Research Article

Cancer Prevention Research

Green Tea Polyphenols Prevent UV-Induced Immunosuppression by Rapid Repair of DNA Damage and Enhancement of Nucleotide Excision Repair Genes

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Abstract

UV radiation–induced immunosuppression has been implicated in the development of skin cancers. Green tea polyphenols (GTP) in drinking water prevent photocarcinogenesis in the skin of mice. We studied whether GTPs in drinking water (0.1-0.5%, w/v) prevent UV-induced immunosuppression and (if so) potential mechanisms of this effect in mice. GTPs (0.2% and 0.5%, w/v) reduced UV-induced suppression of contact hypersensitivity (CHS) in response to a contact sensitizer in local (58-62% reductions; $P < 0.001$) and systemic (51-55% reductions; $P < 0.005$) models of CHS. Compared with untreated mice, GTP-treated mice (0.2%, w/v) had a reduced number of cyclobutane pyrimidine dimer–positive (CPD^+) cells (59%; $P < 0.001$) in the skin, showing faster repair of UV-induced DNA damage, and had a reduced (2-fold) migration of CPD^+ cells from the skin to draining lymph nodes, which was associated with elevated levels of nucleotide excision repair (NER) genes. GTPs did not prevent UV-induced immunosuppression in NER-deficient mice but significantly prevented it in NER-proficient mice ($P < 0.001$); immunohistochemical analysis of CPD^+ cells indicated that GTPs reduced the numbers of UV-induced CPD^+ cells in NER-proficient mice ($P < 0.001$) but not in NER-deficient mice. Southwestern dot-blot analysis revealed that GTPs repaired UV-induced CPDs in xeroderma pigmentosum complementation group A (*XPA*)–proficient cells of a healthy person but did not in *XPA*-deficient cells obtained from *XPA* patients, indicating that a NER mechanism is involved in DNA repair. This study is the first to show a novel NER mechanism by which drinking GTPs prevents UV-induced immunosuppression and that inhibiting UV-induced immunosuppression may underlie the chemopreventive activity of GTPs against photocarcinogenesis. *Cancer Prev Res; 3(2); 179–89. ©2010 AACR.*

Introduction

Green tea is a popular beverage worldwide. Polyphenols isolated from leaves of the green tea plant (*Camellia sinensis*) have several beneficial health effects, including anticarcinogenic activity, which has been shown in various tumor models (1, 2). We and others have shown previously that oral administration of an aqueous extract of green tea or green tea polyphenols (GTP; a mixture of polyphenols) in drinking water inhibits UV radiation-induced skin tumor incidence and multiplicity in mice (3, 4).

Skin exposure to UV radiation initiates a variety of harmful effects on human health, including squamous and basal cell carcinomas of the skin, melanoma, premature skin aging, and a susceptibility to skin infections (5–7). The immunosuppressive effects of solar UV radiation, particularly midwave UV (UVB, 290-320 nm), are well established, most clearly by the effects of UV radiation in inhibiting contact hypersensitivity (CHS), which is a prototypic T-cell–mediated immune response (8, 9). Some of the adverse effects of solar UV radiation on human health, including exacerbation of infectious diseases and initiation of skin cancer, are mediated at least in part by this ability of UV radiation to induce immunosuppression (5–7). Ample clinical and experimental evidence suggests that UV-induced immunosuppression is a risk factor for skin cancer in mice and probably in humans (10, 11). Chronically immunosuppressed patients living in regions of intense sun exposure experience an exceptionally high rate of skin cancer (reviewed in ref. 12), which is consistent with the hypothesis that immune surveillance is an important mechanism of the body in preventing the generation and proliferation of neoplastic cells. Therefore, the inhibition of UV-induced immunosuppression, such as by GTPs in drinking water, is an important potential strategy for managing skin cancer.

UV-induced DNA damage, predominantly the formation of cyclobutane pyrimidine dimers (CPD), is recognized as an important molecular trigger for the initiation

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of UVB-induced immunosuppression and carcinogenesis in the skin (13–15). Reduction of CPDs through application of DNA repair enzymes considerably reduces the risk of UV-induced skin cancer in mice and humans (15, 16). We conducted the present study to determine whether GTPs in drinking water could prevent the skin cancer risk factor of UVB-induced immunosuppression in mouse skin and to assess potential mechanisms of this effect if it occurred. Assessing GTPs in the drinking water of mice should have some relevance to the ready worldwide availability of widely used green tea. We also hypothesized that the rapid repair of UVB-induced DNA damage by GTPs is mediated through the enhanced levels of nucleotide excision repair (NER) genes, postulating further that GTPs in drinking water would be unable to inhibit UVB-induced immunosuppression and DNA repair in NER-deficient mice.

Materials and Methods

Animals

We have used C3H/HeN mice in our experiments as these mice are inbred and therefore considered better for immunologic studies compared with outbred mice, such as SKH-1 hairless mice. Also in our studies, we used xeroderma pigmentosum complementation group A (*XPA*)–deficient mice ($XPA^{-/-}$), which were generated on C3H/HeN background. Because of background of *XPA*-deficient mice, we also preferred to use C3H/HeN mice in the study so that resultant data can be interpreted correctly. The female C3H/HeN mice (6-7 wk old) were purchased from Charles River Laboratory. The $XPA^{-/-}$ mice, which are devoid of NER function, were generated as described previously (17). All mice were maintained under standard conditions of a 12-h dark/12-h light cycle, a temperature of 24 ± 2°C, and relative humidity of 50 ± 10%. The animal protocol used in this study was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Antibodies, chemicals, and real-time PCR primers

The antibody specific for CPDs was obtained from Kamiya Biomedical Co. The manufacturer-supplied standardized real-time PCR primers for NER genes (*XPA*, *XPC*, *RPA1*, *DDB2*, and *DDB1*) and β-actin were obtained from the SuperArray BioScience Corp. All other chemicals of analytic grade were purchased from Sigma Chemical Co.

Green tea polyphenols

The purified mixture of GTPs was obtained from Mitsui Norin Co. Ltd. and contains primarily five major epicatechin derivatives, such as (−)-epigallocatechin-3-gallate, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-epicatechin, and gallocatechin gallate, as described previously (4, 18). This mixture of GTPs was given in the normal drinking water *ad libitum*. In all the animal experiments, mice were given GTPs in drinking water at least 7 d before the start of UVB irradiation. Fresh GTP-containing water was provided every third day. The polyphenolic constituents of samples of the 3-d-old GTP-containing drinking water were analyzed by high-performance liquid chromatography, and this confirmed that the chemical composition of the GTPs in the drinking water was not significantly altered during this time period when compared with the fresh samples (18).

UVB irradiation

The shaved backs of the mice were UVB irradiated as described earlier (4, 18) using a band of four FS20 UVB lamps (Daavlin, UVA/UVB Research Irradiation Unit) equipped with an electronic controller to regulate UV dosage. The UV lamps emit UVB (280-320 nm; ~80% of total energy) and UVA (320-375 nm; ~20% of total energy), with UVC emission being insignificant. This UV unit enables us to enter dose in millijoules, and variations in energy output are automatically compensated so the desired UV dose can be delivered. Mice were kept under the UV lamps at a fixed distance of 24 cm. Monitoring indicated that the majority of the resulting wavelengths of UV radiation were in the UVB (290-320 nm) range with a peak emission at 314 (4, 18).

UVB-induced local or systemic CHS models

The shaved backs of the mice were exposed to UVB radiation (100 mJ per cm^2) for four consecutive days. Twenty-four hours after the last UV exposure, the mice were sensitized by painting 25 μL of 0.5% 2,4-dinitrofluorobenzene (DNFB) in acetone/olive oil (4:1, v/v) either at the UVB-irradiated skin site (local CHS model) or at a shaved non–UVB-irradiated ventral or distant site (systemic CHS model). The CHS response was elicited 5 d later by challenging the 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 swelling was measured 24 h after the challenge using an engineer's micrometer (Mitutoyo) and compared with the ear thickness just before the challenge, as detailed previously (19). Mice that received the same dose of DNFB but were not UV irradiated served as a positive control, whereas the nonirradiated mice that received only ear challenge without sensitization with DNFB served as a negative control. To determine the chemopreventive effect of GTPs against UV-induced immunosuppression, GTPs were given in drinking water of the mice in separate groups of mice. During UV exposure of the mice, the ears of mice were protected from the UV irradiation. The mice that were not exposed to UV radiation were also shaved to maintain the identical regimen. The UV-induced suppression of CHS was determined as described previously (20). Each group consisted of five mice, and each experiment was done at least twice.

To further determine whether GTP-mediated prevention of UVB-induced immunosuppression leads to a long-term immunity, we extended the CHS experiment for a longer period of time. After measuring the ear swelling response to challenge with DNFB (primary challenge), the mice

were rested for 4 wk (i.e., until the ear swelling has regressed to the basal level), and mice were not given GTPs in drinking water after the primary challenge. Mice were then rechallenged (secondary challenge) on the ear skin with the same hapten (DNFB), and the ear thickness was measured before and 24 h after rechallenge.

Immunohistochemical detection of CPDs

Immunohistochemical detection of CPD-positive (CPD^+) cells in the skin or draining lymph node (DLN) samples was done using a procedure described previously (18, 21). Briefly, frozen skin or DLN sections (5 μm thick) were thawed and kept in 70 mmol/L NaOH in 70% ethanol for 2 min to denature nuclear DNA followed by neutralization for 1 min in 100 mmol/L Tris-HCl (pH 7.5) in 70% ethanol. The sections were washed with PBS buffer and incubated with 10% goat serum in PBS to prevent nonspecific binding before incubation with a monoclonal antibody specific for CPDs or its isotype control (IgG1). Bound anti-CPD antibody was detected by incubation with biotinylated goat anti-mouse IgG1 followed by peroxidase-labeled streptavidin. After washing, sections were incubated with 3,3′-diaminobenzidine and counterstained with either H&E or methyl green.

Cells, culture conditions, and UV irradiation

XPA-deficient and *XPA*-proficient human fibroblasts were obtained from the Coriell Institute for Medical Research. These *XPA*-deficient cells originally were obtained from patients suffering from xeroderma pigmentosum and the *XPA*-proficient cells from healthy human donors. Cells were cultured in MEM supplemented with 2 mmol/L L-glutamine and 10% heat-uninactivated fetal bovine serum (Hyclone) and maintained in an incubator at 37°C in a humidified atmosphere of 5% CO_2. The cells were UV irradiated using the same UV source as used for irradiation of the mice. The cells were exposed to the UV radiation through PBS, and on UV irradiation, cells were reincubated with GTPs (0.2%, w/v) for indicated time periods.

Southwestern dot-blot analysis

UVB-induced DNA damage and its repair by GTPs in *XPA*-proficient and *XPA*-deficient human fibroblast cells were determined using Southwestern dot-blot analysis, as described previously (18). Cells were treated with GTPs (0.2%, w/v) for 1 h before irradiation to UVB (20 mJ/cm^2). Cells were harvested 36 h later. Genomic DNA from the cells was isolated following the standard procedures. Genomic DNA (500 ng) was transferred to a positively charged nitrocellulose membrane by vacuum dot blotting (Bio-Dot Apparatus, Bio-Rad) and fixed by baking the membrane for 30 min at 80°C. After blocking the nonspecific binding sites in blocking buffer [5% nonfat dry milk, 1% Tween 20 in 20 mmol/L TBS (pH 7.6)], the membrane was then incubated with the antibody specific to CPDs for 1 h at room temperature. After washing, the membrane was incubated with horseradish peroxidase–conjugated secondary antibody. The CPDs were detected by chemiluminescence using an ECL detection system. The experiments were repeated twice.

Statistical analysis

The results of CPD^+ cells in each group are expressed in terms of either percentage of CPD^+ cells or number of CPD^+ cells per field under microscope and expressed as mean ± SD. The statistical significance of difference between the values of control and treatment groups was determined by ANOVA followed by post hoc test. $P < 0.05$ was considered as statistically significant.

Results

Stability of GTPs in drinking water

We have shown earlier that the chemical composition of GTPs was not significantly changed in drinking water at least for 3 days (18).

GTPs inhibit UVB-induced local immunosuppression

As UVB-induced immunosuppression is considered to be a risk factor for photocarcinogenesis (10, 11), and GTPs given in drinking water prevent photocarcinogenesis in mice (3, 18), we determined whether treatment of mice with GTPs in drinking water protects against UVB-induced suppression of the CHS response to DNFB in a model of local UVB-induced immune suppression in which we measure the CHS response to DNFB. We first confirmed that administration of GTPs in drinking water with various concentrations of GTPs (0.1%, 0.2%, and 0.5%, w/v) did not affect the ability of the mice to generate a local CHS response to DNFB in the absence of UVB irradiation [Fig. 1A, left, compare third to fifth bar from the top with second bar from the top (positive control)]. We then confirmed that, in the absence of treatment with GTPs, the local CHS response in terms of ear swelling was significantly lower (72% suppression; $P < 0.001$; Fig. 1A, left, sixth bar from the top) in those mice that were UVB irradiated than in those mice that were not UVB irradiated (Fig. 1A, left, second bar from the top, positive control), indicating the immunosuppressive effect of the UVB radiation. The group of mice that were treated with GTPs in drinking water at a concentration of either 0.2% or 0.5% before UVB irradiation exhibited a significantly less UVB-induced suppression of CHS (66% lower; $P < 0.001$) than UV-irradiated mice that were not treated with GTPs. Administration of a lower concentration of GTPs (0.1%, w/v) failed to provide significant protection from the UVB-induced suppression of the local CHS response in mice. These data indicate that the treatment doses of 0.2% or 0.5% of GTPs are capable of protecting mice from UVB-induced immunosuppression in a local model of immunosuppression. However, it also has been observed that there was not significant difference in protection of UVB-induced immunosuppression between the doses of 0.2% and 0.5% of GTPs in drinking water.

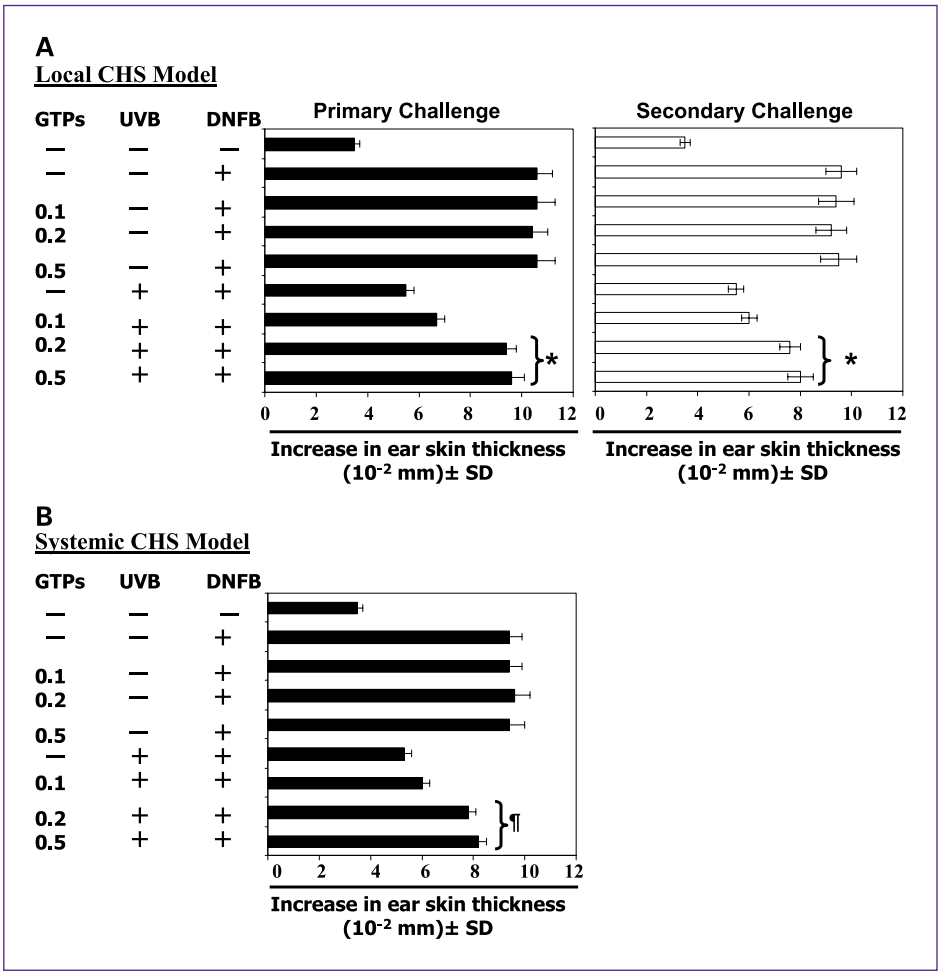

Fig. 1. Drinking GTPs inhibited UVB-induced suppression of the CHS response in a C3H/HeN mouse model of local as well as systemic CHS. A, UVB-irradiated mice that did not receive GTPs did not exhibit a significant response to DNFB challenge when sensitized through the UVB-irradiated skin (local immunosuppression). GTPs given in drinking water before and during the CHS protocol induced a CHS response in mice in a dose-dependent manner (0.1%, 0.2%, or 0.5%, w/v). Mice were rested for 4 wk after the primary challenge and then rechallenged with DNFB (secondary challenge). Right, the change in ear skin thickness in each group was measured. B, GTPs inhibited UVB-induced suppression of CHS response in a C3H/HeN mouse model of systemic CHS. The UVB-irradiated mice that did not receive GTPs did not exhibit a significant response to DNFB challenge when sensitized through the non–UVB-irradiated site of distant abdominal skin (systemic immunosuppression). GTPs given in drinking water before and during the CHS protocol induced a CHS response preferentially at the doses of 0.2% and 0.5% (w/v). A and B, third to fifth bars from the top, GTPs did not affect the ability of the mice to generate a CHS response to DNFB. Columns, mean change in ear swelling response in each group (n = 5 per group); bars, SD. Each experiment was repeated twice with similar observations. *, $P < 0.001$, significant increase in ear skin thickness versus non–GTP-treated (UVB alone) animals; ¶, $P < 0.005$, significant increase in ear skin thickness versus non–GTP-treated (UVB-exposed) animals.

GTPs induce long-term immunity in UVB-exposed mice

To examine whether treatment of mice with drinking GTPs induces long-term immunity in UVB-exposed mice, the mice in local CHS model were rested for 4 weeks after primary challenge with DNFB and were not given GTPs in drinking water during this period. As shown in Fig. 1A (right), again the group of mice that were given GTPs in drinking water earlier (Fig. 1A, left) at a concentration of 0.2% or 0.5% exhibited a significantly greater CHS response (51-55% more; $P < 0.001$) after secondary challenge with DNFB than those UVB-irradiated mice that have not received GTPs at any stage. These data suggest that GTPs have the ability to prevent UVB-induced immune tolerance in mice and can protect for a longer period of time even after ceasing the consumption of GTPs.

GTPs inhibit UVB-induced systemic immunosuppression

We next determined whether administration of GTPs in drinking water induces inhibitory effects in a systemic model of CHS. As in the local model of CHS, treatment of GTPs did not affect the ability of the mice to generate a systemic CHS response to DNFB in the absence of UVB irradiation (Fig. 1B, compare third to fifth bar from the top with second bar from the top). In the systemic model

of CHS, treatment of the lower dose of GTPs (0.1%, w/v) did not result in a statistically significant inhibition of UVB-induced immunosuppression as compared with the positive control. Treatment at the higher doses of GTPs (0.2% and 0.5%) significantly inhibited the immunosuppressive effects of UV radiation in the systemic model of CHS, with GTPs inhibiting UVB-induced immunosuppression by 58% to 62% ($P < 0.005$). The prevention of UVB-induced suppression of systemic CHS response by GTPs may be due to the induction of immune response in animals against UVB-induced adverse effects.

GTPs remove UV-induced DNA damage more rapidly than non–GTP-treated mice

UVB-induced DNA damage in the form of CPDs has been implicated in the UVB-induced immunosuppression (13, 14). Therefore, to determine whether GTPs prevent UV-induced immunosuppression by enhancing DNA repair, we evaluated the formation of CPDs in the UV-exposed skin. As we have found that 0.2% and 0.5% of GTPs in drinking water significantly protect the mice from UVB-induced immunosuppression, and that there was no significant difference in the protection ability of 0.2% and 0.5% of GTPs, in all further experiments we used 0.2% GTPs in drinking water of mice. The shaved backs of C3H/HeN mice were exposed to UVB (60 mJ/cm^2) with and without the treatment of GTPs (0.2%, w/v) in drinking water. Mice were sacrificed either immediately (~30 minutes) or 72 hours later, samples of the skin were obtained, and the presence of CPDs was detected and determined by immunohistochemistry using an antibody directed against CPDs. In skin samples obtained immediately after UV exposure, no differences in the staining pattern of CPDs were observed whether or not the mice were treated with GTPs (Fig. 2A). This observation also eliminated the speculation that drinking GTPs might have significant filtering effect on UV radiation. In contrast, in skin samples obtained 72 hours after UVB exposure, the numbers of CPD$^+$ cells were significantly lower ($P < 0.001$) in the GTP-treated mice than the mice that have not received GTPs in drinking water but were exposed to UVB. It was observed that the skin samples obtained 72 hours after UVB exposure from non–GTP-treated mice also showed a reduction in the number of CPD$^+$ cells, indicating that some endogenous defense mechanism independent to GTPs action may be involved in repair of UV-damaged DNA. The skin samples obtained from the groups of mice that were not exposed to UV (normal skin), including those that were or were not treated with GTPs, were devoid of any CPD$^+$ cells. The numbers of CPD$^+$ cells were counted in at least five to six different places of the sections and are presented as percent of CPD$^+$ cells in different treatment groups and as mean ± SD ($n = 5$; Fig. 2B).

GTPs reduce the number of CPD$^+$ cells in the DLN of UV-exposed mice

UV-induced DNA damage has been recognized as an important molecular trigger for the migration of antigen-presenting cells (i.e., Langerhans cells in the epidermis) from the skin to the DLNs. DNA damage in antigen-presenting cells impairs their capacity to present antigen, which in turn results in a lack of sensitization (22). CPD-containing antigen-presenting cells have been found in the DLN of UV-exposed mice (22). These antigen-presenting cells were identified to be of epidermal origin and exhibited an impaired antigen presentation capacity. As we have found that GTPs have the capacity to induce DNA repair in the UV-exposed skin (Fig. 2), we next determined whether GTPs act to reduce the migration of CPD$^+$ cells from the skin to the DLN. For this purpose, mice were treated with GTPs and UV irradiated. Mice were sacrificed 36 hours later, the DLN was harvested, and the presence of CPDs in the DLN was detected by immunohistochemical analysis. CPD$^+$ cells were not detectable in the DLN of mice that were not UV irradiated whether or not they were treated with GTPs (Fig. 3A). The significant numbers of CPD$^+$ cells in the DLN were found in UV-exposed mice, with the numbers of CPD$^+$ cells in the DLN of the UV-exposed mice being more than 2-fold higher ($P < 0.001$) than in the DLN of GTP-treated mice. The lower number of CPD$^+$ cell in the DLN of GTP + UVB–treated group of mice compared with non–GTP-treated, UVB-exposed mice was not unexpected and may be attributable to the partial removal of the damaged DNA in the migrating cells. Treatment with GTPs resulted in a 59% reduction in the numbers of CPD$^+$ cells in the DLN of UV-exposed mice compared with non–GTP-treated, UV-exposed mice ($P < 0.001$; Fig. 3B).

Microscopic examination suggests that most of the CPD$^+$ cells, in both GTP-treated and non–GTP-treated, were localized in an area extending from the subcapsular sinus to the paracortical region of the lymph nodes, including the interfollicular areas (Fig. 3A). The CPD$^+$ cells were counted in these areas and reported in terms of CPD$^+$ cells per field. As the interfollicular areas are the sites of T-cell localization, the presence of CPD$^+$ cells in these areas may adversely affect the induction of the sensitization response. These data suggest that the ability of GTPs to prevent UV-induced immunosuppression in mice may be due to its capacity to repair UV-induced DNA damage in epidermal antigen-presenting cells.

GTPs enhance the levels of NER genes in mice

As we have found that administration of GTPs in drinking water enhances the removal or repair of UVB-induced thymine dimers or CPDs in the skin of mice, we were interested to determine whether the rapid repair or removal of CPDs in UV-exposed skin by GTPs is mediated through the enhancement in the levels of NER genes. For this purpose, mice were exposed to acute UVB (60 mJ/cm^2) with and without the treatment of GTPs (0.2%, w/v) in drinking water, as detailed in Materials and Methods. Mice were sacrificed at 1 and 3 hours later, skin samples were collected, and epidermal RNA was isolated and subjected to the analysis of mRNA expression of NER genes (e.g., *XPA*, *XPC*, *RPA1*, *DDB2*, and

DDB1) using real-time PCR. As shown in Fig. 4, the acute exposure of the mouse skin with UVB radiation mildly enhances the levels of NER genes (not significantly) compared with non–UVB-exposed skin of the mice. However, the mRNA levels of NER genes, such as *XPA*, *XPC*, and *RPA1*, were significantly enhanced ($P < 0.05$-0.001) in the skin of mice treated with GTPs at both 1- and 3-hour time points after UVB exposure compared with non–GTP-treated, UVB-exposed mouse skin (Fig. 4). GTPs had no significant effect on the stimulation of *DDB2* and *DDB1* NER genes compared with non–GTP-treated mouse skin exposed to UV radiation. Further, the administration of GTPs in drinking water to non–UV-irradiated mice did not affect the steady levels of NER genes (data not shown).

GTPs do not prevent UVB-induced immunosuppression in $XPA^{-/-}$ mice but prevent it in $XPA^{+/+}$ mice

As the enhanced repair of UVB-induced DNA damage in the form of CPDs by GTPs may be associated with the inhibition of UVB-induced immunosuppression in mice, we next determined whether GTPs prevent UVB-induced immunosuppression in *XPA*-deficient or $XPA^{-/-}$ mice, which do not have the ability to repair UVB-induced DNA damage because of absence of functional NER enzymes or genes. For this purpose, $XPA^{-/-}$ and their wild-type counterparts ($XPA^{+/+}$) were subjected to local CHS protocol/experiment with and without the treatment of GTPs in drinking water (0.2%, w/v). Following the local CHS protocol, it was observed that in the absence of treatment with

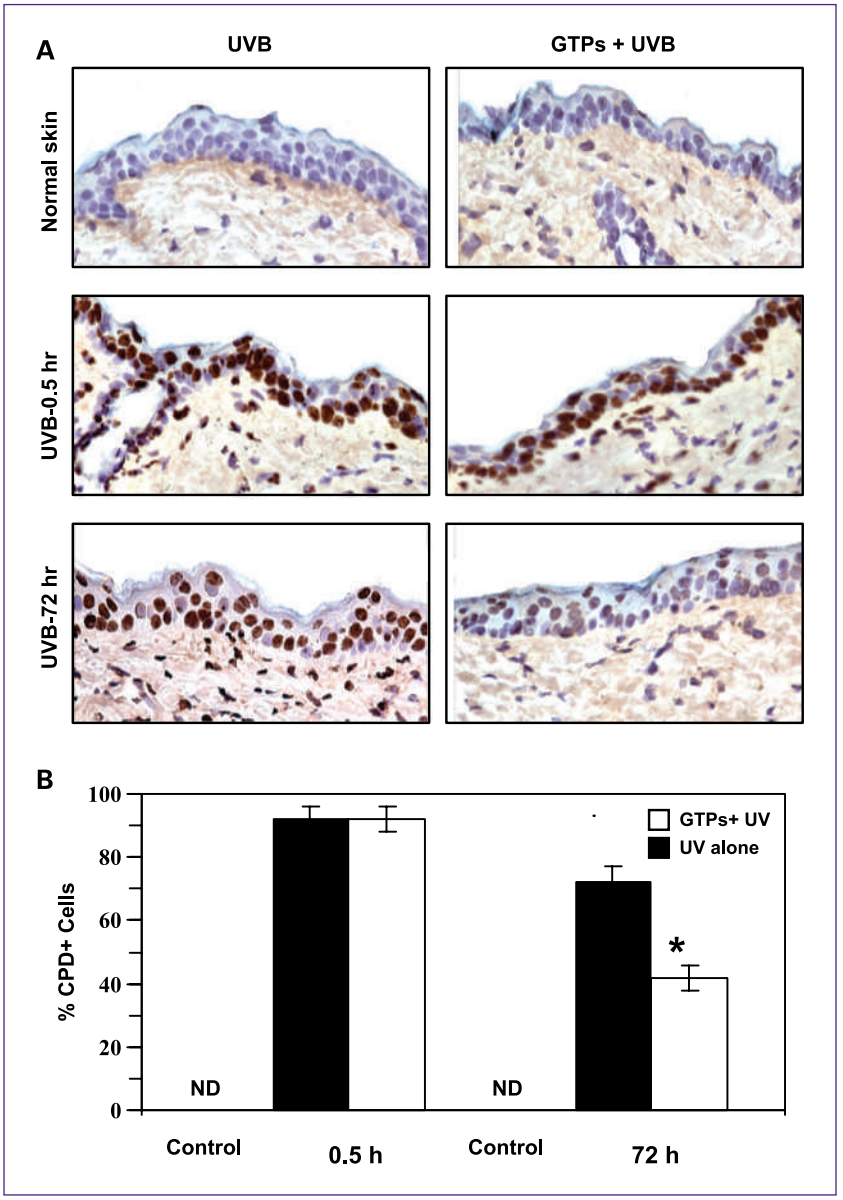

Fig. 2. UV-induced CPDs were repaired or removed more rapidly in GTP-treated than untreated mice. A, mice were exposed to UV (60 mJ/cm^2) radiation with or without treatment of GTPs in drinking water. Mice were sacrificed at 30 min (immediate) or 72 h after exposure, at which times skin samples also were collected and frozen in OCT medium. Dark brown, frozen sections (5 μm thick) were subjected to immunoperoxidase staining to detect CPD$^+$ cells. CPD$^+$ cells were not detected in non–UVB-exposed skin. Magnification, ×40. B, the numbers of CPD$^+$ cells were counted in five to six different areas of the sections under a microscope. Columns, percentage of CPD$^+$ cells in epidermis ($n = 5$); bars, SD. *, $P < 0.001$, significant decrease in number of CPD$^+$ cells versus UV alone. ND, not detectable.

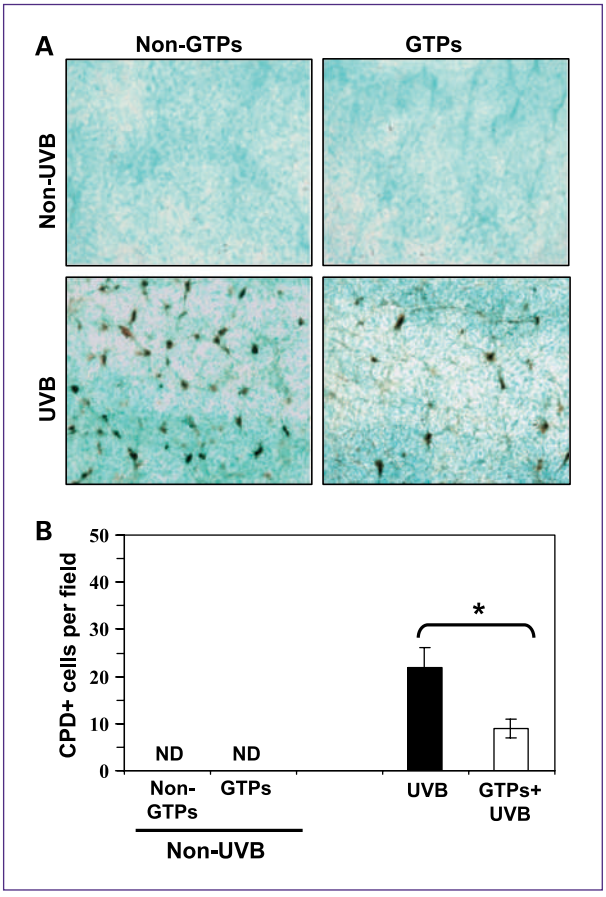

Fig. 3. GTPs in drinking water reduced the migration of CPD$^+$ cells from the skin to DLNs of UV-exposed mice compared with this migration in non–GTP-treated, UVB-exposed mice. A, mice were treated with GTPs and then exposed to UV (60 mJ/cm^2), as detailed in Materials and Methods. Mice were sacrificed 36 h after exposure, and DLNs were harvested and frozen in OCT medium. DLNs obtained from non–UV-exposed mice were used as controls. CPD$^+$ cells were detected by immunoperoxidase staining of frozen sections, as described in Materials and Methods. CPD$^+$ cells were not detectable in DLNs obtained from non–UV-exposed control mice. Five mice were used per group, and the experiment was repeated once. Magnification, ×20. B, the numbers of CPD$^+$ cells were counted in five to six different areas of the sections. Columns, mean number of CPD$^+$ cells per microscopic field within the DLNs (n = 5); bars, SD. *, $P < 0.001$, significant decrease in the number of CPD$^+$ cells in the GTP + UVB–treated group versus the UVB-alone group. ND, not detectable in non–UV-exposed mice.

GTPs, the CHS response in terms of ear swelling was significantly lower (73% suppression; $P < 0.001$; Fig. 5, left, fourth bar from the top) in $XPA^{-/-}$ mice that were UVB irradiated than those $XPA^{-/-}$ mice that were not UVB irradiated (Fig. 5, left, second bar from the top, positive control), indicating the immunosuppressive effect of UVB radiation in $XPA^{-/-}$ mice. The group of mice that were treated with GTPs in drinking water (0.2%, w/v) also exhibited a significant UVB-induced suppression of CHS response ($P < 0.005$), which was similar to non–GTP-treated, UVB-exposed mice (Fig. 5). It suggests that administration of GTPs did not prevent UVB-induced suppression of CHS response to DNFB in $XPA^{-/-}$ mice. In contrast, the administration of GTPs to the wild-type counterparts ($XPA^{+/+}$) significantly induces contact sensitization reaction and ear swelling response to DNFB and was

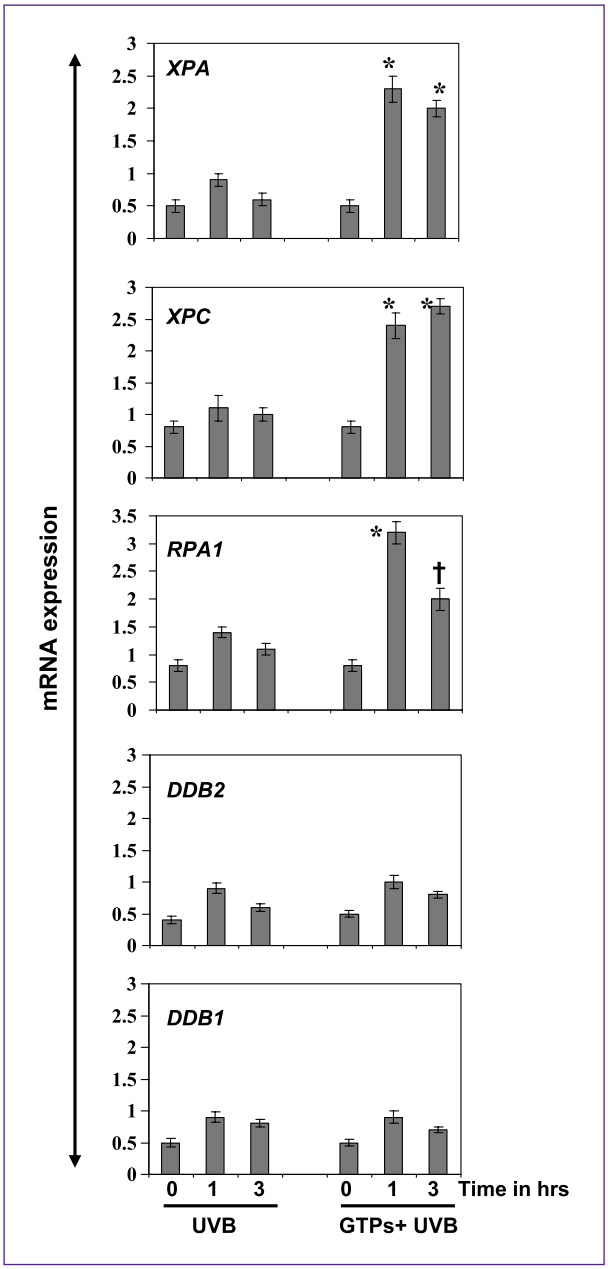

Fig. 4. Levels of mRNA expression of NER genes were enhanced in the UVB-exposed mouse skin of GTP-treated mice versus in non–GTP-treated mice. GTP-treated and untreated mice were sacrificed at 1 and 3 h following exposure to UVB (90 mJ/cm^2). mRNA expression levels were determined using real-time PCR. Columns, mean mRNA expression levels of various NER genes; bars, SD. Five mice were used per group, and the experiment was repeated once. *, $P < 0.001$, significant increase in mRNA levels in GTP + UVB–treated versus UVB alone group; †, $P < 0.05$, significant increase in mRNA levels in GTP + UVB–treated versus UVB alone group.

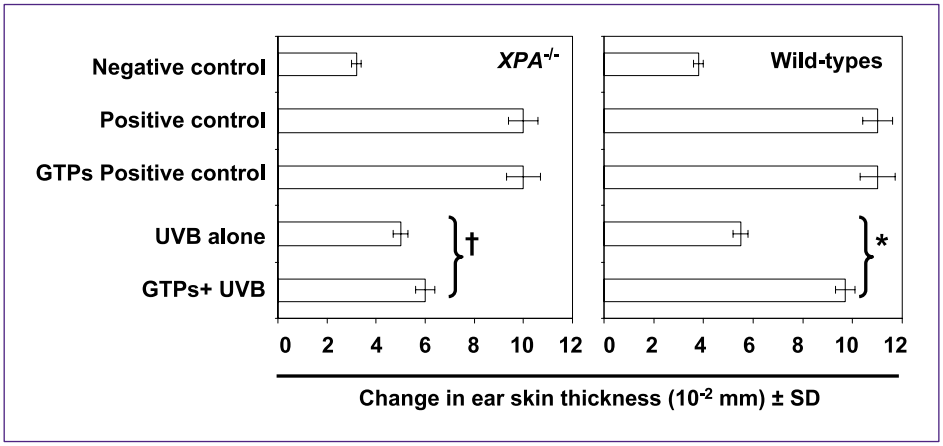

Fig. 5. GTPs given in drinking water did not prevent UVB-induced suppression of the CHS response in $XPA^{-/-}$ mice but did so in wild-type counterparts. The shaved backs of mice were exposed to UVB radiation (100 mJ/cm^2) in GTP-treated [in drinking water, 0.2% (w/v) on four consecutive days] or untreated mice. Twenty-four hours after the last UVB exposure, the mice were sensitized with DNFB through UVB-exposed dorsal skin. Five days after sensitization, the mice were challenged by painting DNFB on the ear, and ear swelling was measured, as detailed in Materials and Methods. Columns, change in ear thickness reported as the mean number of millimeters (mm × 10^{-2}; n = 5 per group); bars, SD. The experiment was repeated twice with similar results. †, $P < 0.001$, significant inhibition in ear skin thickness versus positive control; *, $P < 0.005$, significant increase in ear skin thickness in GTP + UVB group versus non–GTP-treated (UVB alone) wild-type mice.

significantly higher ($P < 0.001$; Fig. 5, right, bottom bar of the panel) than those mice that were not given GTPs in drinking water and exposed to UVB radiation. The ear swelling response in GTP-treated group of wild-type mice was comparable with the mice of control group (Fig. 5, right, second bar from the top). The change in ear skin thickness in $XPA^{-/-}$ mice in response to DNFB sensitization in GTP + UVB was also compared with the change in ear skin thickness in $XPA^{+/+}$ mice in response to GTP + UVB. The increase in ear skin thickness after sensitization to DNFB was greater in the $XPA^{+/+}$ mice treated with GTP + UVB (53%; $P < 0.01$) as compared with increase in ear skin thickness after sensitization to DNFB in $XPA^{-/-}$ mice treated with GTP + UVB. The data from this set of experiments suggest that prevention of UVB-induced immunosuppression by GTPs requires NER genes, which have a role in repair of UVB-induced DNA damage in the form of CPDs.

GTPs enhance repair of UVB-induced DNA damage in $XPA^{+/+}$ mice but do not reduce UVB-induced DNA damage in $XPA^{-/-}$ mice

It has been shown that application of DNA repair enzymes that reduce the numbers of CPD$^+$ cells prevents UV-induced immunosuppression (15, 23). We found that drinking GTPs has the ability to prevent UVB-induced immunosuppression in $XPA^{+/+}$ mice but not in $XPA^{-/-}$ mice (Fig. 5), which are devoid of the NER gene, and that it is necessary for the repair of UV-induced DNA damage in mammalian cells. Therefore, we further examined whether the GTP-mediated repair of UV-induced DNA damage requires NER gene. For this purpose, $XPA^{+/+}$ and $XPA^{-/-}$ mice were exposed to acute UVB exposure (60 mJ/cm^2) with and without the treatment of GTPs in drinking water and sacrificed 72 hours later. Skin samples were collected and subjected to immunohistochemical analysis of CPD$^+$ cells. In skin samples obtained from $XPA^{-/-}$ mice, no significant difference in the staining pattern of CPDs were observed whether or not they were treated with GTPs (Fig. 6A). In contrast, in UVB-exposed skin samples obtained from $XPA^{+/+}$ mice, the numbers of CPD$^+$ cells were significantly lower in the GTP-treated mice ($P < 0.001$) than those mice that were not treated with GTPs (Fig. 6A and B).

GTPs induce repair of UVB-induced damage of DNA in *XPA*-proficient cells but do not reduce UVB-induced DNA damage in *XPA*-deficient cells

To further verify our observations of green tea in $XPA^{-/-}$ and $XPA^{+/+}$ system, we used NER-deficient fibroblasts from *XPA* patients and repair-proficient fibroblasts from healthy persons. The *XPA* gene is an essential component of the NER; thus, cells with a mutated *XPA* gene completely lack a functional NER. Therefore, we examined the effect of GTPs on UV-induced CPDs in *XPA*-proficient and *XPA*-deficient cells using Southwestern dot-blot analysis. For this purpose, *XPA*-deficient and *XPA*-proficient human fibroblasts were exposed to UV radiation in the presence or absence of GTPs. Cells were harvested 48 hours later, and genomic DNA was isolated and subjected to dot-blot analysis. As clearly indicated in Fig. 6C, GTP treatment of *XPA*-proficient cells for 48 hours resulted in remarkable repair or reduction of UV-induced CPDs. However, this DNA-repairing effect of GTPs was not evident in the *XPA*-deficient cells 48 hours after UV irradiation. This may be due to the absence of NER enzymes in these cells. The cells, whether *XPA* deficient or *XPA* proficient and either treated with GTPs or not treated with GTPs, did not show the presence of CPDs as reflected from the absence of dot blot.

Discussion

The results presented here show that GTPs in drinking water inhibited UVB-induced suppression of CHS response to DNFB in C3H/HeN mouse models of both local and systemic CHS. These data provide the first evidence that preventing photocarcinogenesis with GTPs in drinking water (3, 4) may be due, at least in part, to the inhibition of UVB-induced immunosuppression in mice.

Our mechanistic analyses showed that GTP treatment rapidly removed or repaired UVB-induced DNA damage in the form of CPDs in UVB-exposed skin and reduced the migration of CPD^+ antigen-presenting cells from the epidermis to DLNs. There is evidence that UV-induced DNA damage is the molecular trigger for this migration of Langerhans cells (14, 24), which are the only CPD^+ antigen-presenting cells that migrate from the epidermis to DLNs. UV-induced DNA damage to the skin also impairs the antigen-presenting capacity of Langerhans cells, which results in a lack of sensitization to and tolerance of contact sensitizers (22, 24). We found that GTPs in drinking water inhibited the migration of epidermal antigen-presenting cells to DLN in mice, indicating that GTPs may repair UV-induced CPDs. Because the migrating antigen-presenting cells in the epidermis were either repaired or not damaged in GTP-treated mice, we speculate that they were able to present antigen to T cells in DLNs, resulting in T-cell sensitization to DNFB challenge. Furthermore, the numbers of CPD^+ cells were significantly higher in the subcapsular sinus to the paracortical region of the lymph nodes (including interfollicular areas), which are the sites of T-cell localization, in non–GTP-fed versus GTP-fed mice. Therefore, damaged DNA in the lymph nodes of non–GTP-fed

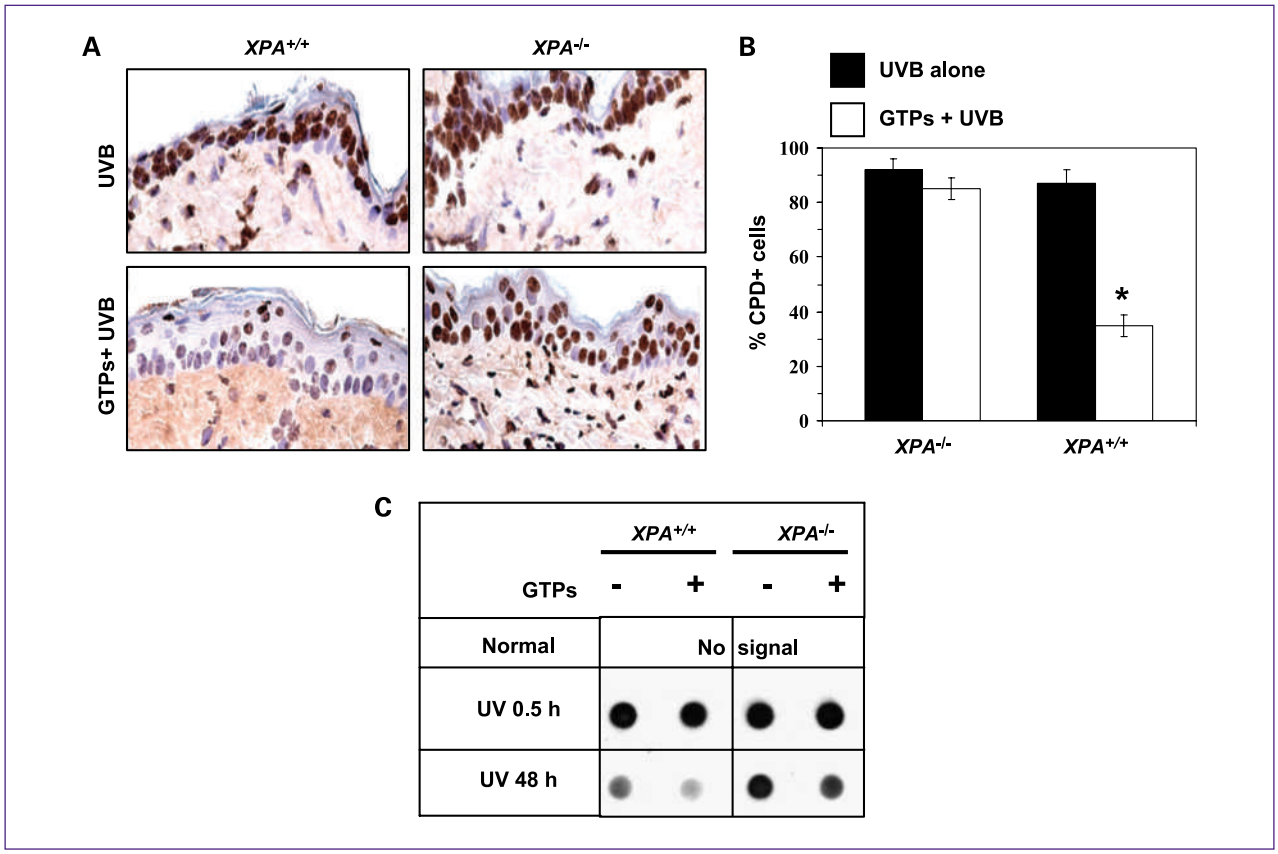

Fig. 6. GTPs reduced or repaired UVB-induced DNA damage in *XPA*-proficient (wild-type) mice but not in *XPA*-deficient mice. A, shaved dorsal skin of the mice was exposed to UVB (50 mJ/cm^2) with or without treatment of GTPs in drinking water, as detailed in Materials and Methods. Mice were sacrificed 48 h after UV irradiation, and skin samples were collected and frozen in OCT medium for immunoperoxidase staining of CPD^+ cells (*dark brown*), as described in Materials and Methods. Magnification, ×40. B, the numbers of CPD^+ cells were counted in five to six areas of the section. Columns, mean percentage of CPD^+ cells ($n = 5$); bars, SD. *, $P < 0.001$, significant decrease in the number of CPD^+ cells in GTP + UVB–treated group versus non–GTP-treated, UVB-irradiated wild-type ($XPA^{+/+}$) mice. C, GTP treatment enhanced the repair of UVB-induced DNA damage in *XPA*-proficient human fibroblast cells but not in *XPA*-deficient cells. *XPA*-proficient cells from a healthy person and *XPA*-deficient cells from a xeroderma pigmentosum patient with a deficiency in *XPA* were exposed to UV (20 mJ/cm^2) in the presence or absence of GTPs (20 μg/mL); cells were harvested 36 h after exposure. Genomic DNA was extracted from these treated cells and subjected to Southwestern dot-blot analysis using an antibody against CPD. $XPA^{-/-}$ and $XPA^{+/+}$ cells treated or not with GTPs but not exposed to UVB did not show the presence of CPDs in the dot blot. Experiments were repeated twice with identical observations.

mice may adversely affect the ability of antigen-presenting cells (to present antigen to T cells), thus abrogating T-cell sensitization to DNFB. The ability of GTPs to reduce the numbers of CPD^+ cells in lymph nodes may be one of the reasons that GTPs prevent UVB-induced immunosuppression in DNFB-challenged mice.

NER is the main mechanism of repair in mammalian cells for the removal of UV radiation-induced DNA damage (25). Because GTPs enhanced the removal or repair of UVB-induced DNA damage, we further examined whether the removal or repair of UV-induced CPDs by GTPs was mediated via induction of NER genes. Our real-time PCR data indicate that GTPs increased the levels of some NER genes (e.g., *XPA*, *XPC*, and *RPA1*) in UVB-exposed skin sites (compared with non–GTP-fed mice) and that this effect may have contributed to the rapid repair of damaged DNA in mouse skin. GTPs had no effect on certain other NER genes (e.g., *DDB1* and *DDB2*), suggesting that GTP function in this setting is NER gene specific. The role of NER was further confirmed by assessing the effect of GTPs on UVB-induced immunosuppression in $XPA^{-/-}$ versus in $XPA^{+/+}$ mice. GTPs in drinking water prevented UVB-induced suppression of CHS response in $XPA^{+/+}$ mice but not in $XPA^{-/-}$ mice, further supporting our observations that GTP inhibition of UVB-induced immunosuppression requires functional NER genes. An important finding is that exposure to UV radiation suppressed CHS response in both $XPA^{-/-}$ and $XPA^{+/+}$ mice, suggesting that UV-induced immunosuppression is mediated through other mechanisms in addition to DNA damage. These mechanisms may include (*a*) UV-induced suppression of interleukin-12 (20), which stimulates the immune system through the development of Th1 cell types, and (*b*) UV-induced stimulation of interleukin-10, which is considered to be an immunosuppressive cytokine (12). To further confirm our hypothesis and verify our present data, we examined NER-deficient cells from *XPA* patients and NER-proficient cells from healthy persons. Cells derived from *XPA* patients either lacked or had reduced DNA repair capacity due to genetic mutations in several components of the NER genes. The *XPA* complementation type represents the most severe phenotype because it lacks or has a mutated *XPA* gene, which is the most crucial component in the repair process, and so cells lacking the *XPA* gene are completely deficient in NER (26, 27). Our dot-blot analysis indicated that GTPs removed UV-induced CPDs in NER-proficient cells ($XPA^{+/+}$) but did not remove or repair such UV-induced damage in NER-deficient ($XPA^{-/-}$) human fibroblast cells. These observations indicate that repair of UV-induced DNA damage by GTPs is mediated through the NER mechanism and requires functional NER.

Our collective data have important implications for the mechanism by which GTPs prevent skin cancer and identify a new mechanism by which GTPs prevent UV-induced immunosuppression. We show that GTP prevention of UV radiation-induced immunosuppression is mediated through rapid repair of UVB-induced DNA damage and requires NER. The importance of these mechanistic results is highlighted by the important role of UV-induced DNA damage and immunosuppression in the risk and development of melanoma and nonmelanoma skin cancers in humans. The magnitude of a protective effect of green tea against UVB-induced immunosuppression may differ in people based on differences in race and other genetic, environmental, and/or metabolic factors and in the intensity and exposure time of UV radiation. Based on the results of this study, however, and a calculation based on protective doses of GTPs we observed in mice, we hypothesize that the consumption of five to six cups (one cup = 150 mL) of green tea (150 mL = 1 g of green tea leaves) per day may provide a level of protection against photocarcinogenic effects in humans similar to that we observed in mice receiving GTPs in drinking water. These results may even support the suggestion that drinking green tea should be further investigated for its potential practical chemopreventive effect against skin cancers in humans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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