Prevention of Ultraviolet Radiation-Induced Suppression of Contact and Delayed Hypersensitivity by Aloe barbadensis Gel Extract

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We investigate the ability of Aloe barbadensis gel extract to prevent suppression of contact hypersensitivity (CHS) and delayed-type hypersensitivity (DTH) responses in mice exposed to ultraviolet (UV) irradiation. Local immune suppression was induced in C57 mice by exposure to four daily doses of 400 J/m² UV-B (280-230 nm) radiation from FS40 trolleys, followed by sensitization with 0.5% fluorescein isothiocyanate (FITC) through the irritated skin. Topical application of 0.167-1.67% Aloe gel after each irradiation significantly reduced this suppression. Aloe treatment partially preserved the number and morphology of Langerhans and Thy-1+ dendritic epidermal cells in skin, compared to those in the skin of mice given only UVR or UVR plus the vehicle. Experiments using a single (2 kJ/m²) dose of UV followed by Aloe treatment showed that the effect of Aloe was not due to screening of the UVR. Systemic suppression of DTH to Candida albicant or CHS to FITC was induced in C3H mice exposed to 5 or 10 kJ/m² UV-B radiation, respectively, on the dorsal skin 3 days later with a subcutaneous injection of formalin-fixed Candida or FITC painted on unirradiated, ventral skin. Treatment of the UV-irradiated skin with Aloe immediately after irradiation prevented suppression of both DTH to Candida and CHS to FITC. Aloe treatment did not prevent the formation of cyclocloty, pyrimidine dimers in the DNA of UV-irradiated skin or accelerate the repair of these lesions. These studies demonstrate that topical application of Aloe barbadensis gel extract to the skin of UV-irradiated mice ameliorates UV-induced immune suppression by a mechanism that does not involve DNA damage or repair. Key words: photo protection/Candida albicant/cyclocloty/ UV-B. J Invest Dermatol 102:197-204, 1994

Chronic exposure of the skin to ultraviolet radiation (UVR) causes skin cancer in humans and laboratory rodents [1-3]. Furthermore, exposing the skin of experimental animals to wavelengths of UVR in the UV-B (280-320 nm) region of the spectrum impairs their ability to develop immune responses to UV-induced skin cancers, contact-sensitizing agents, and a variety of infectious microorganisms [4-7]. Several models have been developed to study the mechanisms of UV-induced immune suppression. In the local suppression model, exposing C3H/HeN mice to low doses of UV-B radiation inhibits the induction of the contact hypersensitivity (CHS) response to hapten applied at the site of irradiation. The depressed immune response is accompanied by a decrease in the number of Langerhans and Thy-1+ dendritic epidermal cells (DETC) in the skin [8-10] and by the appearance of lupus-specific suppressor T cells in the spleen [10-13]. In the systemic suppression model, higher doses of UV-B are used to suppress the induction of CHS and delayed-type hypersensitivity (DTH) responses to antigens introduced at a non-UV-irradiated site [8]. Systemic suppression of CHS and DTH is also accompanied by the appearance of antigen-specific suppressor T lymphocytes [5,6,11,14]. The precise mechanism by which UVR induces immune suppression are unclear; however, a picture is emerging of the sequence of events leading to the downregulation of immune response. Local suppression appears to be mediated by alterations in the activity of epidermal Langerhans cells and involves the release of tumor necrosis factor alpha (TNF-α) [15] and perhaps the formation of cis-urocanic acid (cis-UCA) [16,17] in UV-irradiated skin. Recent evidence suggests that DNA damage is the initial event that triggers systemic suppression of DTH and CHS [18]. Cyclocloty such as TNP and IC [19,20], UV-41 [20], and other soluble factors produced by UV-irradiated keratinocytes [21] have been implicated as mediators of systemic immune suppression and may occupy intermediary status in the cascade of events leading from DNA damage to immune suppression.

Extracts from a number of Aloe species are supported to possess therapeutic properties and have been used to alleviate the pain of burns and in the healing of thermal burns [22]. Aloe extracts, derived primarily from commercially grown Aloe barbadensis, have made their way into a myriad of health and cosmetic products, although scientific evidence for their efficacy is limited. Because of their reported anti-inflammatory effects [23], we wanted to determine whether application of an Aloe extract to UV-irradiated skin would alter the induction of immune suppression. Therefore, we investigated the ability of a standardized sample of Aloe gel extract to inhibit the effects of UV-induced suppression of both the local and systemic immune suppression.

MATERIALS AND METHODS

Microsite-pathogen-free female C3H/HeN(MTV) mice were obtained from the Animal Production Area of the National Cancer Institute.

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Abbreviations: CHS, contact hypersensitivity; cis-UCA, cis-urocanic acid.

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197
of the skin through the action of dermal and epidermal tissues. After exposure, the animal was killed, and the skin was removed. The dermal tissue was sectioned and stained with hematoxylin and eosin for histological examination.

**RESULTS**

The results of this study revealed that UVB radiation significantly increased the number of cells with nuclear DNA damage. This was evident in the form of hyperchromatic nuclei that were visible under the microscope. The control group, which was not exposed to UVB radiation, showed no such changes. These findings support the hypothesis that UVB radiation is a significant factor in the development of skin damage and carcinogenesis.

**CONCLUSION**

The study highlights the importance of protecting the skin from UVB radiation to prevent DNA damage and subsequent health issues. Public health campaigns should encourage the use of sunscreen and other protective measures to reduce the risk of skin damage. Further research is needed to explore the long-term effects of UVB radiation on skin health and the development of more effective protective strategies.

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**Acknowledgments:**

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**References:**


Additional control experiments were performed to determine whether vehicle alone suppressed CHS and whether aleor trop-specifically increased the ear swelling response. Mice were treated with vehicle or 0.5% Ale or vehicle on the day before and additional doses were not sensitized. After 5 d, their ears were painted with FITC, and ear swelling was measured 24 h later. Vehicle-treated mice showed 1.2 ± 0.76 × 10^-2 mm ear swelling, whereas the Ale-treated group had a 0.5 ± 0.65 × 10^-2 mm response (not shown).

These data show that neither vehicle nor Ale had non-specific suppressive or stimulatory effects on the ear swelling response by themselves.

Effect of Aleor on ATPase-, La+, and Thy-1+ Dendritic Epidermal Cells in Mouse Skin. In CHS mice, reduced CHS responsiveness correlates with reduced numbers of dendritic cells in the following UV irradiation. Therefore, we examined the numbers and morphology of Langhan's and Thy-1+ dendritic epidermal T cells (DCT) in skin treated with Ale or low-dose UV. Groups of three CHS mice were treated as described above with four daily doses of 400 J/m^2 UV-B radiation, followed within 3 min by topical application of Ale or vehicle to the irradiated skin. Non-irradiated control groups received vehicle or Ale on their normal skin.

The epidermal sheets were analyzed for numbers of ATPase+, La+, and Thy-1+ cells.

The results from two experiments are presented in Table 1. Unirradiated mice treated with vehicle alone or Ale had numbers of ATPase+ cells similar to those in normal controls. UV-B irradiation reduced the number of ATPase+ cells in skin by 90% compared with the untreated control group. Ale treatment of UV-irradiated skin increased the number of ATPase+ cells by 77 to 112%, compared with the UV-irradiated group. Vehicle alone failed to prevent the loss of ATPase+ cells by UV. The number of La+ cells in epidermal sheets was similar to that of ATPase+ Langhans cells (Table 1). Mice treated with vehicle alone or Ale showed a similar number of La+ cells on the skin at sensitization (Table 1). UV B followed by vehicle treated reduced the number of La+ cells in the UV-irradiated skin by approximately 80%. UV-irradiated skin treated with Ale had approximately 2.5 times more La+ cells than UV-irradiated skin.

We also examined the effects of UV on DCT. Treatment of unirradiated skin with vehicle alone or Ale in vehicle reduced the numbers of Thy-1+ DCT by 10 to 37% compared with naive controls, however, this reduction was not statistically significant. UV irradiation reduced the number of DCT cells by 78 to 90% compared with that in normal skin. Ale treatment following UV irradiation significantly increased the numbers of DCT cells compared with that in the UV-irradiated control group in experiment 2, but in experiment 1 the increment was not statistically significant. The effect of these treatments on the morphology of the dendritic epidermal cells was examined in whole mounts of epidermal sheets. Untreated skin had numerous ATPase+ cells evenly distributed throughout the epidermis. The ATPase+ cells in unirradiated (Fig 2A) or Ale-treated skin (Fig 2B) exhibited an intensely stained cornified layer, which had multiple, branching dendrites. The morphology of the ATPase+ cells in vehicle-treated skin was indistinguishable from that of cells in unirradiated epidermis (not shown). UV injury greatly reduced the number of ATPase+ Langhans cells in skin (Fig 2C). The few remaining cells stains poorly, and their dendritic processes were absent or blurred. In contrast, ATPase+ cells were more numerous in UV-irradiated skin treated with Ale (Fig 2D). The ATPase+ cell bodies were more intensely stained and...
|
|---|
| **Table 1. Effect of Alow Gel Extract on Numbers of Different Epidermal Cells in Low-Dose UVB-Treated C3H Mice** |

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
</tr>
<tr>
<td>Epidermal Sheets</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>797 ± 64</td>
</tr>
<tr>
<td>Lm</td>
<td>781 ± 98</td>
</tr>
<tr>
<td>Thy-1</td>
<td>652 ± 48</td>
</tr>
</tbody>
</table>

**Experiments 1**

**ATPase**

**Lm**

**Thy-1**

**ATPase**

**Lm**

**Thy-1**

**Notes**

1. **Control** groups were treated with normal saline (0.9%).
2. **UV-B+B** groups were treated with UV-B and Alow both, and Alow was applied 24 hours after the last treatment of UV-B. The effects were evaluated for 10 days. Each group consisted of 10 mice, and the data are presented as mean ± standard error (SE). The significance of differences between groups was determined using the Student's t-test.

**Efficacy of Alow and Aquaphor Vehicle on Sunscreening**

The ability of vehicle and Alow to act as sunscreen was tested directly in mice by measuring their ability to reduce the sunburned ears. The ability of agents to prevent UV-induced edema has been used by other investigators as a measure of sunburn efficacy and correlates well with SPF values for commercial sunscreen formulations using human subjects [24]. To test whether Alow alone reduced the irradiance received by skin, Aquaphor vehicle or 0.5% Alow in vehicle was applied to the ears of mice 15 min before exposure to a 5 kJ/m² dose of UV-B. The edema was quantified by measuring the change in ear thickness at 24-h intervals for three consecutive days. The data are presented in Table II. The maximum ear swelling occurred by 48 h after UV irradiation. Neither vehicle nor Alow significantly affects ear swelling in UV-irradiated mice. Non-irradiated control groups, included to determine whether vehicle or Alow by themselves affected ear thickness, showed no effect. These data demonstrate that the effects of UV-induced reductions of CHS was not due to sunscreening activity by the Alow or vehicle. One possible explanation for the ability of Alow's application to prevent sunburn of CHS might be that it blocks UV-Pt generation, thereby decreasing the effective dose of UV-B received. **Figure 2. Effect of Alow barbata gel extract on the number of ATPase-expressing Lm cells in murine skin.** (A) Unirradiated murine skin in C3H/HN mice stained for ATPase activity; (B) 6.0% Alow in vehicle was applied daily for five continuous days (C) skin exposed to low daily doses of 400 mJ/cm² UV-B radiation. D) skin treated with Alow after each of the four daily exposures to UV (T). E) skin exposed to low daily doses of 400 mJ/cm² UV-B radiation. Non-irradiated control groups included to determine whether vehicle or Alow by themselves affected ear thickness, showed no effect. These data demonstrate that the effects of UV-induced reductions of CHS was not due to sunscreening activity by the Alow or vehicle. One possible explanation for the ability of Alow's application to prevent sunburn of CHS might be that it blocks UV-Pt generation, thereby decreasing the effective dose of UV-B received. **Figure 3. Effect of Alow barbata treatment on UV-B induced DNA synthesis in C3H/HN mice.** (A) Normal C3H/HN mice treated with Alow and UV-B, F) 1% Ficoll-anti-rabbit second antibody; (B) Alow-treated mice C) exposed to four daily doses of 400 mJ/cm² UV-B radiation. D) treated with Alow after each of the four daily exposures to 400 mJ/cm² UV-B radiation. Non-irradiated control groups included to determine whether vehicle or Alow by themselves affected ear thickness, showed no effect. These data demonstrate that the effects of UV-induced reductions of CHS was not due to sunscreening activity by the Alow or vehicle. One possible explanation for the ability of Alow's application to prevent sunburn of CHS might be that it blocks UV-Pt generation, thereby decreasing the effective dose of UV-B received.
Table II. Effect of Abe on Vehicle on UV-Induced Edema*  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-B + Vehicle</td>
<td>0.7 ± 0.7</td>
<td>7.9 ± 2.1</td>
<td>5.5 ± 2.4</td>
</tr>
<tr>
<td>UV-B + Abe</td>
<td>1.2 ± 0.8</td>
<td>8.0 ± 1.0</td>
<td>7.6 ± 3.6</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.9 ± 1.7</td>
<td>6.6 ± 1.5</td>
<td>6.0 ± 2.2</td>
</tr>
<tr>
<td>Abe</td>
<td>0.0 ± 0.0</td>
<td>0.8 ± 1.5</td>
<td>0.7 ± 1.3</td>
</tr>
</tbody>
</table>

* Abe in Aquaport vehicle or Aquaport alone was applied to the ears of groups of 5 C57BL/6J mice 15 min before their exposure to 3 kJ/m2 UV-B. The change in ear thickness was determined by subtracting the thicknesses of each ear at 24, 48, and 72 h after treatment from their values before treatment. Values are expressed as the mean ± S.D. Mean ± S.D. of all groups before treatment was 0.4 ± 0.1. UVR-induced ear thickness differences were linear between the control UV-irradiated group and UV-irradiated/vehicle or Abe/vehicle-treated mice at any timepoint (p < 0.05 by Student's t test).

Figure 4. Effects of Abe treatments on UVB-induced local suppression of CHS in FITC. Groups of five C57BL/6J mice were given 2 kg/m² in a single exposure on their shaved ear skin followed immediately by topical applications of 0.5% Abe in vehicle or vehicle alone. Unirradiated control groups were shaved and treated with Abe or vehicle. These data illustrate that mice were sensitized with 0.5% FITC through their vehicle skin. Five days after radiation, the mice were challenged on their ears with 0.2% FITC and swelling measured 24 h later. These data are the mean ± standard deviation of 15 mice from three separate experiments. As in Fig. 1, data were analyzed by ANOVA. Experiment-to-experiment variability was not significant, thus permitting the pooling of data. Percent suppression compared with the appropriate sensitized group is given in parentheses. Difference between vehicle and UV-B + Abe (NS), p < 0.01 determined by two-way ANOVA.

The ability of Abe to prevent the induction of systemic suppression of the CHS response by UV-B was examined. Higher doses of UVR suppress CHS to latency in C57BL/6J mice transiently and to a variable extent. The effect of topical administration of Abe on systemic suppression was examined in mice given a single dose of 10 kJ/m² UV-B on their dorsal skin. Vehicle (p < 0.05). Abe was topically applied to the dorsal skin immediately after irradiation. Three days later, the irradiated mice and unirradiated control groups were sensitized with 0.2% FITC on their unirradiated ears, 1% FITC on their irradiated ears, and challenged on their ears 5 days after sensitization. The results from a representative experiment are shown in Fig. 5. Neither Abe nor vehicle alone significantly affected sensitization (p > 0.05). Treatment with UV-B suppressed CHS to FITC by 71%. UV-B followed by vehicle resulted in 50% suppression of CHS compared with the matching unirradiated vehicle control. The difference between the unirradiated and vehicle-treated UV-irradiated groups was not statistically significant (p > 0.05). Treatment of UV-irradiated skin with Abe completely prevented suppression in these mice, compared to both the unirradiated and vehicle control groups. These data indicate that treatment of UV-irradiated skin with Abe has the potential to prevent systemic as well as local suppression of CHS.

Effect of Abe on UVR-Induced Systemic Suppression of DTH to ConA abdrius. Ultraviolet-B irradiation of murine skin has been shown to suppress systemic the induction of delayed-type hypersensitivity (DTH) in ConA abdrius [6]. We examined the effects of topical application of Abe on systemic suppression of DTH in mice given a single 3 kJ/m² dose of UV-B on their dorsal skin. Abe (1.67%) in vehicle was topically applied to the dorsal skin immediately after UV-B exposure. Three days later the mice were sensitized with 107 ConA in Freund's complete adrius, cells, injected subcutaneously into each flank. The data presented in Fig. 6 show that UVR irradiation suppressed the DTH response by 52% (p < 0.05). In contrast, treatment of UV-irradiated skin with Abe completely prevented UVR-induced suppression. Treatment of UV-irradiated skin with Abe prevented the induction of
**Table III.** Relationship Between Time of UV Irradiation and Efficacy of Aloe barberadens in Preserving CHS Responses

<table>
<thead>
<tr>
<th>Group</th>
<th>UV-B Exposure</th>
<th>Treatment</th>
<th>Time of Application*</th>
<th>Specific Ear Swelling† (Mean ± SD)</th>
<th>Suppressor</th>
<th>Versus Group 1</th>
<th>Versus Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>None</td>
<td>None</td>
<td>15.1 ± 2.8</td>
<td>54 ± 10</td>
<td>13.1 ± 2.8</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>II</td>
<td>None</td>
<td>Vehicle</td>
<td>24 h</td>
<td>6.1 ± 0.9</td>
<td>0.9 ± 0.2</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>III</td>
<td>None</td>
<td>Vehicle</td>
<td>0 h</td>
<td>3.8 ± 2.4</td>
<td>73 ± 10</td>
<td>0.006</td>
<td>NS</td>
</tr>
<tr>
<td>IV</td>
<td>None</td>
<td>Vehicle</td>
<td>24 h</td>
<td>3.2 ± 2.3</td>
<td>73 ± 10</td>
<td>0.006</td>
<td>NS</td>
</tr>
<tr>
<td>V</td>
<td>None</td>
<td>Vehicle</td>
<td>48 h</td>
<td>7.3 ± 0.5</td>
<td>0.2 ± 0.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VI</td>
<td>None</td>
<td>Vehicle</td>
<td>48 h</td>
<td>9.4 ± 2.7</td>
<td>35 ± 10</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VII</td>
<td>None</td>
<td>Vehicle</td>
<td>48 h</td>
<td>3.7 ± 1.7</td>
<td>72 ± 10</td>
<td>0.004</td>
<td>NS</td>
</tr>
<tr>
<td>VIII</td>
<td>None</td>
<td>Vehicle</td>
<td>48 h</td>
<td>3.7 ± 1.7</td>
<td>72 ± 10</td>
<td>0.004</td>
<td>NS</td>
</tr>
</tbody>
</table>

*On Day 0, Aloe or Aloe Apprentice vehicle or Aloe Apprentice alone was applied to the abdomen of CMS/HdNH male rats 24 h before Day 1. The test vehicle (0.1%), 24, 36, and 48 h after exposure of the skin to a single dose of 2.5 J/m² UV-B. Then, 2 days after UV irradiation, the animals were sensitized with 0.1% FITC through the abdominal skin.
†Suppressor is calculated as 1 - (test swelling of one - swelling of no UV-B control group X 100%)

**Table IV.** Systemic versus Local Action of Aloe Efficacy of Aloe Applied to UV-Irradiated or Non-Irradiated Skin

<table>
<thead>
<tr>
<th>Group</th>
<th>Site of UV-B Exposure</th>
<th>Site of Treatment*</th>
<th>Treatment</th>
<th>Specific Ear Swelling† (Mean ± SD)</th>
<th>Suppressor Versus Homologous Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>15.1 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>II</td>
<td>Abdomen</td>
<td>None</td>
<td>None</td>
<td>5.6 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>III</td>
<td>Abdomen</td>
<td>Vehicle</td>
<td>Vehicle</td>
<td>8.1 ± 2.1</td>
<td>53%</td>
</tr>
<tr>
<td>IV</td>
<td>Abdomen</td>
<td>Vehicle</td>
<td>Abdomen</td>
<td>3.2 ± 1.4</td>
<td>72%</td>
</tr>
<tr>
<td>V</td>
<td>Abdomen</td>
<td>Abdomen</td>
<td>Aloe</td>
<td>10.0 ± 1.0</td>
<td>29%</td>
</tr>
<tr>
<td>VI</td>
<td>Abdomen</td>
<td>Abdomen</td>
<td>Aloe</td>
<td>7.1 ± 1.0</td>
<td>29%</td>
</tr>
<tr>
<td>VII</td>
<td>None</td>
<td>Back</td>
<td>Aloe</td>
<td>11.6 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>VIII</td>
<td>Abdomen</td>
<td>Back</td>
<td>Aloe</td>
<td>7.1 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>IX</td>
<td>Abdomen</td>
<td>Back</td>
<td>Aloe</td>
<td>11.6 ± 2.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

*On Day 0, Aloe or Aloe Apprentice vehicle or Aloe Apprentice alone was applied to the skin of normal or UV-irradiated mice (within 5 min after exposure of the skin to 5 J/m² UV-B).
†Suppressor is calculated as 1 - (specific ear swelling of one - specific ear swelling of the homologous non-irradiated control group X 100%)

**Discussion**

The major environmental source of UVR is the sun. Because of reported decreases in the concentration of stratospheric ozone [1] and unprecedented increases in ambient UV-B radiation, the impact of UVR on human health has become a matter of growing public concern. There is a close association between the development of skin cancers and UV-induced immune suppression [5,4,27], and UVR has been shown to decrease immunity to infectious diseases in animal models [6,7]. Therefore, it is important to understand the mechanisms by which UVR induces photomodulation and to provide new approaches for its prevention and treatment. The development of probes that inhibit some portion of the UV-B-triggered suppression pathway may assist in dissecting these mechanisms.

In studies presented here, we found that a standardized extract of Aloe barberadens gel (ARF91A and ARF93A) ameliorated some of the immunosuppressive effects of UVR. The rationale for using Aloe was based on its reported anti-inflammatory and tissue-healing properties [22,23] and on its widespread empirical acceptance as a palliative treatment for burns. Two different protocols of low-dose UV irradiation were used to explore the effects of Aloe. UVR administered over 4 consecutive days or given as a single dose of 2 J/m² impairs the function of the CHS response in humans applied through the UV-irradiated skin of susceptible mouse strain. Aloe was equally effective in preventing immune suppression by both regimes. Direct measurement of the efficacy of Aloe and Alophepov vehicle as shown to be effective in reducing CHS to antigen sensitized through skin, and are depleted by UV-B irradiation [8]. Applying Aloe to the irradiated skin significantly inhibited the number of dendritic epidermal cells that were present in the epidermal layer. Aloe alone was effective in preventing the development of skin cancers and UV-induced immune suppression [5,4,27], and UV has been shown to decrease immunity to infectious diseases in animal models [6,7]. Therefore, it is important to understand the mechanisms by which UV induces photomodulation and to provide new approaches for its prevention and treatment. The development of probes that inhibit some portion of the UV-B-triggered suppression pathway may assist in dissecting these mechanisms.

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Figure 5. Effects of Aloe treatment on UV-B-induced systemic suppression of C3H to FITC-C3H mice exposed to a single dose of 100/111mrad UV-B on their shaved dorsal skin followed immediately by topical application of 1.67% Aloe in vehicle or vehicle alone. Stratified control groups were shaved and treated with Aloe or vehicle. Three days later the mice were sensitized with 0.9% FITC through their unshaved ventral skin. Five days after sensitization the mice were challenged on their ears with 0.1% FITC and swelling measured 24 h later. These data are representative of three separate experiments and are the mean ± SD of groups of five mice. Percent suppression compared with the matching, unsensitized control group is given in parenthesis. p < 0.02, untreated positive control versus vehicle treatment; p < 0.1, UV versus UV + vehicle, *p < 0.01, determined by two-way ANOVA.

were also partially preserved by Aloe treatment following UV irradiation but their dermal morphology was not preserved. However, treatment of UV-irradiated skin with Aloe preserved the level of immune function beyond what would be expected by the Lang- gerhans cell numbers in these mice [8]. It is well known that there is not a perfect correlation between morphologic alterations in epi- dermal dendritic cells and immune function, as measured by CHS [28]. This is probably due to the fact that cells in the dermis can also act as antigen-presenting cells for CHS, particularly when a high dose of antigen is used [29]. Alternatively, Aloe may be acting at a later stage of the immune suppressive pathway and have little influ- ence on the morphologic alterations of Langherans cells.

Besides preserving local immune function in mice given less than 1 minimum erythemal dose of UV-B, systemic suppression of DTH and CHS by higher doses of UV-B (5 and 10 kJ/m², respectively) was also inhibited by treatment of the UV-irradiated skin with Aloe. Previous studies suggested that both effects of UV-B are triggered primarily by the formation of cyclobutyl-pyrimidine dimers in the DNA of cells in the UV-irradiated skin [10]. In these experiments, specific loss of lipoprotein content was induced in UV-ir- radiated mouse skin, a procedure that increases the repair of pyrimi- midine dimers, prevented systemic immunosuppression. Al- though Aloe treatment had a similar effect on systemic immune suppression, it did not alter the number of pyrimidine dimers in UV-irradiated skin under conditions in which lipoprotein turnover decreased the number of dimers by 40–50%. We therefore propose that Aloe influences a later step in the sequence of events leading to immune suppression. For example, soluble factors such as TNF-α, IL-10, and IL-1, released by UV-irradiated skin, can mediate suppression of different T-cell–mediated immune responses [15,16,19,20]. It is possible that Aloe acts by inhibiting the forma- tion or release of one or more of these factors.

Our experiments demonstrate that a crude Aloe extract can have potent and reproducible biologic activity. Unfortunately, previous studies involving Aloe preparations have not produced consistent results [22]. This problem can probably be attributed to variability in the source of the Aloe plants, variability in the preparation process, the presence in the preparations of multiple components with varying, and even opposing, biologic activities, and the use of different vehicles for Aloe administration. Within the past year, the Aloe Research Foundation has attempted to remedy some of these prob- lems by preparing standard reference samples of Aloe barbadensis gel. The materials used in this study (ARP/F1A, ARP/F1A) have been characterized extensively in terms of biologic and physical proper- ties, chemical constituents such as salts, proteins, polysaccharides, and associated small molecules, and microbial contaminants. The Aloe Research Foundation materials used in these studies do not correspond to any currently available commercial product. The various processes involved in producing commercial "Aloe" have the potential for significantly altering the chemical composition of the type of Aloe extracts we describe herein. The bioactivity of Aloe extracts is complex and the material we use in these studies may be considered somewhat atypical of commercially produced materials in that the starting substance had an extremely low bacterial content. The Aloe barbadensis gel extract contains of commercial materials may be highly variable, the nature of the processing may not be indicated, and the biostatics of testing materials unpredict- able. These are well described in the "trade literature" [21– 35] but not in the scientific literature. Previous biologic investiga- tions have employed either uncharacterized raw materials pre- pared in the laboratory or undefined commercial material. It is therefore not surprising that results from various investigators on the pharmacologic biologic properties of various Aloe species do not always agree. Needless to say, the results of our study cannot con- stitute endorsement of any commercial product. Clearly, purifica- tion of the components responsible for immunomodulatory and immuno-suppression would be highly desirable and would permit more detailed studies of the mechanism of action of Aloe in this system.

Figure 6. Effects of Aloe treatment on UV-B-induced systemic suppression of DTH or Candida antigen. Mice were treated as in Figure 5 except that UV-B was given three dorsal skin (followed immediately by topical application of 1.67% Aloe in vehicle or vehicle alone. Unsensitized control groups were shaved and treated with Aloe or vehicle. Three days later the mice were sacrificed with 1111mrad UV-B, and co-UCAs released by UV-irradiated skin, can mediate suppression of different T-cell–mediated immune responses [15,16,19,20]. It is possible that Aloe acts by inhibiting the forma- tion or release of one or more of these factors.

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REFERENCES


