dilution of OKIa and low toxicity rabbit complement (Cedarlane Laboratories Ltd., Hornby, Ontario). Treatment in this manner resulted in a T-lymphocyte population that, without the addition of exogenous accessory cells, was completely unresponsive to concanavalin A and OKT3. The number of Ia-positive cells was less than 0.5% as monitored on a FACS EPICS V fluorescence activated cell sorter (Coulter EPICS Division, Hialeah, FL), which was at the limit of sensitivity of the assay.

Preparation and UVB-irradiation of accessory cells. Accessory cells were prepared by gently scraping off and removing the plastic-adherent cells during the first step of the T-lymphocyte purification procedure. They were then centrifuged, resuspended in HBSS (KC Biological, Lenexa, KS), and counted. This population was 85–90% nonspecific esterase positive, and for the purposes of this paper will be called MN. Two $\times 10^6$ MN in 1.5 ml HBSS without phenol red were placed in 35 $\times$ 10 mm culture dishes (Falcon) and exposed to various doses of UVB from 4 FS20 sunlamp bulbs (Westinghouse Electrical Corp., Bloomfield, NJ). The UVB output was monitored by means of an IL 700 Research Radiometer and SEE 240 UVB Photodetector (International Light, Newburyport, MA) and was $\sim 12.2 \times 10^{-5}$ J/s per cm^2 at a tube to target distance of 22 cm. To obtain an homogeneously irradiated cell population, culture dishes were gently shaken every 15 s during UVB exposure. MN were recovered from the dishes by scraping with a rubber policeman, centrifuged, resuspended in RPMI 1640 medium (Whittaker, Walkersville, MD) and counted. UV-irradiated [MN(UV)] and non-UV-irradiated MN [MN(no UV)] were treated in an identical fashion. Viability of irradiated MN, as assessed by trypan blue exclusion was >90% and did not differ from that of unirradiated cells during the 72-h culture period.

OKT3-induced T cell blastogenesis. The MAb OKT3 served as the mitogenic stimulus. 1×10^5 highly purified T cells were co-cultured with 2×10^4 autologous, UV-irradiated MN and OKT3 in a total volume of 200 μl of culture medium. OKT3 and MN were used in concentrations which, in preliminary experiments, gave optimal proliferative responses. Culture medium consisted of RPMI 1640, supplemented with 10% pooled human serum, penicillin (10,000 U/ml), streptomycin (10,000 μg/ml), L-glutamine (2 mM), and Hepes buffer (10 mM). In some experiments cells from a PPD reactive T cell line (1×10^5) were used as responder cells instead of highly purified T cells (15). Cultures were set up in 96-well microtiter plates (Corning Glass Works, Corning, NY) and incubated for 72 h at 37°C in 5% CO_2 humidified air. All variables were performed in triplicate wells. 1.0 μCi of *methyl*-[^{3}H]thymidine (New England Nuclear, Boston, MA) was added to each well for the last 18 h of culture. Wells were harvested onto glass filter paper using a Multiple Automatic Sample Harvester (M. A. Bioproducts, Walkersville, MD) and tritiated thymidine content per well assessed by scintillation spectrophotometry. Data were expressed as cpm $\times 10^{-3} \pm$SD.

Attachment of anti-mouse IgG to microtiter wells. The method of Walker et al. (32) was employed to attach anti-mouse IgG (aMIgG) to the bottom of 96-well microtiter plates. Affinity purified goat aMIgG was diluted in Tris buffer at a pH of 9.5 to a final concentration of 5 μg/ml. 200 μl of this solution was added to each well. Plates were incubated for 40 min at room temperature. Coated wells were then washed three times with PBS and once with PBS containing 1% fetal calf serum to remove unbound antibodies.

Fc-receptor and ICAM-1 staining. Presence of p72 Fc-receptors on MN was assessed using the MAb FcR32. Staining of MN was performed as described by Anderson et al. (28). Briefly, 1×10^6 MN were incubated for 2 h at 4°C in 50 μl of MAb FcR32 diluted in RPMI 1640 supplemented with 10% fetal bovine serum. Cells were washed twice in HBSS with 0.3% BSA, resuspended and incubated for 2 h at 4°C in 100 μl of a 1:20 dilution of FITC-aMIgG. Cells were then fixed with 1% paraformaldehyde and stored at 4°C before analysis. Stained cells were analyzed on an EPICS V fluorescence activated cell sorter. Data are shown as histograms of log fluorescence intensity (x-axis) and cell number (y-axis) of 1×10^5 counted cells.

For ICAM-1 staining, RR1/1 was employed as the primary antibody at a concentration of 10 μg/ml for 30 min (29). Cells were washed and then stained with a 1:20 dilution of FITC-aMIgG (Chemicon International).

Cell-cluster formation assay. Cultures were set up as described for OKT3-induced T-lymphocyte blastogenesis, but in flat-bottom microtiter plates (Falcon Labware). Plates were centrifuged for 4 min at 500 rpm before incubation at 37°C in 5% CO_2 humidified air. Cultures were examined for cell-cluster formation after 0, 12, and 18 h. One cell cluster was defined as an aggregation of 3 or more cells when examined under a tissue culture microscope (American Optical, Buffalo, NY). Preliminary experiments in which clusters were stained with monoclonal antibodies demonstrated that they contained both T cells and monocytes. The number of clusters was determined at a magnification of 200 by examining six randomly chosen view fields using an ocular grid. Variables were set up in triplicate. No cluster formation could be detected in cultures without MN. However, cell clusters were detectable in cultures without OKT3, indicating that there was a component of OKT3 independent cell to cell interaction. This non-OKT3 dependent cell cluster formation was always < 30% of that occurring in cultures containing OKT3. In some experiments, the monoclonal anti-ICAM-1 antibody RR 1/1 or an isotype control monoclonal antibody was added to cultures at a 1:100 (42 μg/ml) or 1:1,000 dilution (4.2 μg/ml). Data are expressed as the mean number of clusters per 200× field.

Results

Effects of in vitro UVB irradiation on MN accessory cell function for OKT3-induced T cell proliferation. Our previous studies had shown that in vitro exposure of MN to UVB radiation

resulted in an inability of those cells to act as stimulator cells in accessory cell dependent T lymphocyte proliferation assays for the soluble antigen tetanus toxoid and for the mitogen PHA (15). In the experiments presented here (Table I and Fig. 1), we examined whether the inhibitory effect of UVB on accessory function extended to assays in which OKT3 served as the mitogenic stimulus. Fig. 1 shows that a dose dependent decrease in T cell proliferation resulted when MN were exposed to various doses of UVB (0–100 J/m^2). The UVB doses were not lethal for MN over the entire duration of the culture period, as assessed by trypan blue exclusion. Similar results were obtained when the responding cells were T lymphocytes from a PPD-specific T cell line that proliferated in response to OKT3 as well (data not shown). Furthermore, no inhibition of proliferation was observed in mixing studies in which MN(UV) were placed in culture with MN(no UV), OKT3, and T cells (data not shown), indicating that the defect in accessory cell function following UVB exposure was intrinsic to the irradiated cell population. Thus, similar to its effect on the accessory function for PHA and for soluble antigen, UVB inhibited the function of accessory cells required for OKT3-induced T cell proliferation (15).

Effects of UVB irradiation on MN-derived accessory signals required for OKT3-induced T cell proliferation. The accessory signals required for activation of resting T-cells by anti-CD3 antibodies are well-defined. Accessory cells secrete the cytokine IL-1 (23–25) and they provide an immobile matrix to which OKT3 molecules attached to T lymphocytes are able to bind (24, 25). The finding that UVB inhibited the function of accessory cells in this system suggested that it might be an excellent model for study of the effects of UVB on specific accessory signals.

In previous studies we had shown that UVB-irradiation of MN was associated with a greater than 95% reduction in their ability to produce IL-1 (15). Our initial attempts to reconstitute the accessory function of MN(UV) consisted of adding exogenous IL-1 to cultures. Neither recombinant IL-1α nor IL-1β, was able to provide even partial reconstitution of the proliferative response when MN(UV) were used as the stimulator cells (Table II). Although the data presented in Table II employed 10 U of IL-1α and IL-1β, essentially identical results were obtained with a wide range of IL-1 doses (0.1–100 U).

Table I. Inhibition of OKT3-induced T Lymphocyte Proliferation by In Vitro Exposure to UVB Radiation

Cultures containing	Proliferative response	
	Exp. 1	Exp. 2
	(cpm × 10^{-3})±SD	
T cells	358±89	180±6
T cells + OKT3	509±111	217±25
T cells + OKT3 + MN (no UV)	51,502±4,831	52,240±2,100
T cells + OKT3 + MN (100 J/m^2 UV)	302±33	472±141
T cells + MN (no UV)	430±75	459±99
T cells + MN (100 J/m^2)	282±38	319±70

T lymphocytes (1×10^5) were cultured with or without OKT3 and either MN (no UV) or MN (100 J/m^2) for 72 h as described in Methods.

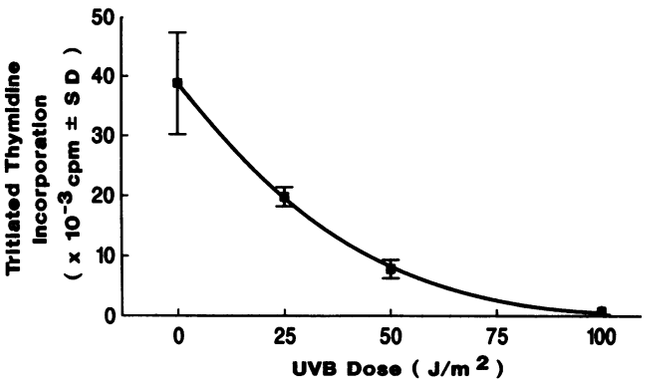

Figure 1. Dose-dependent inhibition of OKT3-induced T cell proliferation by UVB irradiation of MN. MN were exposed in vitro to various doses of UVB (0–100 J/m^2) and then co-cultured with autologous, accessory cell–dependent T lymphocytes as described in Table I.

The inability of biologically active, exogenous IL-1 to overcome the UVB-induced defect in accessory cells indicated that altered IL-1 production was not the sole accessory cell signal modulated by UVB.

Because Fc receptor-mediated binding to the antibody molecule represented another accessory signal required for T cell activation by OKT3, studies were next performed to analyze the effects of UVB on this monocyte-derived activity. This was assessed in a cell binding assay in which the number of clusters of T cells around MN(UV) was compared to clusters of T cells around MN(no UV). Table III shows a representative experiment. No cell clusters were detected in any culture at 0 h. A significant number formed after 12 h, and this increased further after 18 h (see also Fig. 2 *A*). Thus, proliferation in cultures with highly purified T cells, MN(no UV) and OKT3 was accompanied by an increase in cell cluster formation. Cluster formation was strictly dependent on the presence of MN; no clusters formed in cultures with T cells and OKT3 or T cells alone. A small number of cell clusters formed in cultures containing T cells and MN in the absence of OKT3. The data in Table III demonstrate that a dose-dependent inhibition of cell cluster formation occurred when MN were exposed to UVB doses ranging from 0 to 100 J/m^2. 100 J/m^2 was sufficient to completely inhibit cluster formation (Fig. 2 *B* and Table III). The UVB doses that inhibited cluster formation

Table II. Failure of Exogenous IL-1 to Reconstitute Accessory Cell Activity of MN (UV)

Cultures containing	Proliferative response
	(cpm × 10^{-3})±SD
T + MN (no UV) + OKT3	89,778±2,150
T + MN (UV) + OKT3	1,298±206
T + MN (UV) + OKT3 + IL-1α	978±242
T + MN (UV) + OKT3 + IL-1β	997±341

Cultures were prepared as described in Table I. 10 U of biologically active, human recombinant IL-1α or IL-1β were added. IL-1 α and IL-1 β did not augment the proliferative activity of cultures containing MN (no UV) or cultures containing no MN.

Table III. Inhibition of OKT3-induced Cell Cluster Formation by UVB Radiation

OKT3	MN	UVB dose	Number of cell clusters		
			0 h	12 h	18 h
		J/m^2		*(Mean±SD)*	
+	+	0	0.0±0.0	24.3±3.8	33.3±4.3
+	+	25	0.0±0.0	8.3±2.5	10.3±2.5
+	+	50	0.0±0.0	2.6±1.5	1.6±0.6
+	+	100	0.0±0.0	1.0±1.0	0.7±0.6
−	−	—	0.0±0.0	0.0±0.0	ND
+	−	0	0.0±0.0	0.3±0.6	ND
−	+	0	0.0±0.0	5.0±1.4	10.0±2.6

T lymphocytes, MN (0–100 J/m^2) and OKT3 were cultured in flat-bottom microtiter plates and number of cell clusters determined after 0, 12, and 18 h incubation at 37°C as described in Methods.

were identical to those that produced inhibition of T cell blastogenesis observed in Table I. These results indicate that MN(UV) were unable to form a physical interaction with the responding lymphocyte population.

We reasoned that if an alternative source for binding of the OKT3 molecule was present, restoration of the proliferative response might be observed. This was performed by attaching anti-mouse IgG molecules to the bottom of microtiter wells and adding purified T cells, OKT3 antibody and UVB-irradiated monocytes. Representative experiments are shown in Table IV. Cultures containing MN(no UV) showed a normal proliferative response (line *a*), which was reduced to background levels when MN were exposed in vitro to 100 J/m^2 UVB (line *b*). Attachment of aMIgG to the bottom of microtiter wells in cultures with MN(UV) completely restored proliferation (line *c*). This was a consistent and reproducible finding ($n = 6$). It has been demonstrated that immobilization of anti-CD3 antibodies is sufficient to induce proliferation of purified resting T lymphocytes (24, 31, 33, 34) and this observation was confirmed in these experiments (line *e*). Restoration could not be achieved when OKT4 or OKT8 was added to cultures instead of OKT3. When soluble aMIgG was added to cultures in lieu of using it to coat microtiter wells, no reconstitution of blastogenesis in cultures with MN(UV) occurred, suggesting that an immobilized matrix was required for proliferation (35, 36). Addition of biologically active recombinant IL-1 alpha to cultures with T-lymphocytes, OKT3, MN(UV) and attached aMIgG failed to further augment the response (data not shown). Taken together, these results suggest that the primary effect of UVB was to inhibit the interaction between T cells and MN, and that by providing an alternative mechanism for binding with solid-phase bound aMIgG, T lymphocyte proliferation could be reestablished.

Effect of UVB radiation on the surface membrane expression of MN receptors involved in binding to T cells. OKT3 antibodies bind to the p72 Fc receptor, and it is through this receptor that monocytes mediate their binding function (37, 38). The next series of studies investigated whether UVB irradiation of monocytes depleted or inhibited the expression of this type of Fc receptor. This was examined by determining the reactivity of MN(UV) with monoclonal antibody FcR32, whose reactivity is specific for the p72 Fc receptor (28). Fluorescence intensity and number of stained MN(UV) was compared to that of MN(no UV) immediately after UVB exposure and after a 24-h incubation period. As shown in Fig. 3, there was no difference in the number of UV-irradiated or unirradiated MN at either time point nor was there a detectable difference in fluorescence intensity between the two cell populations. Thus, an inability of MN(UV) to bind OKT3 molecules was not due to a lack of p72 Fc receptors.

Because MAb32 does not recognize the ligand binding site of the p72 Fc receptor (28), it was possible that although the p72 Fc receptor was present on the monocyte surface it was unable to bind to the OKT3 molecule. To exclude this possibility, the binding of FITC-conjugated OKT3 antibodies was determined cytofluorometrically with a window selecting for the monocyte population. No difference in binding of FITC-OKT3 between MN(no UV) and MN(UV) could be detected immediately after UVB exposure or after 24, 48, or 72 h.

Monocytes express ICAM-1 on their cell surface which binds to LFA-1 on the surface of T cells (29, 39). This receptor ligand interaction provides a stimulus independent mechanism for T cell binding to monocytes. Since alterations in Fc receptor expression or function did not appear to be responsible for the inability of T lymphocytes to bind to monocytes, studies were carried out to determine whether ICAM-1 expression was necessary for OKT3-induced T cell binding and proliferation, and, if so, whether monocyte expression of

Table IV. Restoration of OKT3-induced T Cell Proliferation in Cultures with MN (UV) by the Addition of Immobilized MIgG

Panel	Additions to cultures				Proliferative response		
	aMIgG	OKT3	MN (no UV)	MN (UV)	Exp. 1	Exp. 2	Exp. 3
a	−	+	+	−	21,983±2,089	59,697±10,558	55,630±15,659
b	−	+	−	+	201±39	552±132	350±30
c	+	+	−	+	34,672±6,422	77,847±5,611	50,442±15
d	+	−	−	−	ND	255±14	310±15
e	+	+	−	−	23,871±749	48,642±10,912	44,763±9,886
f	−	−	−	−	354±154	486±109	ND

Cultures were prepared as described in Table I. They were plated in microtiter wells, that had been coated with aMIgG or that had been left uncoated, as described in Methods. UVB dose was 100 J/m^2.

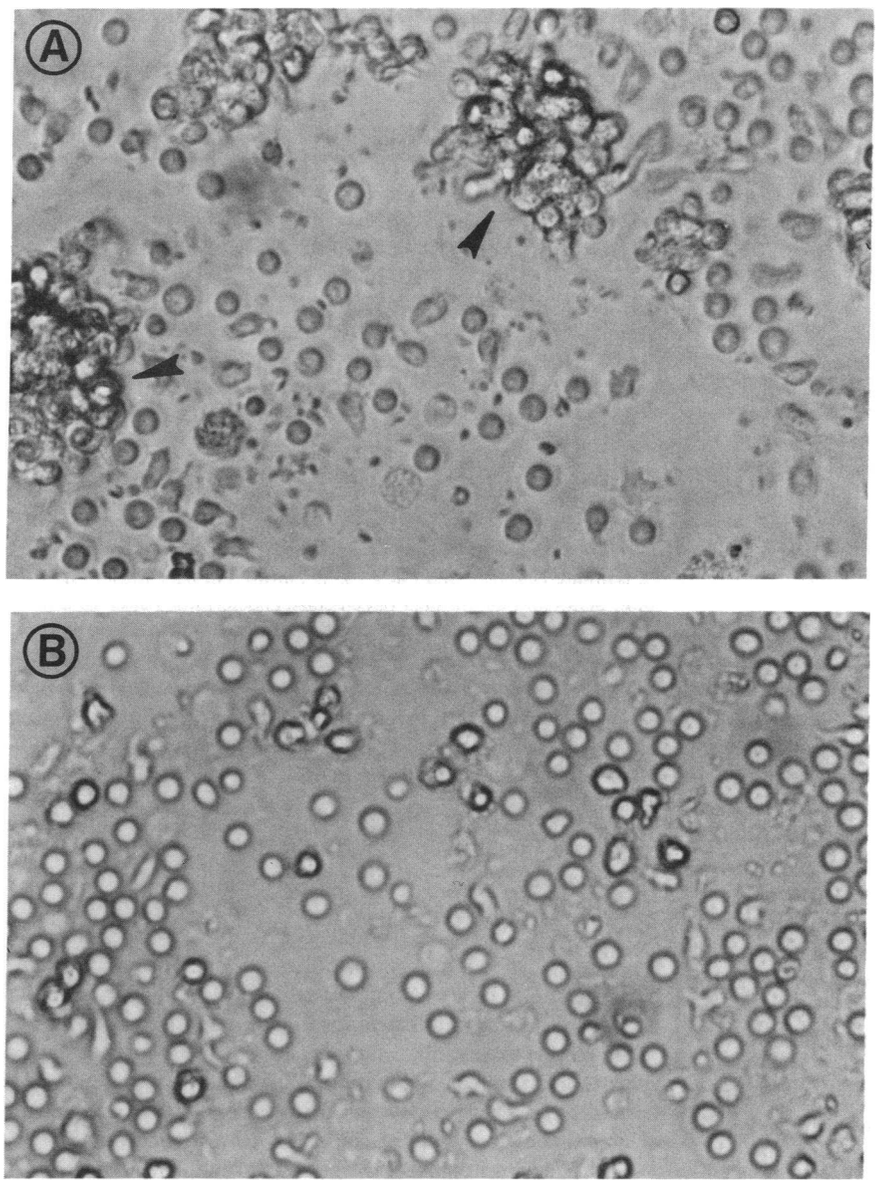

Figure 2. Photomicrographs (×200) of cultures containing T cells, OKT3 and MN(no UV) (*A*) or MN(UV) (*B*). Arrows in *A* indicate cell clusters.

ICAM-1 was affected by UVB exposure. When the anti-ICAM-1 antibody RR 1/1 was added continuously to cultures containing unirradiated monocytes, T cells and OKT3, OKT3-induced T cell proliferation was inhibited by greater than 80% (Table V). Isotype specific control antibodies did not affect the T cell proliferative response (data not shown). In cell binding assays, the addition of anti-ICAM-1 antibodies inhibited the development of clusters around unirradiated monocytes (Table VI). Isotype specific monoclonal antibodies did not inhibit binding of T cells to monocytes (data not shown). The results of these experiments indicated that ICAM-1 expression was essential for T cell interactions with monocytes in the OKT3 assay.

To assess the effects of UVB on ICAM-1 expression, monocytes were UV-irradiated, incubated overnight, and then stained with the anti-ICAM-1 monoclonal antibody. In contrast to the lack of an effect on Fc receptor expression, UVB irradiation of monocytes produced a dose-dependent decrease in ICAM-1 expression (Fig. 4). 100 J/m^2 UVB inhibited ICAM-1 expression by 82%, indicating that UVB radiation had a major effect on MN surface membrane expression of ICAM-1.

Discussion

Our data indicate that UVB radiation modifies monocyte cell membrane function. With respect to accessory function for OKT3-induced T cell activation, this was manifest as an inability of UV-irradiated monocytes to provide the necessary signals for T cell proliferation. Direct cell to cell contact between MN(UV) and T cells was markedly decreased. T lymphocyte proliferation could be completely restored by adding immobilized goat anti-mouse IgG to cultures thereby providing an alternative source for binding of the Fc portion of the OKT3 molecule.

The UVB-induced inhibition of accessory function was associated with a decrease in MN surface membrane ICAM-1 expression. Recent interest has focused on receptor ligand in-

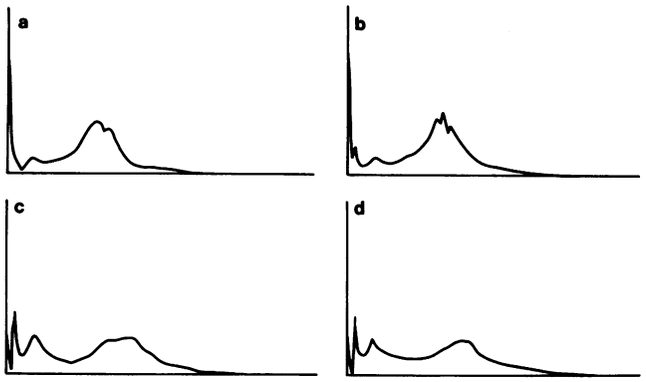

Figure 3. Normal numbers of p72 Fc receptors on the surface of UVB-irradiated MN. MN(UV) were exposed in vitro to 100 J/m^2 UVB. Cells were stained with MAb FcR32 and FITC-anti-mouse IgG immediately after UVB exposure (0 h) or after 24 h incubation in complete medium supplemented with 10% PHS. Histograms of cell-number (*y*-axis) and fluorescence intensity (*x*-axis) are (*a*) MN(no UV) stained at 0 h, (*b*) MN(UV) stained at 0 h, (*c*) MN(no UV) stained at 24 h, (*d*) MN(UV) stained at 24 h.

teractions between T cells and monocytes as additional accessory signals involved in antigen- and mitogen-induced activation of T lymphocytes (40–42). In particular, T cells express LFA-1 and CD2 on their cell surface which bind to ICAM-1 and LFA-3, respectively, on monocytes. T cell proliferative responses to PPD have been shown to be inhibited by antibodies to ICAM-1 (40). The finding in this study that UVB radiation inhibited ICAM-1 expression on monocytes provides evidence to suggest that this form of radiant energy impairs accessory cell-T cell interactions that lead to T cell activation through an effect on this molecule. It is also possible that other membrane proteins including other adhesion molecules could be additional contributing factors to the deficient binding of T cells to UVB-irradiated monocytes.

The effect of UVB radiation on IL-1 production by MN and keratinocytes is controversial. While some investigators have found that UVB-irradiated murine peritoneal exudate cells (43) and keratinocytes produce increased amounts of IL-1 and contain increased levels of IL-1 mRNA (44) others have found precisely the opposite (15, 19, 21). The reason for such divergent results is unclear although differences in the UVB doses actually delivered may provide a partial explanation. In our hands, supernatants from MN exposed to 50-200 J/m^2 UVB contain reduced amounts of IL-1 (15). Nonetheless, exogenous IL-1 failed to reconstitute the T cell proliferative response to OKT3. The ability of other investigators to partially reconstitute immunological responsiveness with this cytokine may be due to a small number of accessory cells contaminating the responding cell population. This appears likely since less rigorous procedures were used in these studies to obtain an accessory cell dependent responder cell population (20, 21, 22). When immobilized aMIgG was provided to bind OKT3 attached to T cells, complete restoration of the response occurred, which could not be further increased by the addition of IL-1. These results might suggest that IL-1 is not required for T

Table V. Inhibition of OKT3-induced T Lymphocyte Proliferation by Anti-ICAM-1 Antibodies

Cultures containing	Proliferative response	
	Exp. 1	Exp. 2
	cpm × 10^{-3}±SD	
T cells	268±167	363±384
T cells + OKT3	201±73	443±491
T cells + OKT3 + MN (no UV)	116,584±1,049	81,838±6,786
T cells + OKT3 + MN (no UV) + ICAM-1 (1:100)	35,143±866	ND
T cells + OKT3 + MN (no UV) + ICAM-1 (1:1,000)	ND	49,979±6,758
T cells + MN (no UV)	939±116	1,012±743
MN	333±139	198±171

T lymphocytes (1 × 10^5) were cultured with or without OKT3 and MN (no UV) for 72 h as described under Methods. ICAM-1 was added at the indicated dilution.

Table VI. Inhibition of OKT3-induced Cell Cluster Formation by Anti-ICAM-1 Antibodies

OKT3	MN	Antibody added	Number of cell clusters	
			Exp. 1	Exp. 2
+	+	—	14.4±6.7	29.3±4.8
+	+	ICAM-1 (1:100 dilution)	1.1±1.2	ND
+	+	ICAM-1 (1:1,000 dilution)	ND	11.7±1.5
−	−	—	0.1±0.2	0.2±0.5
+	−	—	0.0±0.0	0.0±0.0
−	+	—	3.7±2.0	7.4±2.2
−	+	ICAM-1 (1:100 dilution)	0.5±0.7	ND
−	+	ICAM-1 (1:1,000 dilution)	ND	3.6±2.2

T lymphocytes, MN and OKT3 were cultured in flat-bottom microtiter plates and the number of cell clusters determined after 16 h incubation at 37°C as described in Methods.

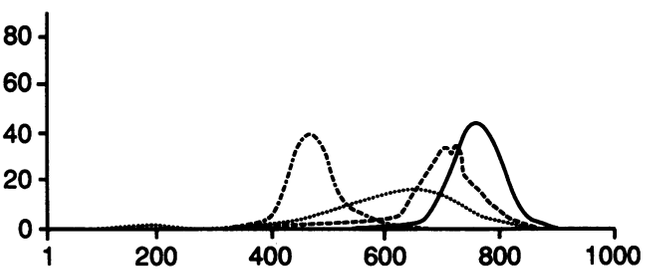

Figure 4. Deficient ICAM-1 expression on the surface of UVB-irradiated MN. Cells were stained with MAb RR1/1 as described in Methods and were then examined by flow cytometry. The *x*-axis represents the log of the fluorescence intensity on arbitrary units. The *y*-axis represents the cell number. – – –, negative control; · · · · ·, MN exposed to 100 J/m^2 UVB; -----, MN exposed to 50 J/m^2; ——, MN exposed to 0 J/m^2 UVB.

cell activation by OKT3. However, it is possible that the target of IL-1 in this assay system is the accessory cell rather than the responding T lymphocyte. This would be consistent with the findings of Koide and Steinman (45) who reported that the accessory function of dendritic cells was markedly enhanced by preculture with IL-1, indicating a direct effect of this cytokine on the dendritic cell population. If that is the case, then an additional mechanism by which UVB might exert its effect on cells would be by inhibiting their response to soluble mediators. In this regard, we have shown that human natural killer cells are less responsive to IL-2 and interferon-gamma following UVB exposure (46). This, too, could be mediated by UVB-induced inhibition of signals transmitted through the membrane.

In summary, the present study demonstrates that UVB-induced modulation of human MN accessory cell function is associated with a decrease in expression of MN membrane ICAM-1. To our knowledge, this is the first report describing the cell membrane as a major target structure in UVB effects on accessory cells. Further studies are under way to examine whether the cell membrane is the actual chromophore and which membrane components are altered by UVB.

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