A-4. 2 UV ray-mediated immunomodulation and resulting changes in viral infection

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[A]. Effect of UV ray irradiation on humoral immunity of mice; Early promotion of antibody response

Abstract

In a series of studies about the UV effect on the humoral immunity of mice using influenza virus vaccine, we found that the antibody response was not suppressed even by prolonged UV-B irradiation. To examine this more precisely, herein, we used purified Tetanus toxoid and introduced ELISA to determine antibody production. In the early stage, one week postimmunization, the humoral immune response was extensively promoted compared to that in control mice. Herein, the conditions for irradiation were examined.

Key Words: Humoral immunity, Early promotion, Up-regulation,

Introduction

UV ray irradiation causes immuno-suppression but the activation or promotion of immune functions has not been studied detail [1)2)3)].

Many viral infections induce a potential humoral immune response with which the host animal recover from the disease. Any suppression of the humoral immunity by UV-B irradiation would alter the profiles of viral diseases in human society. However, few studies has been made concerning the effect of UV-B rays on humoral immunity. We examined this interesting theme.

Materials and Methods

Mouse: Female ddy mice (SLC, Hamamatsu, Japan) were used at 8 weeks old. Seven to 10 mice were grouped by experiment.

UV irradiation: Dorsal hair was clipped and the remaining hair was cleaned by depilatory cream. On the next day, 230 mJ/cm^2 (Toshiba FL20S.E, 20 W UV-B lamp, 40 cm distance for 60 min) was irradiated once.

Immunization: One day after irradiation, purified Tetanus toxoid (TTd) 5 Lf units (10.5 μg) was injected within the irradiated extent, subcutaneously. To examine the effect by a different routes, immunization was also performed by the intracutaneous, intraperitoneal and intravenous routes.

Determination of antibody titer: Serum samples were diluted by half-log and ELISA was performed according to the standard procedure with alkaline phosphatase (AP) labelled antibody to mouse IgG. The antibody titer was determined as serum dilution at OD_{410}=0.15 cut-off value and the geometrical mean and 95% confidence limits were calculated.

Results and Discussion

Figure 1 shows the antibody response in UV-B irradiated mice subcutaneously immunized with TTd. At one week after immunization, the mean antibody titer of irradiated mice was 8 fold higher than that of unirradiated control being significant difference. Within 2 weeks after immunization, thereafter, the humoral immune response of control mice promoted rapidly and no significant difference was seen in the antibody titer between the two groups of mice.

Herein, several conditions of irradiation were examined such as with different route of immunization, schedule of irradiation and UV dose. Those were summarized in Table I. The early promotion in antibody response by UV-B irradiation was not only detected with subcutaneous injection of TTd but also intracutaneous shot. This was of interest because many researchers have reported the depression of antigen presenting activity of Langerhans cells in the epidermis by UV irradiation [4)5)6)]. As to the test of time schedule of irradiation, the early promotion in humoral immune response at one day pre-irradiation or the same day irradiation to antigen injection were effective but less after irradiation. Sixty mJ, of which same degree of UV dose might be included in the sun light, was the lowest effective dose. This suggests that prolonged or frequent exposure to solar UV rays might promote the humoral immune response in humans.

This early promotion in humoral immunity suggests that a helper T cell, Th 2, is involved in this phenomenon and UV-B ray activate selectively Th 2 cells.

Figure 1

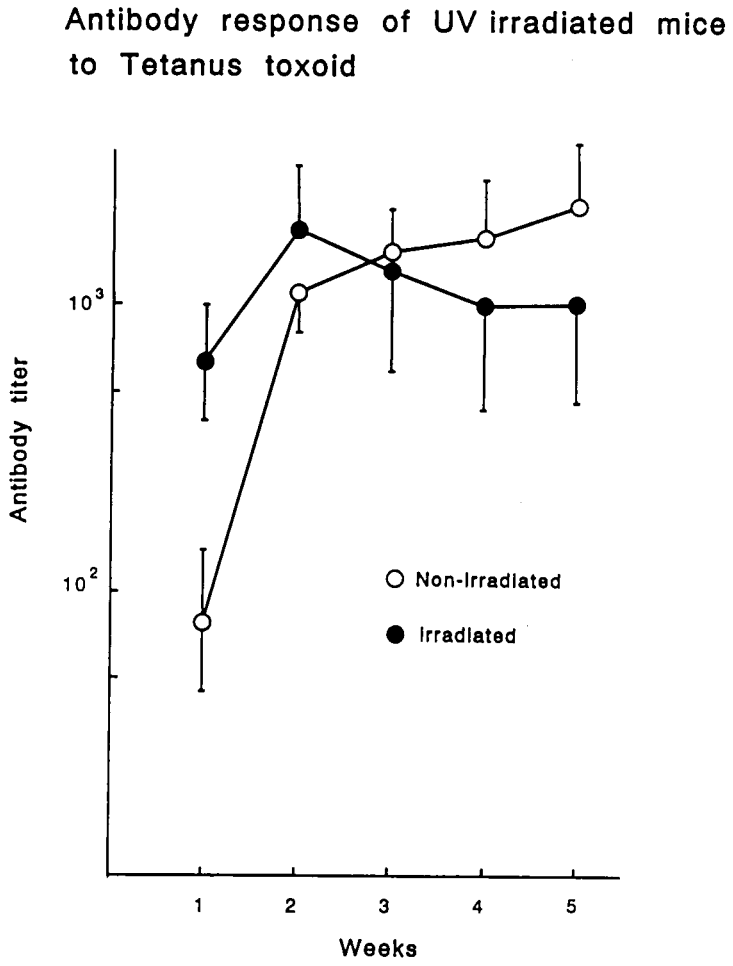

Table 1. Summary on early promotion of humoral immune response* by UV effects

Category of Test Condition		Degree of UV effect
1. Test of injection route		
Injection route	Correlation with UV (230 mJ/cm^2) irradiated lesion	
Subcutaneous	Same (Dorsal)	+ + + **
Intracerebral	Same (Dorsal)	+ + + **
Subcutaneous	Different (Inguinal)	−
Intraperitoneal	Different (Peritoneal)	−
Intravenous	Different (tail vein)	+ + **
Subcutaneous	non-irradiated (Control)	+ **
2. Time schedule		
Irradiation (230 mJ/cm^2)--24 hr--Immunization		+ + + **
UV--0 hr--Immunization		+ + + **
Immunization--UV		+ **
3. UV dose		
230 mJ/cm^2		+ + + **
115		+ + + **
58		+ **
0		−

+++: 7 fold or more than that of control IgG titer
++ : 3 to 6 fold
+ : less than 3 fold
* Verdict at one week after immunization
** Significantly different ($p<0.05$)

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[B]. Acute Phase response and inflammatory cytokine induction in mice following UV irradiation

Abstract

Expression of cytokines related to inflammation was studied in UV-irradiated skin. IL-1 β and IL-6 mRNAs which was not detected in normal skin, were progressively expressed in the skin on day 2 after UV-irradiation. ELISA showed that IL-6 protein but not IL-1 β was produced in the UV-irradiated skin, followed by an increase in its amount circulating in plasma.

Key words: Cytokine, IL-6, Acute phase response, Sialic acid

Introduction

Recently, we reported that UV-irradiation to mice resulted in an increase of the plasma sialic acid (Yamamoto et al. 1995)[1], which indicated induction of an acute phase response in vivo following inflammation. In such mice, various cytokines related to inflammation expressed, were released from and acted on various organs or tissues [2][3][4][5]. In our phenomenon, it was suggested that some cytokines were released from irradiated skin to liver because plasma sialic acid was a component of serum glycoproteins synthesized in the liver.

In this study, we examined the correlation between cytokine production and plasma sialic acid increase in UV-irradiated mice.

Materials and Methods

Mouse and UV: followed as described in part (A).

Assay of plasma sialic acid: Mouse plasma was bled from heart or suborbital genus. Sialic acid was determined with commercially available kit; Determiner SA (Kyowa Medex, Tokyo, Japan).

Determination of cytokines: The cytokine levels in plasma and skin tissue homogenate (10% in PBS) were determined using the commercially available ELISA kit for mouse cytokines, IL-1, IL-6 and TNF (Perceptive Diagnostics Inc., Cambridge. MA).

Reverse transcriptase polymerase chain reaction (RT-PCR): Total RNA in UV-irradiated mouse skin was extracted with a commercial regent, Isogen (Nippon Gene, Toyama, Japan). RT-PCR was performed as described previously (Kameoka et al., 1995)[6]. The PCR product was visualized by ethidium bromide staining after agarose gel-electrophoresis.

Results and Discussion

We examined cytokine mRNA expression in the UV-irradiated dorsal skin by RT-PCR (Fig.1). IL-1 α mRNA was not detected in either normal skin or that following UV-irradiation. On the other hand, IL-1 β mRNA was not detected in normal skin as well, but was extensively detected in the UV-irradiated skin at an early stage (5 hrs postirradiation), followed by a high level expression through the experimental period of 3 days. IL-6 mRNA was expressed slightly later than IL-1 β mRNA. IL-6 mRNA ,which is undetectable in normal skin, was detected as an intense band with RT-PCR on day 2 after UV-irradiation. The TNF α mRNA was not detected either with or without UV-irradiation. Our findings are in potential contrary to the previous reports, in which the mRNA or cytokine protein of L-1 α were detected in the skin under normal conditions [7][8].

We next analyzed the production of two cytokines by ELISA whose the mRNAs were detected by RT-PCR, using 10% homogenates of UV-irradiated skin. One day postirradiation, both cytokine proteins were detected (data not shown). Meanwhile, only IL-6 was detected in

Figure 1

Detection of cytokine m-RNA in dorsal skin of UV irradiated mouse.

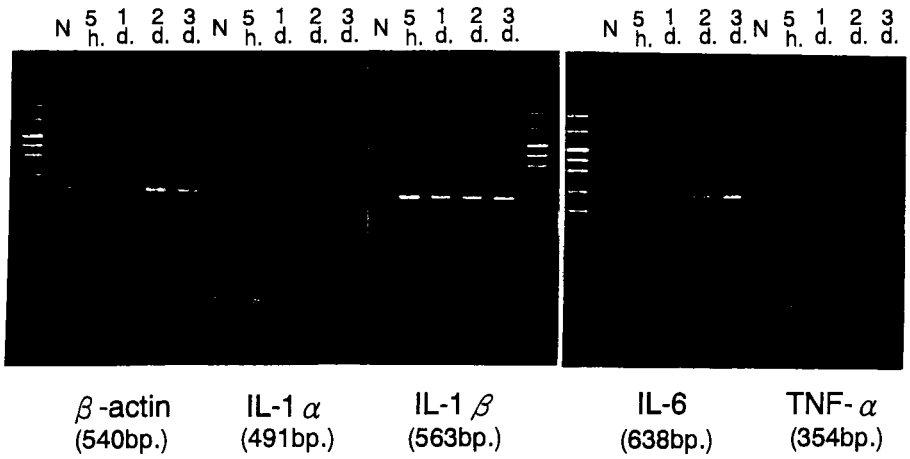
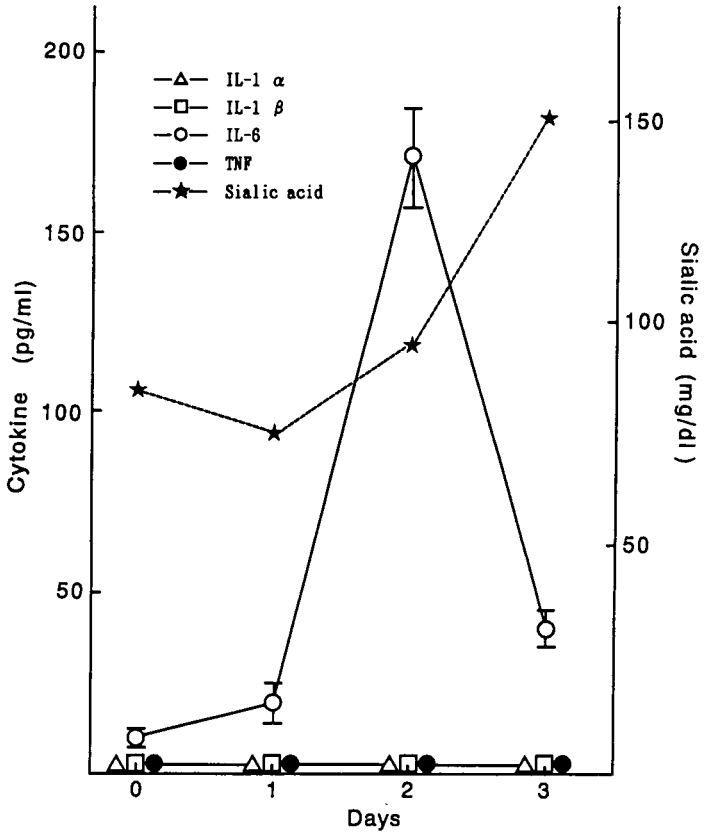


Figure 2

Cytokine release into and sialic acid increase in mice blood after UV irradiation.



the circulating plasma, IL-1 β beeing below the detectable level of 1pg/ml by the ELISA(Fig.2). IL-6 appeared in circulating blood with a sharp peak on day 2 postirradiation preceding the sialic acid increase in plasma, but on day 3 its level was rapidly decreased to the background level.

It is known that IL-1 and IL-6 are mediators of the acute phase response in which those released from inflamed tissues act onto liver and synthesize acute phase proteins [5]. Our findings in this study suggest that the IL-6 cytokine network is involved in the increase of plasma sialic acid level following UV-irradiation.

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[C] Alteration of cytokine production in lymphocytes from herpes simplex virus-infected mice by UV-B irradiation

Abstract

To determine whether the production of T-cell-derived cytokine (IFN- γ, IL-2 and IL-4) by immune cells from irradiated mice is also suppressed, we examined the production of cytokines by lymph node cells and spleen cells taken from UV-B irradiated, herpes simplex virus type 1 (HSV-1)-infected mice. UV-B irradiation prior to HSV-1 infection was found to markedly suppressed IFN- γ but less in IL-2 production compared to that of the non-irradiated control and IL-4 production slightly enhanced by UV-B irradiation.

Key ward: Cytokines, IFN- γ, IL-2, IL-4, Herpes simplex virus

Introduction

Previously, we investigated the effects of ultraviolet (UV)-B irradiation on immunity to herpes simplex virus (HSV) infection [1)2)] in mice in order to elucidate the possible mechanism(s) that might lead to a recrudescence of HSV infection. The findings showed that UV-B irradiation on the site of HSV injection results in a higher incidence of zosteriform lesions in mice and that suppressor T cells are induced in irradiated mice [1)].

In this study, we examined the effect of UV-B irradiation on the production of T-cell-derived cytokines of immune lymphocytes from UV-B irradiated, HSV type1 (HSV-1) infected mice to elucidate the possible involvement of an alteration of cytokine production in the pathogenesis of cutaneous HSV infection.

Materials and Methods

UV irradiation and HSV inoculation of mice: The chest fur of female BALB/c mice at a the age of 6-7 weeks were shaved and then exposed to 120 mJ/cm^2 of UV-B ray.

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Virus inoculation and immune lymphocyte preparation: Mice were inoculated with 5X10^4 PFU of HSV-1 at the site of UV-B irradiation. On day 8 of inoculation, cells collected from draining lymph nodes (LN) and spleen were suspended in RPMI 1640 medium with 10% fetal calf serum and inactivated HSV-antigen. The culture supernatants were collected as samples of cytokine on day 1 to 5.

Cytokine assay: IFN- γ was measured by ELISA method using home-made ELISA-plate coated with 1.5 μg of rat anti-murine IFN- γ monoclonal antibody (LEE Biomolecular Research Inc., San Diego, CA). Further steps were performed according to the standard indirect method. IL-2 and IL-4 were measured by the bioassay-method with CTLL-2 cells of which growth was depend on the IL-2/IL-4 activity. The cells were cultured in the presence of a cytokine sample and the incorporated radioactivity of [^{3}H]-thymidine was counted in a liquid scintillation counter.

Results and Discussion

In the control mice, 100 U/ml of IFN- γ was detected on day 1 in the supernatant of lymph node cells and reached a peak on day 3. On the other hand, the culture fluid of LN cells from UV irradiated mice showed a lower amount on any day compared to that of the control mice. In spleen cell culture, IFN- γ which was evident in normal mice on day 5 were suppressive in that of UV-irradiated mice (Fig.1).

Table 1 summarizes a part of the results of IL2/IL-4. IL-2/IL-4 activity in the supernatants of the culture of lymph node cells from UV-irradiated mice was significantly suppressed, especially on days 1 and 5, in comparison to that of control mice. The suppression of the cytokine production by spleen cells from UV-irradiated mice was not evident until day 3 but then became significant by day 5.

The experiment using anti-IL-2 monoclonal antibodies showed that culture with spleen cells from control mice abrogated CTLL-2 cell growth by 75% whereas that with spleen cells from UV-irradiated mice revealed only 54% abrogation and 46 % of the proliferative response depending on IL-4 was restored.

In this study, we demonstrated that UV-B irradiation altered the cytokine production by immune cells of HSV infected mice. The production of IFN- γ and IL-2 was decreased, whereas that of IL-4 was enhanced in the early stage. These findings suggest the preferential activation of Th2 and the selective suppression of Th1 helper T cells in UV-B irradiated mice followed by HSV infection. Finally, alteration in cytokine production profile may be involved in the development of severe skin lesions caused by HSV infection in UV-B irradiated mice since HSV is cleaned from the site of infection mainly by cellular immune responses such as delayed type hypersensitivity reaction, a Th1 cell function [3)4)].

Reference

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Figure 1

Activity of IFN-γ in the supernatant from cultures of HSV-stimulated immune lymphocytes.

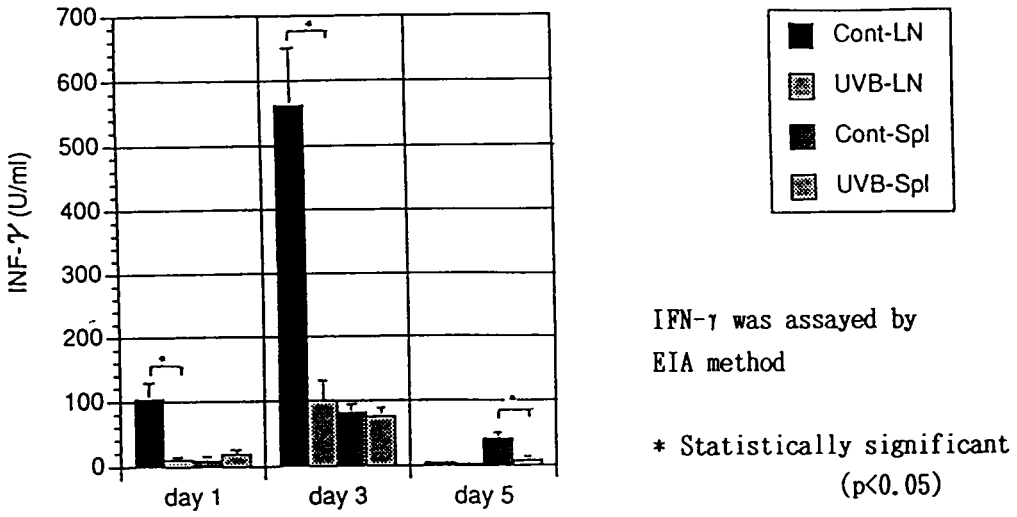

Table 1. IL-2 and IL-4 activity in the supernatant from culture of HSV-immune cells

		[^{3}H] thymidine uptake by CTLL-2 cells (x10^{3}cpm) in					
		LN			Spleen		
Cytokine		Culture day					
Source	UV	1	3	5	1	3	5
Control	−	1.7	2.4	1.8	1.8	2.1	1.4
	+	2.1	2.1	1.9	1.9	2.3	2.3
Supernatant	−	97.1	133.9	88.7	69.8	117.6	122.7
(1:2 dilution)	+	36.3*	108.5	14.2*	53.1	110.8	46.4*

*Statistically significant (P<0.05) by Student's t-test compared to that of non-irradiated control.