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# Prevention of ultraviolet radiation-induced suppression of contact hypersensitivity by *Aloe vera* gel components

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**Abstract**

We have recently reported that *Aloe vera* gel contains small molecular weight immunomodulators, G1C2F1, that restore ultraviolet B (UVB)-suppressed accessory cell function of epidermal Langerhans cells (LC) in vitro. In the present study we evaluated the UVB-protective activity of G1C2F1 in vivo. Exposure of the shaved abdominal skin of mice to 2.4 KJ/m$^2$ of UVB radiation resulted in suppression of contact sensitization through the skin to 41.1%, compared to normal unirradiated skin. Topical application of G1C2F1 immediately after irradiation reduced this suppression significantly. The percentage recovery of UVB-suppressed contact hypersensitivity (CHS) response was 52.3, 77.3, and 86.6% when the irradiated skin was treated once with 0.1, 0.5, and 2.5 mg/ml of G1C2F1-containing cream, respectively. G1C2F1 did not show nonspecific stimulatory activity on CHS response. The present study, together with the previous observation, show that *Aloe vera* gel contains small molecular weight immunomodulators that prevent UVB-induced immune suppression in the skin by restoration of UVB-induced damages on epidermal LC. 

*Keywords: Aloe vera*; Immunomodulator; Ultraviolet B; Contact hypersensitivity

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## 1. Introduction

Exposure to UVB (280–320 nm) has been shown to generate an immunosuppressive microenvironment in the skin. UVB irradiated skin was unable to elicit sensitization to contact allergens, but rather induced tolerance to them (Toews, Bergstresser, & Streinlein, 1980; Elmet, Bergstresser, Tigelaar, Wood, & Streilein, 1983). UVB irradiated skin was also unable to reject syngeneic UVB-induced tumors, although the same tumors were strongly rejected when transplanted into non-UVB-exposed syngeneic mice (Kripke, 1984).

Local immunosuppression by UVB radiation appears to be closely related to damages of epidermal LC, which are accessory cells delivering costimulatory signals as well as antigens to T cells (Streilein, Grammar, Yoshikawa, Demiden, & Bermeer, 1990). UVB radiation was shown to abrogate the accessory cell function of LC (Stingl, Gazze-Stingl, Aberer, & Wolff, 1981; Austaad & Braathen, 1985; Cruz, Nixon-Fulton, Tigelaar, & Bergstresser, 1989; Simon, Tigelaar, Bergstresser, Edelbaum, & Cruz, 1991), probably through distortion or deletion of costimulatory molecules such as ICAM-1 from LC (Krutmann et al., 1990; Tang & Udey, 1992a). Functional inactivation of costimulatory signals by UVB radiation would result in conversion of the function of LC from immunogenic to tolerogenic (Simon, Krutmann, Elmets, Bergstresser, & Cruz, 1992). In higher doses, UVB radiation was shown to exert more severe toxic activity for LC leading ultimately to elimination (Bergstresser, Toews, & Streilein, 1980; Lynch, Gurish, & Daynes, 1981; Tang & Udey, 1992b).

An immunomodulator that prevents UVB-irradiated LC from functional alteration or elimination would reduce some of the immunosuppressive effects of UVB in the skin. Such an immunomodulator would be invaluable in preventing UVB-induced immune suppression in the skin, because currently used sunscreens such as *para*-aminobenzoate (Padimate) and 2-ethylhexyl-*p*-methoxycinnamate (2-EHMC) were shown to be unable to reduce local immune suppression, although they could reduce sunlight-induced erythema (Mommaas, van Praag, Bavinck, Out-Luiting, & Vermeer, 1990; Ho, Halliday, & Barnetson, 1992).

To search for an immunomodulator that prevents UVB-induced functional alteration of LC, we developed an in vitro screening system (Lee et al., 1997). Briefly, freshly isolated murine epidermal LC were irradiated with UVB (180 $J/m^2$), and then cultured in a cell culture medium containing the sample(s) to be tested for 2 days. Accessory cell function of the UVB-irradiated and then sample-treated LC was measured by assessing their ability to support anti-CD3 monoclonal antibody-primed T cell mitogenesis. With this in vitro functional assay method, we identified and purified UVB-protective immunomodulators from the gel of *Aloe vera* (Lee et al., 1997). The activity-guided sequential fractionation of the gel of *Aloe vera* showed that it contains at least two small molecular weight immunomodulators that prevent UVB-induced suppression of LC accessory cell function in vitro. In the present study we evaluated the UVB-protective activity of the immunomodulators in vivo.

## 2. Experimental

### 2.1. Mice

Specific pathogen-free male C57BL/6 mice were purchased from Korea Research Institute of Chemical Technology (Daejeon, Korea), and used between 8–14 weeks of age.

### 2.2. Isolation of Aloe component, G1C2F1

The *Aloe* component, G1C2F1, was isolated from the gel of *Aloe vera* as described previously (Lee et al., 1997). Briefly, the lyophilized *Aloe vera* gel, provided from The Aloe Research Foundation (Fort Worth, Tx, USA), was dissolved in distilled water, and then fractionated according to the molecular size. Components in the range of 500–1000 Da were collected, and then further separated into five subfractions on a Bio-Gel P-2 (Bio-Rad) column. The second fraction, G1C2F1, was able to protect UVB-induced damages on LC. G1C2F1 could be further divided into two UVB-protective components, but in the present study we used G1C2F1 to investigate UVB-protective activity in vivo due to quantity limitations.

### 2.3. UVB irradiation

The abdominal fur of mice was clipped and then shaved with razor blades one day before UVB irradiation. On the next day, the shaved skin was exposed to UVB radiation after anesthetization with methoxyfluorane (Pitman-Moore Inc., Washington Crossing, NJ) and wrapping the other portion of the mice with aluminum foil. The UVB source was a bank of three FS-20 sun lamps (Westinghouse Co., Bloomfield, NJ). UV exposures were measured using a UVX radiometer (UVP, San Gabriel, CA) equipped with a UVX-31 sensor.

### 2.4. Treatment

The irradiated skin was treated immediately after irradiation with G1C2F1-cream. G1C2F1-cream was prepared by emulsifying G1C2F1, which was dissolved in distilled water to three times the final concentration, into twice volume of polyethylene glycol (PEG) cream in a mortar. The PEG cream was a 1:2 mixture of PEG 8000 (Sigma) and PEG 400 (Junsei Chemical Co., LTD, Japan), which were melted and then fused at 60°C water bath. Control cream was prepared by emulsifying distilled water into twice volume of PEG cream in a mortar. Each of the creams was applied to the irradiated skin in a volume of 0.1 ml per mouse by spreading gently with a gloved finger. Usually, 95% of the cream (i.e., 95 μl) was spread on the irradiated skin as measured by the residual cream on the gloves.

### 2.5. Contact hypersensitivity (CHS)

The abdominal skin of mice was irradiated with UVB, and then treated the irradiated skin immediately after irradiation with G1C2F1-cream or control cream as described in the above section. Three days after irradiation and treatment, mice were sensitized with 100 μl of 3% trinitrochlobenzene (TNCB) dissolved in acetone through the irradiated skin. Six days after sensitization, mice were challenged by painting 10 μl of 1% TNCB dissolved in 4:1 mixture of acetone and olive oil on both the dorsal and ventral surfaces of right ear. Ear thickness were measured by a dial thickness gauge (Ozaki MFR, Co., LTD, Japan) immediately before challenge and 24 h after challenge. Specific ear swelling (Δear thickness) was determined by substracting the average ear thickness measured immediately before challenge from that measured 24 h after challenge for each group (at least seven mice per group), and the

percentage recovery of CHS response was calculated as follows:

$$\frac{[\Delta\text{ear thickness of G1C2F1-treated group} - \Delta\text{ear thickness of irradiated control group}]}{[\Delta\text{ear thickness of unirradiated control group} - \Delta\text{ear thickness of irradiated control group}]} \times 100$$

## 3. Results

### 3.1. Determination of UVB dose

To determine optimal dose of UVB radiation to suppress CHS response to TNCB, the shaved ventral skin was exposed to different doses of a single UVB radiation, rested for 3 days, and then painted with TNCB. Six days later, right ears of the mice were challenged with TNCB. The CHS response is illustrated by the net ear swelling (Fig. 1). Exposure to a single UVB radiation suppressed CHS response in a dose dependent manner reaching plateau of suppression at around 3.0 KJ/m$^2$ of UVB radiation (some of the data is not shown). At a dose of 2.4 KJ/m$^2$ of UVB radiation, the average ear swelling was reduced from 31.4 to 16.0 mm $\times$ $10^{-2}$ (57.9% suppression; $P < 0.001$). From this result, we decided to irradiate the shaved ventral skin with a single dose of 2.4 KJ/m$^2$ of UVB radiation.

### 3.2. Restoration of UVB-suppressed CHS response by G1C2F1

The UVB-protective activity of G1C2F1 was determined through four independent experiments, and the representation of all the experiments is shown in Fig. 2. A single dose of

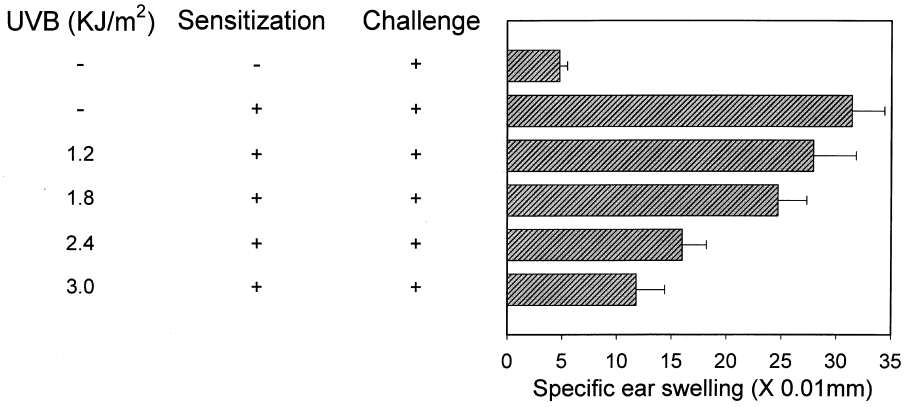


Fig. 1. Suppression of CHS response by UVB radiation. The shaved abdominal skin of C57BL/6 mice was exposed to different doses of a single UVB radiation or sham-radiation. Three days after irradiation, mice were sensitized with 100 μl of 3% trinitrochlobenzene (TNCB) through the abdominal skin. Six days after sensitization, mice were challenged by painting 10 μl of 1% TNCB on both sides of right ear. Ear thickness was measured immediately before challenge and 24 h after challenge. Specific ear swelling was determined by substracting the average ear thickness measured immediately before challenge from that measured 24 h after challenge for each group. The data are expressed as mean $\pm$ SE of at least seven mice per group.

2.4 KJ/m$^2$ of UVB radiation followed by treatment with cream base alone suppressed the CHS response to 44.3% compared to the unirradiated, cream base-treated group. Topical application of G1C2F1-cream (0.1 m1/mouse) immediately after irradiation reduced this suppression significantly. The percentage recovery of UVB-suppressed CHS response was 52.3, 77.3 and 86.6% when the irradiated skin was treated once with 0.1, 0.5, and 2.5 mg/ml of G1C2F1-cream, respectively.

### 3.3. Nonspecific stimulatory activity of G1C2F1 on CHS response

To examine whether G1C2F1 by itself has nonspecific stimulatory or suppressive activity on CHS response, G1C2F1-cream or cream base alone was applied topically to shaved, but not irradiated, abdominal skin of mice. Three days later, mice were sensitized and then challenged with TNCB by the same experimental procedures used to examine UVB-protective activity of G1C2F1. As shown in Fig. 3, treatment of the shaved skin of mice with G1C2F1 did not increase the specific ear swelling, suggesting that it does not have nonspecific stimulatory activity on CHS response. Rather, G1C2F1 appeared to reduce CHS response, but the decrease was not significant when compared to the cream base-treated control group in Student's *t*-test.

## 4. Discussion

Previously, we reported that *Aloe vera* gel contains small molecular weight immunomodulators, G1C1F1, that restore UVB-suppressed accessory cell function of

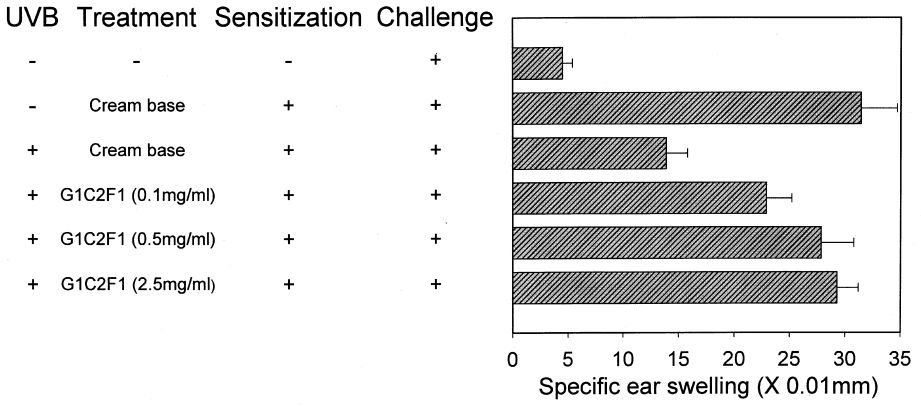


Fig. 2. Prevention of UVB-induced suppression of CHS response by *Aloe vera* gel components. The shaved abdominal skin of C57BL/6 mice was exposed to a single dose of 2.4 KJ/m$^2$ of UVB radiation or sham-radiation. The irradiated skin was treated immediately after irradiation with 0.1 ml of G1C2F1-cream (final concentration = 0.1, 0.5 or 2.5 mg/ml) or cream base alone by gentle spreading with a gloved finger. Three days after treatment, mice were sensitized and then challenged with TNCB, and then specific ear swelling was determined by the same methods described in Fig. 1. The data are expressed as mean ± S.E. of at least five mice per group. This experiment was performed four times, and these data are representative of all results.

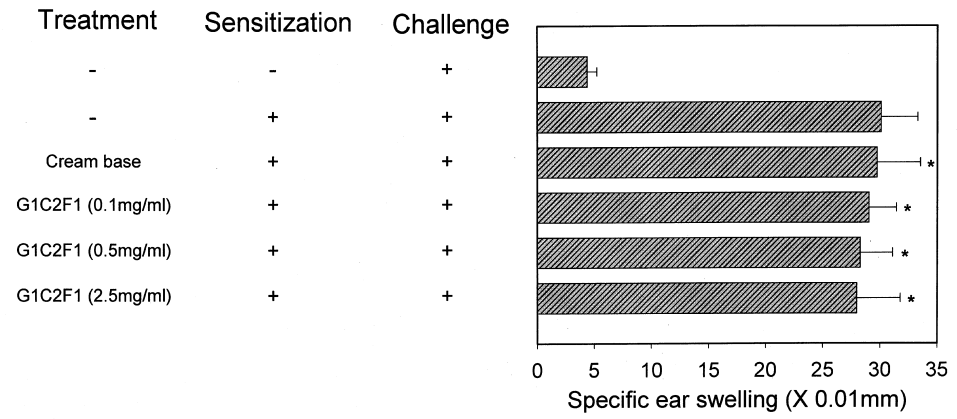


Fig. 3. Effects of *Aloe vera* gel components on the CHS response in normal unirradiated mice. The shaved abdominal skin of C57BL/6 mice was treated with 0.1 ml of G1C2F1-cream (final concentration = 0.1, 0.5 or 2.5 mg/ml) or cream base alone by gentle spreading with a gloved finger. Three days after treatment, mice were sensitized and then challenged with TNCB, and then specific ear swelling was determined by the same methods described in Fig. 1. The data are expressed as mean ± S.E. of at least nine mice per group.*$P > 0.05$.

epidermal LC in vitro (Lee et al., 1997). In the present study we evaluated the UVB-protective activity in vivo. In evaluating the UVB-protective activity in CHS model in mice, we decided to induce local immune suppression by a single radiation exposure instead of several daily radiation exposures. The irradiated skin was then treated immediately after irradiation once with G1C2F1-cream. A single radiation exposure followed by treatment ensures that the UVB-protective activity of G1C2F1 is not due to sunscreen activity by the residual G1C2F1 from the previous treatment, which may happen in several daily UVB exposures followed by treatment for each exposure.

We believe that the present study has at least two important implications related to the prevention of UVB-induced suppression of skin's immune responses. Firstly, the gel of *Aloe vera*, which has long been used to ameliorate sun burns, has been confirmed to contain small molecular weight immunomodulators that prevent UVB radiation-induced suppression of contact hypersensitivity response. The immunomodulators prevent UVB-induced suppression of skin's cellular immune response in a dose-dependent manner, and this prevention is not due to non-specific stimulatory activity of the cellular immunity in the skin. Secondly, searching for an immunomodulator which restores accessory cell function of UVB-damaged LC in vitro would be an appropriate method for the screening and development of UVB-protective immunomodulators. With this in vitro screening method, we have identified several soil microorganisms producing UVB-protective immunomodulators (Mo et al., 1995). The present study confirms the predictive value of the *in vitro* screening technique that we have previously developed (Lee et al., 1997).

We conclude that G1C2F1 may exert UVB protection through functional alteration or the prevention of elimination of UVB-irradiated LC activity because G1C2F1, isolated from the gel of *Aloe vera,* restores accessory cell function, suppressed by UVB-irradiation, of LC in vitro. In addition, topical application of *Aloe* gel extract has previously been reported to

preserve the number and morphology of UVB-irradiated LC in the skin (Strickland, Pelly, & Kripke, 1994). Additionally, G1C2F1 may also prevent secretion of immunosuppressive cytokines such as tumor necrosis factor-α (Yoshikawa & Streilein, 1991) and interleukin-10 (Ulrich, 1994) from UVB-irradiated keratinocytes. Another interesting observation through this experiment was that, although treatment with G1C2F1 prevented UVB-induced suppression of CHS response in a dose-dependent manner, the irradiated skin of G1C2F1-treated groups showed similar levels of sun burn when examined on the day of sensitization or challenge. This may indicate that G1C2F1 prevents UVB-induced elimination or functional alteration of epidermal LC through a mechanism that does not alter the initial inflammatory response and release of pro-inflammatory cytokines.

In summary, topical application of G1C2F1, which was isolated from the gel of *Aloe vera* by its activity to restore UVB-induced suppression of accessory cell function of LC in vitro, to the UVB-irradiated skin of mice prevented UVB-induced suppression of CHS response to TNCB. As confirmed in the present study, searching for an immunomodulator that restores accessory cell function of UVB-irradiated LC would be an appropriate in vitro screening method to find out UVB-protective immunomodulators.

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