Dietary grape-seed proanthocyanidin inhibition of ultraviolet B-induced immune suppression is associated with induction of IL-12

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We have shown previously that dietary grape seed proanthocyanidins (GSPs) inhibit UVB-induced photocarcinogenesis in mice. As UVB-induced immune suppression has been implicated in the development of skin cancer risk, we investigated whether dietary GSPs can modulate the effects of UVB on the immune system. We found that the UVB-induced (180 mJ/cm²) ear swelling response (inflammatory reaction) was significantly lower in mice fed with a GSP-supplemented (0.5 and 1.0%, w/w) diet than mice fed with the standard AIN76A diet. Dietary GSPs markedly inhibited UVB-induced (180 mJ/cm²) suppression of contact hypersensitivity responses in a local model of immunosuppression but had only moderate inhibitory effect in a systemic model of immunosuppression. Dietary GSPs reduced the UVB-induced increase in immunosuppressive cytokine interleukin (IL)-10 in skin and draining lymph nodes compared with mice that did not receive GSPs. In contrast, GSPs enhanced the production of immunostimulatory cytokine IL-12 in the draining lymph nodes. Intraperitoneal injection of GSPs-fed mice with a neutralizing anti-IL-12 antibody abrogated the protective effects of the GSPs against UVB-induced suppression of the contact hypersensitivity response. These data indicate for the first time that GSPs modulate UVB-induced immunosuppression and suggest that this may be one of the possible mechanisms by which they prevent photocarcinogenesis in mice.

Introduction

Solar ultraviolet (UV) radiation, particularly UVB (290–320 nm), can act as a tumor initiator (1), tumor promoter (2) and co-carcinogen (3,4). Exposure of skin to UVB radiation results in a variety of biological effects, including inflammation, sunburn cell formation, immunologic alterations and induction of oxidative stress, all of which play important roles in the generation and maintenance of UV-induced neoplasms (5–7). Although the skin possesses an elaborate defense system consisting of enzymatic and non-enzymatic components that can protect against the adverse biological effects of UV radiation, excessive exposure to UV radiation overwhelms and depletes the cutaneous defense system leading to the development of various skin disorders including the risk of skin cancer (2,7–9).

UVB radiation has multiple effects on the immune system (10,11) and there is a considerable body of clinical and experimental evidence that suggests that immune factors contribute to the pathogenesis of solar UV-induced skin cancer in mice, and probably in humans (12,13). Chronically immunosuppressed patients living in regions of intense sun exposure experience an exceptionally high rate of skin cancer (14). Furthermore, the incidence of skin cancers, especially squamous cell carcinomas (SCCs), is increased among organ transplant recipients (15–17). The increased frequencies of SCC in transplant patients are presumably attributable to a long-term immunosuppressive therapy (18), although non-immune mechanisms may also play a role (19). All these implicate UV-induced immunosuppression in the development of non-melanoma skin cancer.

In recent years, there has been a great interest in the use of naturally occurring botanicals for the prevention of UV-induced skin photodamage, including cancer. Botanical supplements, specifically dietary botanicals that possess anti-inflammatory, immunomodulatory and anti-oxidant properties are among the most promising group of compounds that can be exploited as ideal chemopreventive agents for skin cancer prevention. Recently, we have shown that inclusion of grape seed proanthocyanidins (GSPs) in the diet of mice prevents UVB-induced photocarcinogenesis in terms of tumor incidence, tumor multiplicity and tumor size (9). Dietary GSPs also prevented the malignant conversion of papillomas into carcinomas (9). However, the mechanisms underlying the chemopreventive effects of dietary GSPs have not been elucidated. As UVB-induced immunosuppression has been implicated as a risk factor for the development of skin cancer (10,13), in the present study, we determined whether inclusion of GSPs in the diet inhibits the UVB-induced skin edema, which is a surrogate marker of sunburn and inflammation. The UV-induced immunosuppression was assessed in vivo through measurement of allergic contact hypersensitivity (CHS) responses in both low- and high-dose models of UVB radiation-induced immunosuppression. Exposure of murine skin to UV radiation suppresses the development of allergic CHS, a prototypic T-cell mediated immune response (20,21), through both local and systemic effects (22). Local immunosuppression is defined as the decreased CHS response observed when hapten is applied directly to UV-irradiated skin and systemic effects are defined as a decreased CHS response when the hapten are applied at a distant, non-UV-irradiated, site (23). There is evidence to indicate that local suppression is mediated by alterations in the activity of epidermal Langerhans cells and that it involves the UV-induced release of TNF-α by skin cells (24). Cytokines, such as TNF-α (24), interleukin (IL)-10 (25) and IL-1 (26), and other soluble factors produced by UV-irradiated keratinocytes (27) have

Abbreviations: CHS, contact hypersensitivity; DNFB, 2,4-dinitrofluorobenzene; GSP, grape seed proanthocyanidin; IL, interleukin; UV, ultraviolet.
been implicated as mediators of systemic immune suppression. IL-10 has been implicated in immunosuppressive effects, and IL-12 has been demonstrated to play an important role in the induction and elicitation of contact sensitization reactions (28) and to augment antitumor cell-mediated immune responses (29–31). We therefore examined the effects of dietary GSPs on UVB modulation of the immunoregulatory cytokines IL-10 and IL-12 in skin and draining lymph nodes.

Materials and methods

Animals and experimental diet

Pathogen-free female C3H/HeN mice (6–7 weeks old) were purchased from Charles River Laboratory (Wilmington, MA) and were housed in the animal resource facility of the University of Alabama at Birmingham. The animals were acclimatized for at least 1 week before the start of experiments, and were maintained under the following conditions: 12 h dark/12 h light cycle, 24 ± 2°C temperature and 50 ± 10% relative humidity. The mice were fed a standard AIN76A diet (Harlan Teklad, Madison, WI) with or without GSPs (0.2, 0.5 or 1.0%, w/w) and water ad libitum. The experimental animal protocol was approved by Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

GSPs extracted from grape seeds used in this study was provided by Kikkoman (Japan) and had the chemical composition shown in Table I. Experimental diets containing GSP (0.2, 0.5 and 1.0%, w/w) were prepared separately in AIN76A powdered control diet, as detailed previously (9). Briefly, the ingredients were mixed for at least 4 h in a rotating pan to obtain a uniform mixture. Water was then added (approximately one-tenth of the diet) to the ingredients further mixed by hand and spread uniformly in shallow pans such that a compact texture was achieved. The diet was then cut into small pieces and air dried at room temperature. The experimental diet was prepared once a month and stored at 4°C.

Chemicals, enzymes and antibodies

Monoclonal antibodies to mouse IL-10 (rat IgG2b, Clone JES5-16E3) and IL-12 (rat IgG1, Clone C15:6) were purchased from Pharmingen (San Diego, CA). 2,4-Dinitrofluorobenzene (DNFB) was purchased from Sigma Chemical (St Louis, MO), Cytoscreen USTM mouse IL-10 and IL-12 ELISA kits were obtained from BioSource International (Camarillo, CA). The trypsin, hyaluronidase and DNase enzymes were purchased from Sigma Chemical and dispersease from Collagen Research (Bedford, UK). The diamide-dependent substrate kit was purchased from Kirkegaard & Perry (Gaithersburg, MD).

UVB light source and irradiation of mice

Mice were exposed to UVB as described previously (9). In case of determining the effect of GSPs on UVB-induced edema or inflammatory responses, the mice were exposed to UVB (180 mJ/cm²) to induce immunosuppression. Three days after UVB exposure, the mice were treated topically with 25 μl of 0.5% DNFB in acetone/olive oil (4:1, v/v) on the UVB-irradiated site (local suppression of CHS) or on shaved non-UV-irradiated ventral skin (systemic suppression of CHS). The CHS response was elicited 5 days later by challenging both surfaces of the ears of each mouse with 20 μl of 0.2% DNFB in acetone/olive oil (4:1, v/v). The ear skin swelling was measured 24 h after the challenge using an engineer’s micrometer (Mitutoyo, Tokyo, Japan) and was compared with the ear thickness just before the challenge. Mice that received the same dose of DNFB but were not UVB irradiated served as a positive control, whereas the non-UVB-irradiated mice that received only ear challenge without sensitization with DNFB served as negative controls. The percent suppression of CHS was determined using the following formula, as described previously (21).

Percent suppression = \( \frac{\text{Ear swelling (ve control) } - \text{ear swelling (experimental) } \times 100}{\text{ve control}} \)

The percent protection of UVB-induced suppression of CHS by GSP was determined by the following formula (23): [1 – (GSP/–GSP)] × 100, where +GSP represents the percent suppression of CHS in GSP-treated plus UVB-irradiated mice, and –GSP is the percent suppression in UVB-irradiated mice without GSP treatment.

In vivo treatment of anti-IL-12 antibody

To assess the effect of anti-mouse IL-12 antibody on GSP-induced prevention from UVB-induced suppression of CHS response in mice, anti-IL-12 antibody was diluted in sterile endotoxin-free saline and injected intraperitoneally (i.p.).

Preparation of single-cell suspensions

Single-cell suspensions of epidermal cells were prepared, as described previously (32). Briefly, after removal of subcutaneous tissues, the skin samples from different treatment groups were incubated with 0.25% trypsin (Sigma Chemical) for 60 min at 37°C. The epidermis was then separated from the dermis and placed into HBSS buffer containing 0.025% DNase (Sigma Chemical) and 10% heat-inactivated fetal bovine serum (FBS). The epidermal cell suspension was filtered through 50 μm nylon mesh (Tetko, Elmford, NY) to obtain a single-cell suspension. The cells were then resuspended (1 million cells/0.5 ml media), kept in an incubator for 24 h and thereafter centrifuged at 14,000 r.p.m. for 5 min and supernatants were collected for ELISA assays.

Chemical composition of GSPs

Table I. Chemical composition of GSPs

<table>
<thead>
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<th>Components</th>
<th>Percent of total GSP</th>
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<td>Total proanthocyanidins</td>
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<tr>
<td>Dimers</td>
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<tr>
<td>Trimmers</td>
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<td>Total monomeric flavanols</td>
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<tr>
<td>(−)-Epigallocatechin-3-gallate</td>
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<td>Minerals</td>
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</table>

Immunoistochemical detection of intracellular IL-12 in lymph nodes

Immunostaining was performed to detect the intracellular presence of IL-12, as described previously (32). Briefly, 5 μm thick frozen sections of the draining surfaces of the ears of each mouse with 20 μl of 0.2% DNFB in acetone/olive oil (4:1, v/v). The ear skin swelling was measured 24 h after the challenge using an engineer’s micrometer (Mitutoyo, Tokyo, Japan) and was compared with the ear thickness just before the challenge. Mice that received the same dose of DNFB but were not UVB irradiated served as a positive control, whereas the non-UVB-irradiated mice that received only ear challenge without sensitization with DNFB served as negative controls. The percent suppression of CHS was determined using the following formula, as described previously (21).

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To assess the effect of anti-mouse IL-12 antibody on GSP-induced prevention from UVB-induced suppression of CHS response in mice, anti-IL-12 antibody was diluted in sterile endotoxin-free saline and injected intraperitoneally (i.p.). The mice received two doses (500 ng each) 24 and 3 h before DNFB sensitization. Control mice were injected i.p. with equal volumes of rat IgG1 (isotype control of anti-IL-12) in saline, which was found to have no effect on the outcome of the sensitization procedure or on the suppressive effect of UV irradiation.

Preparation of single-cell suspensions

Single-cell suspensions of epidermal cells were prepared, as described previously (32). Briefly, after removal of subcutaneous tissues, the skin samples from different treatment groups were incubated with 0.25% trypsin (Sigma Chemical) for 60 min at 37°C. The epidermis was then separated from the dermis and placed into HBSS buffer containing 0.025% DNase (Sigma Chemical) and 10% heat-inactivated fetal bovine serum (FBS). The epidermal cell suspension was filtered through 50 μm nylon mesh (Tetko, Elmford, NY) to obtain a single-cell suspension. The cells were then resuspended (1 million cells/0.5 ml media), kept in an incubator for 24 h and thereafter centrifuged at 14,000 r.p.m. for 5 min and supernatants were collected for ELISA assays.

Single-cell suspensions from dermis were prepared, as described previously (32). Briefly, skin biopsies were incubated with dispase overnight at 4°C. Dermis was separated from the epidermis incubated with RPMI 1640 (Gibco BRL, Grand Island, NY) containing 10 mM HEPES (Irvine Scientific, Santa Ana, CA), 0.01% DNase, 1000 U/ml of hyaluronidase and collagenase (Sigma Chemical), and digested for 1 h at 37°C. The digested dermal suspension was filtered through 50 μm nylon mesh (Tetko, Elmford, NY) to obtain a single-cell suspension. The cells were then resuspended (1 million cells/0.5 ml media), kept in an incubator for 24 h and thereafter centrifuged at 14,000 r.p.m. for 5 min and supernatants were collected for ELISA assays.

Single-cell suspensions from dermis were prepared, as described previously (32). Briefly, skin biopsies were incubated with dispase overnight at 4°C. Dermis was separated from the epidermis incubated with RPMI 1640 (Gibco BRL, Grand Island, NY) containing 10 mM HEPES (Irvine Scientific, Santa Ana, CA), 0.01% DNase, 1000 U/ml of hyaluronidase and collagenase (Sigma Chemical), and digested for 1 h at 37°C. The digested dermal suspension was filtered through 50 μm nylon mesh, and the cells resuspended in media and incubated as described for the epidermal cells.

Inguinal lymph nodes or draining lymph nodes were collected aseptically from different treatment groups 48 h after UVB irradiation. The draining lymph nodes from non-UVB-exposed mice served as control. Single-cell suspensions were prepared by placing the lymph nodes in HBSS buffer containing 5% FBS, the connective tissues teased out by using fine sterile scissors and the lymph nodes ruptured to release the lymph node cells. The cells were then filtered, resuspended and incubated as described above.

Immunoistochemical detection of intracellular IL-12 in lymph nodes

Immunostaining was performed to detect the intracellular presence of IL-12, as described previously (32). Briefly, 5 μm thick frozen sections of the draining...
Dietary GSPs inhibit the UVB radiation-induced edema or ear swelling

To determine whether addition of GSPs to the diet is capable of inhibiting the UVB-induced inflammatory response in terms of ear swelling, C3H/HeN mice were fed either a GSP (0.2, 0.5 and 1.0%, w/w)-supplemented AIN76A diet or a standard AIN76A diet for at least 2 weeks before exposure to UVB irradiation (180 mJ/cm²). As shown in Figure 1, the inclusion of 0.5 or 1.0% GSPs in the diet resulted in a reduction in the UVB-induced ear swelling or edema by 28% (P < 0.05) and 40% (P < 0.01), respectively, compared with mice fed a standard control diet (non-GSP). Inclusion of a lower amount of GSPs (0.2%) in the diet did not result in a reduced UVB-induced ear swelling response. As the UVB-induced edema is a surrogate marker of UVB-induced inflammatory reactions, the ability of dietary GSPs to inhibit these responses indicates that the GSPs have an anti-inflammatory effect.

ELISA assay for IL-10 and IL-12

Single-cell suspensions obtained from the epidermis, dermis or draining lymph nodes were kept in incubator for 24 h in RPMI 1640 containing 10% FBS (1 million cells/0.5 ml media) as described above. The cells were then removed by centrifugation and the supernatants collected and filtered through a 0.2 μ cellulose acetate membrane filter for determination of IL-10 or IL-12 protein by ELISA (BioSource International, Camarillo, CA), following the manufacturer’s protocol.

Statistical analysis

The statistical significance of difference in ear swelling response in CHS and inflammatory experiments among different treatment groups were analyzed using the two-tailed Student’s *t*-test for unpaired samples. A *P*-value < 0.05 was considered significant.

Results

Dietary GSPs inhibit the UVB radiation-induced edema or ear swelling

Fig. 1. Dietary GSPs reduce UVB-induced ear skin edema in mice. Mice in each treatment group were exposed to a single UVB dose of 180 mJ/cm². The ear skin swelling response was calculated as the difference in ear skin thickness 24 h after UVB irradiation as compared with prior to UVB irradiation of the mice. Mice fed with the 0.5 and 1.0% (w/w) GSP-containing diet showed a significantly lower ear edema response (*P < 0.05 and **P < 0.01) after UVB exposure than mice fed a diet (non-GSP). The mice fed 0.2% GSPs in the diet did not inhibit UVB-induced edema and was similar to non-GSP fed control mice. The change in ear skin thickness after UVB irradiation in each group is reported in millimeter (mm x 10⁻²) as the mean ± SEM. n = 5 per group.

GSP (0.2%) alone, P < 0.05. *Significant versus UVB alone, P < 0.05. **Significant versus non-GSP treated animals, P < 0.05.

Fig. 2. Dietary GSPs inhibit UVB-induced suppression of the CHS response in mice. The UVB-irradiated mice that received the standard diet without GSP supplementation did not exhibit a significant response on DNFB challenge whether sensitized through the UVB-irradiated site (A, local immunosuppression) or through the non-UVB-irradiated abdominal skin (B, systemic immunosuppression). Mice that received a diet supplemented with GSPs and that were exposed to UVB were able to mount a CHS response in a dose-dependent manner. The magnitude of CHS response was higher in the model of UVB-induced local suppression than UVB-induced systemic suppression. Dietary GSPs did not affect the ability of the mice to generate CHS response to DNFB (third to fifth bars from the top). The change in ear swelling response in each group is reported as mean±SEM. n = 5 per group. Similar results were obtained when the experiment was repeated. *Significant response versus non-GSP (UVB alone) treated animals, P < 0.001. **Significant versus non-GSP treated animals, P < 0.05.

Proanthocyanidin inhibits UVB-induced immunosuppression

We then determined whether dietary GSPs protect against UVB-induced suppression of the CHS response to DNFB in a model of local UV-induced immune suppression. Provision of GSPs in the diet of control mice did not affect the ability of the mice to generate a local CHS response to DNFB in the absence of UVB irradiation (Figure 2A, third to fifth bar from the top) compared with mice that did not receive GSPs and were not irradiated (Figure 2A, positive control, second bar from the top). The local CHS response in those mice that did not receive GSPs and that were UVB irradiated was significantly lower (75% suppression, *P < 0.001; Figure 2A, fourth bar from the top) than those mice that were not irradiated.
(Figure 2A, second bar from the top), confirming the immunosuppressive effect of the UVB irradiation. In contrast, the mice that received 0.5 and 1.0% GSPs (w/w) in the diet and were UV irradiated showed significant prevention of UVB-induced suppression of CHS by 73 and 89% (P < 0.001), respectively. However, a diet containing a lower amount of GSPs (0.2%) did not provide the mice with significant protection from the UVB-induced suppression of the local CHS response. These data indicate that supplementation of the diet with GSPs at a concentration of 0.5 or 1.0% is capable of protecting mice from UVB-induced immunosuppression in a local model of immunosuppression.

Dietary GSPs inhibit UVB-induced systemic immunosuppression, but are not fully protective

Additional experiments were performed to examine whether dietary GSP confers protective effects in the systemic model of UVB-induced immunosuppression. Again in the systemic model of CHS, supplementation of GSPs (0.2, 0.5 or 1.0%, w/v) in control diet did not affect the ability of the mice to generate a systemic CHS response to DNFB in the absence of UVB irradiation (Figure 2B, third to fifth bar from the top). As shown in Figure 2B, supplementation of 0.5% and 1.0% (w/w) GSPs to the diet resulted in significant protection of the mice from UVB-induced suppression of CHS response (33 and 45%, respectively, P < 0.05) in the systemic model of CHS response. As observed in the local CHS model, addition of 0.2% GSPs did not result in a statistically significant protection compared with the positive control (second bar from the top). Thus, dietary GSP at doses of 0.5 and 1.0% (w/w) inhibits the deleterious effects of UV radiation in the systemic model of UV-induced immunosuppression; however, the photoprotective efficacy was lower than that observed in the model of the local CHS response.

Effect of dietary GSPs on the high-dose model of UV-induced immunosuppression

We further evaluated the effect of dietary GSPs on the immune suppression of the local CHS response induced by a higher dose of UVB radiation in C3H/HeN mice (Figure 3). The shaved dorsal skin of the mice was exposed to a single UVB dose of 1000 mJ/cm², which is more than five times higher than the low-dose UVB irradiation used in the local suppression of CHS. Otherwise the protocol was identical. In this case, a significantly reduced ear swelling response in local CHS model was observed in the UVB-irradiated mice, including those mice fed 0.2, 0.5 or 1.0% (w/w) GSPs in AIN76A control diet at 24 h after challenge to DNFB compared with the positive control (Figure 3, second bar from the top). These observations suggest that dietary GSPs, at least under the present experimental conditions, do not protect the mice from the consequences of abnormally high exposure to UVB radiation.

Dietary GSPs inhibit UVB-induced IL-10 production in the skin and draining lymph nodes

It has been shown that UVB irradiation of the skin results in increased production of IL-10 (25), and IL-10 has been implicated in the UVB-induced suppression of the CHS response (33,34). Therefore, we examined whether inclusion of GSPs in the diet has any effect on UVB-induced IL-10 production in the skin and draining lymph nodes. The studies described above showed that supplementation of the diet with 0.5% GSPs conferred significant protection against UVB-induced suppression of CHS in local as well as in systemic mouse models. Therefore, in the studies of the mechanistic basis for the alleviation of the immunosuppressive effects, we used this dose (i.e. 0.5% GSP in diet, w/w). As shown in Figure 4, UVB irradiation of mouse skin resulted in enhanced production of IL-10 by epidermal as well as dermal cells compared with non-UVB-irradiated control mice (Figure 4A). Supplementation of the diet with GSPs (0.5%, w/w) resulted in significant inhibition of UVB-induced enhanced production of IL-10 by the epidermal and dermal cells of mouse skin, by 56% (P < 0.01) and 54% (P < 0.005) (n = 5), respectively. Supplementation of the diet with GSPs (0.5% in diet, w/w) did not alter the levels of IL-10 in the epidermal or dermal cells of un-irradiated mice (data not shown). As IL-10 is thought to act as an immunosuppressive cytokine, the inhibition of IL-10 in UVB-irradiated skin of mice that had been fed GSPs may play a role in the prevention of UVB-induced suppression of the CHS response.

We also determined the production of IL-10 by the cells of the draining lymph nodes of the mice in the different treatment groups. Very low levels of IL-10 were produced by the cells of the draining lymph nodes obtained from mice that were not UVB-irradiated, whether they were fed a standard diet, as shown in Figure 4B, or a GSP-supplemented diet (data not shown). UVB irradiation of mouse skin resulted in enhancement of IL-10 production (~8-fold) in the draining lymph node cells of mice that were not fed with GSPs. The provision of GSPs in the diet resulted in a 50% inhibition of the UVB-induced IL-10 production by the draining lymph node cells (n = 5, P < 0.005), as shown in Figure 4B. These data further indicate that inhibition of UVB-induced suppression of CHS by dietary GSPs may be associated with the inhibition of IL-10 production.

Dietary GSPs increase the production of IL-12 in draining lymph nodes

IL-12 has been shown to stimulate T-cell mediated immune responses (20,31), and has been shown to prevent
UVB-induced suppression of CHS response in mice (35); therefore, we also examined the effect of GSP on the induction of IL-12 in mice. By intracellular immunostaining, as shown in Figure 5, IL-12\(^+\) cells were not detectable in frozen sections of draining lymph nodes obtained from mice that were not exposed to UVB (Figure 5A). However, IL-12\(^+\) cells were detected in the draining lymph nodes obtained from UVB-exposed mice (Figure 5B). The numbers of IL-12\(^+\) cells as well as the intensity of the IL-12 immunostaining was higher in the draining lymph nodes obtained from the mice that received the GSP-supplemented diet (Figure 5C) compared with those mice that were not provided dietary GSPs (Figure 5B). Most of the IL-12\(^+\) cells, particularly in the mice that received GSPs, were localized in an area extending from the subcapsular sinus to the paracortical region of the lymph nodes, including the interfollicular areas, which are the sites of T-cell localization (Figure 5C).

Quantitative analysis of IL-12 by ELISA indicated that exposure of the skin to UVB results in increased production of IL-12 in the draining lymph node cells (19 ± 4 pg/million cells) compared with non-UVB-irradiated control mice (5 ± 1 pg/million cells), and that provision of GSPs in the diet (0.5%, w/w) of UVB-irradiated mice enhanced IL-12 production (44 ± 5 pg/million cells) (Figure 5D) by >2-fold. Because of supersensitive ELISA kit (0.2 pg/ml) for IL-12, it was detectable, but at low concentration (5 ± 1 pg/million cells), in lymph node cells obtained from control mice (Figure 5D). Dietary GSPs did not affect the basal level of IL-12 in the draining lymph nodes (data not shown).

Treatment of mice with anti-IL-12 antibody inhibits the ability of dietary GSPs to prevent the UVB-induced suppression of the CHS response in local model of CHS

As IL-12 augments immune responses and possesses antitumor activity, we examined whether GSP-induced IL-12 production contributes to the prevention of the UVB-induced suppression of the CHS response in mice. As shown in Figure 6, UVB
irradiation induced significant suppression of the CHS response to DNFB (third bar from the top) on local CHS model compared with the positive control (second bar from the top), and dietary GSP resulted in significant inhibition of the UVB-induced suppression of the CHS response in mice (fourth bar from the top, \( P < 0.001 \)). Intraperitoneal injection of anti-IL-12 mAb of GSP-treated mice (fifth bar from the top) resulted in significant inhibition of the GSP-induced prevention of UVB-induced suppression of CHS response \( (P < 0.01) \) compared with GSP + IgG + UVB-treated mice (fourth bar from the top). This observation supports the concept that the prevention of UVB-induced suppression of CHS by dietary GSPs is mediated, at least in part, through enhanced production of IL-12 in mice.

Discussion

Excessive exposure to UV radiation has a variety of adverse effects on human health and can lead to basal cell and squamous cell carcinoma, melanoma, photocaging and immune suppression (10,12). Studies with immune suppressed transplantation recipients and biopsies of proven skin cancer patients have confirmed that UV-induced immune suppression is a risk factor for the development of skin cancer in humans (10,13). Therefore, development of effective chemopreventive agents that can inhibit the UVB-induced suppression of immune responses is required to address this issue. Dietary manipulation as well as pharmacologic intervention can be used as chemoprevention strategies.

In earlier studies, we showed that inclusion of GSPs in the diet prevents UVB-induced photocarcinogenesis (9). Since UVB-induced suppression of immune responses has been implicated in the development of non-melanoma and melanoma skin cancers, we extended our studies to examine the effect of dietary GSPs on UVB-induced suppression of immune responses. In initial studies, we observed that dietary feeding of GSPs inhibits the UVB-induced edema in ear skin (Figure 1), which has been commonly used as a marker of inflammation and is considered to be a marker of tumor promotion (36). The chemopreventive effect of GSP on UVB-induced suppression of immune response was then determined in two CHS models, local and systemic. Our CHS data demonstrate that diet supplemented with GSPs inhibited concentration-dependently the low-dose UVB-induced immunosuppression in both local and systemic models of immunosuppression in mice (Figure 2). Therefore, dietary GSPs do not prevent the deleterious effects of UV radiation in the high-dose model of UV-induced (1000 mJ/cm²) immunosuppression under the experimental conditions used in this study (Figure 3).

UVB irradiation has been shown to increase IL-10 production in the skin and regional lymph nodes (32), which has been implicated in UVB-induced suppression of CHS response (33,34). Therefore, we examined the effect of GSPs on UVB-induced changes on the immunoregulatory cytokines IL-10 and IL-12. Our data demonstrate that supplementation of GSPs in the diet inhibits the UVB-induced increases in IL-10 production, both in the skin and in the draining lymph nodes (Figure 4). Simultaneously, dietary GSPs increases the production of IL-12 in the draining lymph nodes (Figure 5). The inhibition of immunosuppressive cytokine IL-10 production and induction of IL-12 may have a crucial role in prevention of UVB-induced suppression of CHS response by GSPs in mice. It has been shown that i.p. administration of IL-10 inhibits the ability of mice to be sensitized to trinitrophenyl-coupled spleen cells for a delayed type hypersensitivity response (34). The same study also showed that i.p. injection of IL-10 into sensitized mice 24 h before challenge resulted in a significant suppression of ear swelling response, suggesting that IL-10 has the ability to block the effector phase of CHS in vivo. Furthermore, administration of neutralizing antibodies to IL-10 largely, but not totally, inhibited the ability of UV radiation to suppress sensitization to alloantigens (33). In agreement with these observations, our data suggest that prevention of UVB-induced immunosuppression by dietary GSPs may be mediated, at least in part, through the inhibition of IL-10 production in the skin and in the draining lymph nodes.

It has been proposed that IL-12 plays a crucial role in vivo in the induction phase of the CHS response. CHS appears to be a Th1 type cell-mediated immune response (37) and Langerhans cells, critical antigen presenting cells in the induction phase of CHS (38), have been reported to be an additional source of IL-12 production. After UV exposure, the antigen presenting cells in the skin migrate to the regional lymph nodes and initiate sensitization. The supplementation of GSPs in the diet increases the production of IL-12 in the draining lymph nodes (Figure 5). It may be due to the possibility that the number of cells that migrate from the skin to the regional lymph nodes is higher in the UVB-irradiated mice that received GSPs in the diet than those that did not. IL-12 regulates the development and function of T-cells, particularly the development of Th1 type cells by stimulating the production of IFN-γ (39–41). It has been demonstrated that i.p. injection of recombinant IL-12 prevents UV-induced immunosuppression (42) and overcomes UV-induced hapten-specific tolerance (43). In our UVB-induced immunosuppression models, we found that dietary GSPs increase IL-12 production in T-cell specific areas of the draining lymph nodes and is thus placed to tilt the immune response in favor of the development of Th1 type cells. Therefore, a dietary GSP-induced shift in the cytokine balance appears to be a potential mechanism by which GSP may reverse or inhibit UVB-induced suppression of CHS in mice. The role of enhanced IL-12 production in the
Proanthocyanidin inhibits UVB-induced immunosuppression was further confirmed using local CHS model, in which we show that i.p. injection of anti-IL-12 antibody before sensitization resulted in the GSP-treated mice exhibiting UVB-induced suppression of the CHS response to DNB (Figure 6).

These studies indicate that inhibition of immunosuppressive cytokine IL-10 production and induction of IL-12 may play a crucial role in the inhibition of UVB-induced suppression of CHS response by GSPs in mice. However, the mechanism by which GSPs exert these effects is unknown. Current studies of the bioavailability of GSPs and the distribution pattern of GSPs to various organs of the body, including the skin, should provide further insight into the mechanisms by which dietary GSPs provide protection against UVB-induced photodamage to the skin. Together, our data for the first time suggest that supplementation of GSPs in the diet has the capability to provide further insight into the mechanisms by which dietary GSPs provide protection against UVB-induced photodamage to the skin. Together, our data for the first time suggest that supplementation of GSPs in the diet has the capability to protect the skin from UVB-induced photodamage, and further suggest that the protection from UVB-induced immunosuppression afforded by GSPs may be associated with the protection from UVB-induced photocarcinogenesis in mice.

Acknowledgements

This work was supported by the funds from USPSH Grant, CA104428, the Merit Review Award from Veterans Administration and the UAB Skin Diseases Research Center (AR050948-01).

Conflict of Interest Statement: None declared.

References


Received May 14, 2005; revised June 15, 2005; accepted June 18, 2005