Acta Derm Venereol (Stockh) 1993; 73: 270–272

Effect of a Single Exposure to *In vivo* UVB Radiation on the Allogeneic Mixed Lymphocyte Reaction of Spleen Cells

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We investigated the effect of a single exposure to in vivo UVB radiation on the splenic T cell alloreactivity and antigen presenting cell (APC) function needed for alloantigen presentation. Splenic T cells from UVB-irradiated C57BL/6 mice were used as responders, and spleen cells from UVB-irradiated BALB/c mice were used as stimulators for a source of APCs in mixed lymphocyte culture. A single UVB radiation suppressed T cell alloreactivity, although the proliferative response to T cell mitogens was still normal. Moreover, UVB radiation impaired APC function. FACS analysis revealed a reduction not in the number of APCs but the intensity of class II alloantigen expression on APCs. Our findings suggest that, unlike repeated UVB exposure which impairs splenic APC function by the decrease in the number of APCs, a single UVB exposure impairs APC function by decreasing class II alloantigen expression. ***Key words: Immunosuppression; Ultraviolet light; Alloreactivity; Antigen presenting cell.***

(Accepted March 1, 1993.)

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Ultraviolet (UV) radiation has been reported to affect various immune status factors (1). Not only directly irradiated skin as an immune organ but also other lymphoid organs, such as spleen and lymph nodes, are immunologically affected by UV radiation. There are two well-described immunological effects of UV radiation on spleen. Repeated UV exposure causes splenic antigen presenting cell (APC) function deficiency by migration of splenic APC to lymphoid tissues (2), and, antigen sensitization after UV radiation causes the generation of antigen-specific suppressor cells in spleen (3). On the other hand, general splenic immune states such as antibody production, proliferative response to antigenic and mitogenic stimulations, and the induction of cytolytic cells are normal after UV exposure (4).

One-way allogeneic mixed lymphocyte reaction (MLR) is mainly a proliferative response of T cells against the class II alloantigen expressed on APCs (5). Using this well-defined system, we investigated whether a single (not repeated) UV radiation alters the splenic immune states. We used spleen cells from mice that had received a single dose of UVB radiation for responder cells and stimulator cells in MLR, and examined the alloreactivity of T cells and the alloantigen presenting capacity of APCs.

MATERIALS AND METHODS

Mice

Pathogen-free female C57BL/6 (B6) and BALB/c mice were purchased from Clea, Inc, Tokyo, Japan, and maintained in the Animal Center of Kyoto University. Mice were used at 6 to 8 weeks of age.

UV radiation

The source of UV was FL20S.E-30 sunlamps (Toshiba Electric, Tokyo, Japan), emitting mainly UVB (280–320 nm) with a peak emission at 305 nm. The dorsal hair of the mice was shaved off and the mice were exposed to UVB radiation for 1 h. The total UVB dose received by the mice was approximately 30 kJ/m^2, which caused edematous erythema on the back. Another group of mice was exposed to the lower UVB dose (3, 10 kJ/m^2). Three days after UVB radiation, the mice were killed and their spleen was removed.

Mitogen response

Spleen cells (2×10^5 cells) from UVB-irradiated and normal B6 mice were cultured in the presence of mitogens (Con A (Pharmacia Fine Chemicals, Uppsala, Sweden), 2.5 μg/ml; LPS (Difco, Detroit, MI), 25 μg/ml) in a microculture well in 0.2 ml of RPMI-1640 medium (Nissui Seiyaku, Tokyo, Japan), supplemented with 10% FCS and 5×10^{-5} M 2-mercaptoethanol for 48 h at 37°C in a humidified atmosphere of 5% CO_2 in air. During the last 4 h, the cells were pulsed with 0.5 μ Ci/well of ^{3}H-thymidine. The cells were harvested and ^{3}H-thymidine incorporation was determined by liquid scintillator counting.

Mixed lymphocyte cultures

Nylon-wool-passed splenic T cells (6) from UVB-irradiated and normal B6 mice were used as responder cells. Spleen cells from UVB-irradiated and normal BALB/c mice were used as stimulator cells for a source of APCs. Responder cells (5×10^5 cells) and 2,000 rad-irradiated stimulator cells (5×10^5 cells) were mixed and cultured similarly, as mentioned above, for 4 days. During the last 12 h, the cells were pulsed with 0.5 μ Ci/well of ^{3}H-thymidine. The cells were harvested and counted.

FACS analysis

To examine if the number of T and B cells is affected by UVB radiation, spleen cells from UVB-irradiated and normal B6 mice were stained with fluoresceinated anti-Thy 1.2 (PharMingen, San Diego, CA) and goat anti-mouse Ig (Cedarlane, Ontario, Canada) antibodies.

The major MLR is provided by allogenic H-2 class II molecules on APCs (5). Therefore, we examined the effect of UV radiation on the number of APCs (class II positive cells), and the intensity of class II expression. Spleen cells from UVB-irradiated and normal BALB/c (H-2^d) mice were stained with rat antibody M1/42 (H-2 class I, all allele, ATCC, Rockville, MD) and antibody M5/114.15.2 (I-A^{b,d,q}, I-E^{d,k}, ATCC), followed by fluoresceinated goat anti-rat Ig antibody (Caltag, San Fransico, CA).

All samples were then analyzed with an FACScan flow cytometer (Becton Dickinson, Mountain View, CA), gated to exclude non-viable cells by propidium iodide.

Statistical analysis

Student's *t*-test was employed to determine the statistical significance.

RESULTS

As shown in Tables I and II, the number of T cells (Thy-1.2$^+$ cells) and B cells (mouse Ig$^+$ cells) and the mitogenic reactivities of spleen cells to T cell mitogen Con A and B cell mitogen LPS were not affected by a single exposure to UVB radiation.

Table I. *Effect of UVB radiation on the number of splenic T and B cells*[a]

Treatment	T cells (Thy-1.2$^+$)	B cells (sIg$^+$)
NR[b]	33±2.7	53±5.0
UV[c]	31±2.2	50±3.1

[a] Spleen cells were obtained from UVB (30 kJ/m^2)-irradiated and normal B6 mice. Five thousand cells were totally counted, and unstained cells were used as controls. Each value is % positive/total spleen cells ± SD from four mice.
[b] Non-irradiated.
[c] UVB-irradiated.

Table III shows the effect of UVB radiation on the alloreactivity of splenic T cells. The proliferative response of responder cells from UVB-irradiated mice was suppressed in a dose-dependent manner. On the other hand, Table IV shows the effect of UVB radiation on the stimulatory capacity of APCs. The data indicates that the allogeneic responses of responder cells from both UVB-irradiated and normal mice were suppressed by in vivo UVB treatment of APCs. These findings suggest that UVB radiation diminishes both the stimulator and responder functions of spleen cells.

Fig. 1 shows the staining pattern of class I and class II antigens on spleen cells from UVB-irradiated and normal mice. The left shift of class II flourescence intensity peak (Fig. 1B) indicates that the intensity of class II alloantigen expression on the APCs from UVB-irradiated mice was significantly reduced compared to normal mice ($p < 0.001$), assuming that the size of the APCs was unchanged after UVB radiation. On the other hand, the number of APCs (class II antigen positive cells) was not changed by UVB radiation. The class I antigen was normal in both expression intensity and positive cell number (Fig. 1A).

DISCUSSION

In the present study, we show the suppressive effect of a single UVB exposure on the splenic T cell ability to respond to alloantigens and the ability of APCs to present alloantigen.

Ullrich has reported that antigen stimulation to UV-irradiated mice induces antigen-specific suppressor cells, resulting in the suppressed MLR to the specific antigen and that UV radiation alone is not sufficient for the induction of suppressor

Table II. *Effect of UVB radiation on the mitogen response of spleen cells*[a]

Treatment	ΔCPM		
	Con A	LPS	(−)
NR	87189±14331	83883±6786	(2750±414)[b]
UV	93410±15780	90942±9865	(2539±694)[b]

[a] Spleen cells were obtained from UVB (30 kJ/m^2)-irradiated and normal B6 mice. Each value is mean ΔCPM ± SD from four mice.
[b] The background response.

Table III. *Effect of UVB radiation on the alloreactivity of splenic T cells*[a]

UVB dose (kJ/m^2)	ΔCPM		% inhibition[b]
NR	70215±8792	(5635±442)[c]	–
3	59174±5162	(4225±457)[c]	16
10	42607±10264[d]	(6642±1253)[c]	39
30	29151±6045[e]	(4443±890)[c]	58

[a] Nylon-wool-passed T cells (Thy1$^+$ cells ≥ 90%) from UVB-irradiated and normal B6 mice served as responder cells. Each value is mean ΔCPM ± SD from three or four mice.
[b] (1-ΔCPM/control ΔCPM) × 100.
[c] The background response.
[d] $p < 0.02$, vs NR.
[e] $p < 0.001$, vs NR.

cells (3). On the other hand, we show that a single UV exposure without antigen stimulation also suppresses the MLR, although the extent of suppression is low compared to that by UV radiation followed by antigen sensitization (3). The findings that the number of T cells and the proliferative ability to respond to the other type of stimulation by T cell mitogens are normal indicate that the suppressive effect of UV radiation is not general. For the elucidation of the mechanism of the suppressed alloreactivity of T cells caused by a single UVB radiation alone, further investigations are needed.

Repeated exposure of mice to UV radiation induces an impairment in splenic APC function by a decrease in the number of APCs (2, 7, 8). However, we show that suppressed APC function by a single UVB radiation is not due to the decrease in the number of APCs but related to the decreased intensity of class II alloantigen expression. Treatment of spleen cells with in vitro UV radiation or with various inhibitors of RNA and protein synthesis causes a defect in the stimulatory capacity in allogeneic MLR (5, 9). However, there is no evidence that class II molecules themselves are affected. The suppressive mechanism of such treatment is due to the

Table IV. *Effect of UVB radiation on the stimulatory capacity of spleen cells*[a]

Treatment		ΔCPM	
Responder	Stimulator	Exp. 1	Exp. 2
NR	NR	56756±3132	83980±2546
NR	UV	37409±1348[b]	41340±3532[b]
NR	(−)	(5621±753)[d]	(7571±1772)[d]
UV	NR	29513±2372	39365±3233
UV	UV	15770±1483[c]	25850±641[c]
UV	(−)	(4734±767)[d]	(6622±1123)[d]

[a] B6 responder (splenic T cells) and BALB/c stimulator (spleen cells) were obtained from UVB (30 kJ/m^2)-irradiated and normal mice. Experiments were repeated 4 times, and similar results were obtained. Two representative data are presented. Each value is ΔCPM ± SE from triplicate samples.
[b] $p < 0.001$, vs the NR stimulator.
[c] $p < 0.01$, vs the NR stimulator.
[d] The background response.

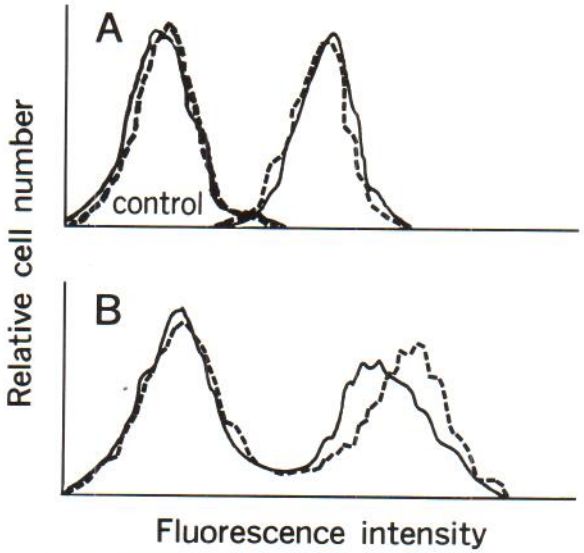

Fig. 1. FACS analysis pattern of class I (M1/42) (A) and class II (M5/114.15.2) (B) against spleen cells from UVB (30 kJ/m^2)-irradiated and normal BALB/c mice. Five mice from each group were examined. 4–6% of cells were weakly stained with FITC anti-rat Ig antibody alone (control).
(———), UV; (-----), NR.

Class I:	peak channel	UV, 139±6.4;	NR, 137±4.3
	% total	UV, 100;	NR, 100
Class II:	peak channel	UV, 172±3.8;	NR, 190±4.5 ($p < 0.001$)
	% total	UV, 49±5.4;	NR, 51±4.5

disturbance of certain metabolic activities (5), which may differ from in vivo UV treatment, because in vivo UV radiation does not penetrate to the spleen (10).

UV-irradiated keratinocytes release soluble factors involved in systemic immunosuppression (11, 12). On the other hand, prostaglandins, glucocorticoids, or α-fetoprotein can down-regulate class II antigen expression in vitro (13, 14). Our findings suggest that a single in vivo UV exposure causes the release of mediators involved not only in suppressed T cell alloreactivity but also in the decreased expression of class II alloantigen.

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