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Photobiologic and Photoimmunologic Characteristics of XPA Gene-Deficient Mice

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Xeroderma pigmentosum group A (XPA) gene-deficient mice cannot repair UV-induced DNA damage and easily develop skin cancers by UV irradiation. Just like human XP patients, homozygous (–/–) mice developed stronger longer-lasting acute inflammation than did wild-type mice after a single irradiation with UVB. Moreover, the model mice showed more severe UV-induced damage of keratinocytes and Langerhans cells than did the control mice. UVB-induced local and systemic immunosuppression was greatly enhanced in the (–/–) mice. Treatment with indomethacin, an inhibitor of prostaglandin (PG) synthesis, inhibited UV-induced inflammation and abrogated immunosuppression. In XPA-deficient mice, the amount of PGE_2 and the expression level of COX-2 mRNA greatly increased after UVB irradiation compared with wild-type mice. These results suggest that the excess DNA photoproducts remaining in XPA-deficient cells after UV radiation induce COX-2 expression and subsequently produce a high amount of PGE_2, which causes the enhancement of inflammation and immunosuppression. In XPA-deficient mice, the natural killer cell activity significantly decreased after repeated exposures to UVB. Our experimental data indicate that cancer development in XP patients involves not only mutagenesis due to the defect in DNA repair, but also the enhanced UV-immunosuppression and intensified impairment of natural killer function. ***Key words: excision repair/immunosuppression/prostaglandin/sunburn. Journal of Investigative Dermatology Symposium Proceedings 6:58–63, 2001***

Xeroderma pigmentosum (XP) is clinically characterized by extremely high sensitivity to sunlight and a more than 1000-fold increased risk of developing cancers on the sun-exposed skin (Robbins *et al*, 1974). To clarify the mechanisms of ultraviolet (UV)-induced carcinoma in XP patients, extensive investigations have been undertaken at the cellular and molecular levels. The development of skin cancer is initiated when UV-induced DNA damage, including cyclobutane pyrimidine dimers (CPD) and pyrimidine-pyrimidone (6–4) photoproducts, are not properly repaired. It is now well known that cultured cells from patients with XP have a defect in an early step of the nucleotide excision repair process of the DNA lesions, and therefore have a greatly increased sensitivity to the lethal effects of UV radiation (Cleaver, 1968).

Clinically patients with XP show acute UV-induced inflammation and chronic photoaging before developing skin cancers. It has not been elucidated if or how the defective excision repair is involved in these photosensitive reactions. To investigate photobiologic behaviors *in vivo*, an animal model is a useful tool. Among various complementation groups, group A XP (XPA) shows the most severe clinical symptoms. The cloning of the XPA gene and biochemical characteristics of the encoded protein have been determined. By gene targeting we have generated XPA gene-deficient mice, which are defective in nucleotide-excision repair and show a high incidence of skin cancers initiated by UVB irradiation (Nakane *et al*, 1995) (**Fig 1**). Similarly to the XP patients, these XPA mice develop stronger and longer-lasting inflammation after UVB exposure. Furthermore, we have demonstrated that UVB radiation greatly suppresses cell-mediated immunity in XPA mice in comparison with wild-type mice (Miyauchi-Hashimoto *et al*, 1996). These findings strongly suggest that not only the mutation caused by the defect in nucleotide-excision repair but also the enhanced UVB-induced immunosuppression is involved in the development of skin cancers in XP. We further investigated the mechanisms by which UVB radiation enhances immunosuppression in XPA model mice.

MATERIALS AND METHODS

Animals The XPA gene-deficient mice with CBA, C57BL/6, and CD^{-1} chimeric genetic background were backcrossed with hairless albino mice of the inbred strains Hos/HR-1, and the resultant hairless XPA (–/–) and (+/+) mice were used in these studies. The mice were 6–12 wk old at the beginning of each experiment, but within a single experiment all mice were sex- and age-matched.

UV irradiation The UVB source was a bank of seven fluorescent sunlamps (FL.20SE.30; Toshiba Medical Supply, Tokyo, Japan) with an emission spectrum of 275–375 nm peaking at 305 nm. The irradiance of UVB was measured by a radiometer (UVR-305/365D(II); Toshiba Medical Supply, Moriguchi, Osaka, Japan).

Manuscript received June 14, 2001; accepted for publication June 14, 2001.

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Abbreviations: CHS, contact hypersensitivity: COX, cyclo-oxygenase; DNFB, dinitrofluorobenzene; PG, prostaglandin; XP, xeroderma pigmentosum.

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Figure 1. UV-induced skin tumors in the XPA-deficient mouse.

Ear swelling response to UVB radiation The mice were anesthetized by intraperitoneal injection of sodium pentobarbital to keep them immobile during exposure. The ears were irradiated with 250 mJ per cm^2 of UVB. Twenty liters of 1% (wt/vol) indomethacin in acetone was applied epicutaneously immediately after UV irradiation. Ear thickness was measured with a dial thickness gauge (Peacock, Tokyo, Japan) immediately before irradiation and 1–4 d after irradiation. Ear swelling response represented an increment in thickness above the value before irradiation.

Histologic examination and sunburn cell counting The depilated abdominal area of mice was exposed to UVB. Biopsy specimens were taken 24 h after irradiation and processed histologically and stained with hematoxylin and eosin. Histologic changes induced by UVB radiation were observed, and the number of sunburn cells (SBC) in the interfollicular epidermis was counted in three different sections under a 10× ocular lens and grid. The average number of SBC per section was calculated.

ADPase staining The epidermal sheets were prepared from skin samples using ethylenediamine tetraacetic acid and stained with adenosine-5′-diphosphate (ADP). Stained Langerhans cells were counted in 30 randomly selected interfollicular fields per sheet with the aid of a calibrated ocular grid.

Sensitization and elicitation of contact hypersensitivity (CHS) The mice were sensitized by epicutaneous application of 25 μL of 1% dinitrofluorobenzene (DNFB) solution in acetone:olive oil (4:1) on depilated abdominal skin. CHS was elicited by application of 20 μL of 0.2% DNFB solution on the surface of each left ear 6 d after sensitization. Ear thickness was measured before and 24 h after application of the challenge dose, and the difference between the two readings was recorded as the ear swelling.

Assay for UVB-induced immunosuppression of CHS To assay local immunosuppression induced by UVB, the depilated abdominal area of mice was irradiated with UVB on day 0, and sensitization was done at the irradiated site on day 1. Elicitation at the left ear was done on day 6.

To assay systemic immunosuppression induced by UVB, the depilated dorsal skin of mice was irradiated with UVB on day 0, and the mice were sensitized at the nonirradiated depilated abdominal skin on day 5. On day 10, CHS was elicited on the left ear.

During irradiation, the ears of mice were protected from UV irradiation by black adhesive tape, which was removed after exposure. Suppression of the ear swelling response was calculated as follows: 100 − (ear swelling of test mice/ear swelling of no-UVB control mice) × 100%.

Determination of PG content in mouse ears The amounts of PGD_2, PGE_2, and PGF_2 in mouse ears at 0, 24, 48, and 72 h after irradiation with 250 mJ per cm^2 or 500 mJ per cm^2 of UVB were determined by enzyme immunoassay. In brief, both ears of each mouse were cut and immediately dropped into liquid nitrogen. Four frozen ears were combined into one sample, and then weighted and homogenized with a polytron homogenizer in 10 ml ethanol containing 0.1 ml of 5 N HCl, which was precooled at −20°C. $[^3H]PGD_2$, $[^3H]PGE_2$, and $[^3H]PGF_2$ (10000 dpm each) were added to the homogenates as tracers for estimation of the recovery. After centrifugation, PG in the ethanol extract were applied onto SEP-PAK C18 cartridges (Waters Associates, Milford, MA) and purified by using high pressure liquid chromatography. The amounts of PGD_2, PGE_2, and PGF_2 were measured in duplicate by enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI).

Expression of mRNA by reverse transcriptase-polymerase chain reaction (RT-PCR) The expression of the genes COX-1, COX-2, cytosolic phospholipase A2 (cPLA2), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were analyzed by using the RT-PCR method. Total RNA was isolated from UV-irradiated mouse ears using the standard guanidine thiocyanate method. Messenger RNA was extracted from the total RNA using QuickPrep Micro mRNA Purification kit (Amersham Pharmacia Biotech, Buckinghamshire, U.K.). Messenger RNA (0.5 g) was reverse transcribed using RNA PCR kit (AMV) ver. 2.1 (Takara shuzo, Kyoto, Japan) according to the manufacturer's protocol. Briefly, RT products were used for PCR with 0.5 units of Taq DNA polymerase (Takara shuzo) and 100 pmol of each primer using the Perkin-Elmer Gene Amp PCR system 2400 (Perkin Elmer Cetus, Foster City, CA). PCR was performed as follows: denature at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s for 30 cycles. The intensity of mRNA expression was determined with a densitometer and calculated the relative density of PCR bands against GAPDH in the same sample.

Hematologic and lymphocyte phenotype analysis Peripheral blood was obtained by venipuncture and anticoagulated with heparin. Total white blood cell (WBC) counts and lymphocyte counts were obtained for each sample. Peripheral blood mononuclear cells were isolated using standard Ficoll-Isopaque gradient centrifugation. The number of T cells and natural killer cells were measured by flow cytofluorometric analysis. Fluorescein isothiocyanate-labeled antimouse CD4 (L3T4) and antimouse natural killer cells (2B4) were purchased from PharMingen (San Diego, CA). Fluorescein isothiocyanate-labeled antimouse CD8 (Lyt 2) and anti-Thy 1, 2 were purchased from Becton Dickinson (Bedford, MA). As controls, fluorescein isothiocyanate-labeled mouse IgG2a and IgG2b (Dako, Glostrup, Denmark) were used.

***In vivo* induction of natural killer cell activity** The natural killer cell activity was enhanced by injecting polinosinic:polycytidylic acid (poly I:C) (100 g per 20 g K.W.) (Sigma, St. Louis, MO) i.p. 18 h before harvesting the spleen cells.

Natural killer cell assays Cell cultures and assays were performed in Dulbecco's minimal essential medium (DMEM), supplemented with 10% fetal calf serum, 100 IU penicillin per ml, and 100 g streptomycin per ml. As a target for natural killer cytolysis, the YAC-1 cell line (a DBA/2 lymphoma) was used. The natural killer cell activity was assessed in the whole spleen of mice. These were removed aseptically, gently crushed, and then suspended in DMEM. Lymphocyte suspensions were washed twice and adjusted to 110^7 viable cells per ml. The YAC target cells (5×10^6 cells per 100 liters) were labeled with $Na_2{}^{51}CrO_4$ (Amersham, Arlington Heights, IL) for 1 h at 37°C (1 mCi per ml to 5×10^6 cells in 0.1 ml of DMEM). The ^{51}Cr-release assay for the target was performed in 96-well, U-bottomed microtiter plates for each sample, in triplicate. Aliquots containing 1×10^4 target cells were added to each well containing the appropriate number of effector cells in a final volume of 200 liters, resulting in effector-to-target cells rations from 25:1 to 200:1. All plates were incubated for 4 h in a humidified atmosphere of 5% CO_2 in air. One hundred milliliters of the supernatants were harvested and the radioactivity was counted by a gamma counter (Packard, Tilburg, the Netherlands) as counts per minute (cpm). The maximum release by lysis of the target cells was estimated by incubating the labeled target cells with 2 N HCl and was 80%–90% of the total ^{51}Cr incorporated. Background ^{51}Cr release was estimated by incubating the labeled target cells in DMEM without the lymphoid effector cells, which yielded a release of between 5% and 15% of incorporated ^{51}Cr. The percentage specific lysis of the target cells was calculated as (sample cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm) × 100%.

Statistical analysis A Student's t test was employed to determine the statistical significance.

RESULTS AND DISCUSSION

The (−/−) mice developed severe inflammation after UVB, which was inhibited by indomethacin A single exposure to 250 mJ per cm^2 of UVB induced significant ear swelling in the XPA gene-deficient (−/−) mice 24 h after irradiation and the edema was still increasing at day 5 (**Fig 2**) (Miyauchi-Hashimoto *et al*, 1996).

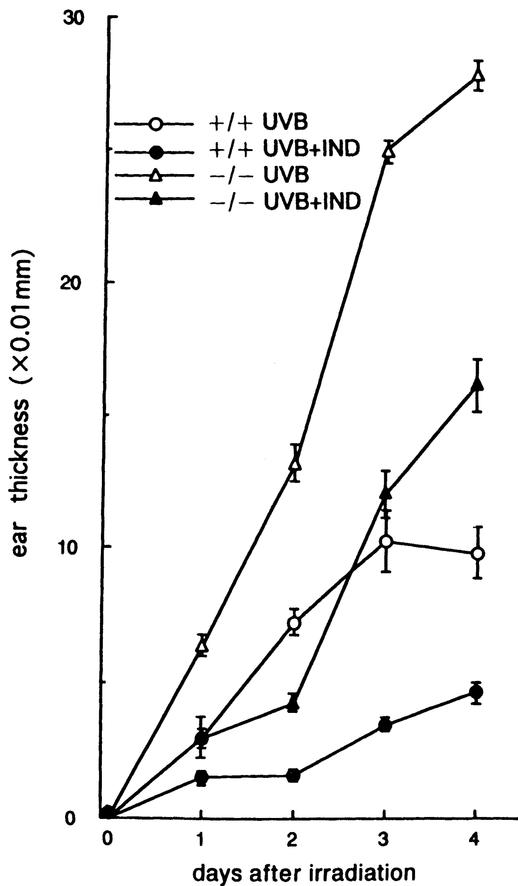

Figure 2. Effect of indomethacin on UV-induced ear swelling. Ears of mice were exposed to 250 mJ per cm^2 UVB with or without topical application of 1% indomethacin (IND). Significant inhibition of ear swelling was observed in (+/+) and (–/–) mice with IND at all times after UVB radiation: $p < 0.005$ as compared with the respective irradiated-mice without IND treatment.

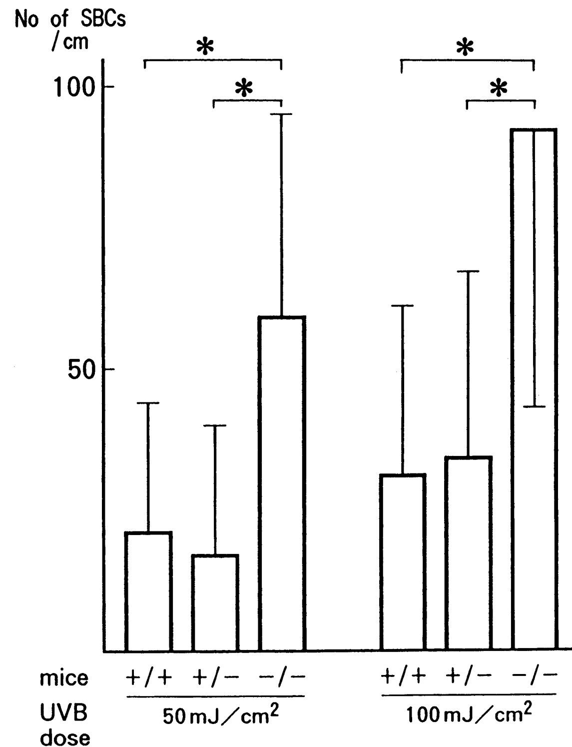

Figure 3. More SBC were formed in XPA-deficient mice. Biopsy specimens were taken 24 h after irradiation with 50 or 100 mJ per cm^2 of UVB. The number of SBC was counted in three haematoxylin and eosin-stained sections. Data are expressed as mean number of SBC (±SD) per 1 cm of the epidermis. *$p < 0.0005$.

To determine whether the UV-induced edema is mediated by PG, which are inflammatory mediators, we examined the effect of indomethacin, a potent inhibitor of PG-biosynthesis, on UV-induced inflammation. Topical application of 1% indomethacin significantly inhibited ear swelling in both (+/+) and (–/–) mice (Kuwamoto *et al*, 2000).

The (–/–) mice showed stronger histologic changes after UVB irradiation At 24 h after 500 mJ per cm^2 UVB irradiation, abdominal skin samples from (–/–) mice showed intracellular edema and necrosis in the epidermis and subepidermal bullae. In contrast, the samples from (+/+) mice revealed only a little change in the epidermis. Moreover (–/–) mice showed marked inflammatory infiltrates of lymphocytes, pronounced edema, vasodilatation, and extravasation of erythrocytes in the dermis.

The (–/–) mice developed enhanced sunburn cell formation after UVB radiation At 24 h after 50 mJ per cm^2 of UVB irradiation significantly enhanced SBC, (apoptosis) formation was induced in the skin of (–/–) mice (59.6 ± 36.2 per cm, mean ± SD) compared with that in the (+/+) and (+/–) mice (20.6 ± 23.8 per cm and 17 ± 22.8 per cm, respectively) (**Fig 3**). Similarly, SBC induced by UVB irradiation at a higher dose (100 mJ per cm^2) in (–/–) mice were almost three times as numerous as those in (+/+) or (+/–) mice.

The (–/–) mice developed stronger reduction in number of APDase(+) Langerhans cells after UVB radiation Because keratinocytes of (–/–) mice were severely damaged by UVB radiation as described above, the alteration of Langerhans cells, another resident in the epidermis, was also examined. In nontreated skin, approximately the same numbers of ADPase(+) Langerhans cells were found in the (–/–), (+/+), and (+/–) mice (516.8 ± 85.8 per mm^2, 553.6 ± 100.48 per mm^2, and 601.6 ± 138.9 per mm^2, respectively). In the (–/–) mice, the number of ADPse(+) Langerhans cells decreased by 59% of the preirradiated level 24 h after irradiation with 25 mJ per cm^2 of UVB. On the other hand, the percentage reductions in the (+/+) and (+/–) mice were 33% and 38%, respectively. ADPase(+) Langerhans cells remaining after 25 mJ per cm^2 UVB radiation were rounded and lacked dendrites in the (–/–) mice, but those in (+/+) or (+/–) mice had multiple branched dendrites. UVB irradiation at a higher dose (100 mJ per cm^2) reduced the number of ADPase(+) Langerhans cells by almost 100% in the (–/–) mice, but by only 62% in (+/+) mice.

In another group of experiments, we investigated the kinetics of ADPase(+) Langerhans cells after exposure to 25 mJ per cm^2 of UVB. The recovery of ADPase(+) Langerhans cells was slower in the (–/–) mice than in the (+/+) or (+/–) mice. In the (+/+) and (+/–) mice, the number of ADPase(+) Langerhans cells after UVB radiation reached a minimum 3 d after irradiation and then rapidly recovered, returning nearly to preirradiation levels by day 19. On the other hand, the density of ADPase(+) Langerhans cells in the (–/–) mouse skin promptly declined to a minimum 24 h after irradiation and recovered more slowly than in control mice. By day 19, the density of ADPase(+) Langerhans cells in (–/–) mice skin reached only 52% of that of preirradiation levels.

The (–/–), (+/+), and (+/–) mice developed similar CHS without UVB irradiation We compared the ear swelling response in the elicitation of DNFB-CHS among the groups of (–/–), (+/+), and (+/–) mice. The ear swelling measured 24 h after

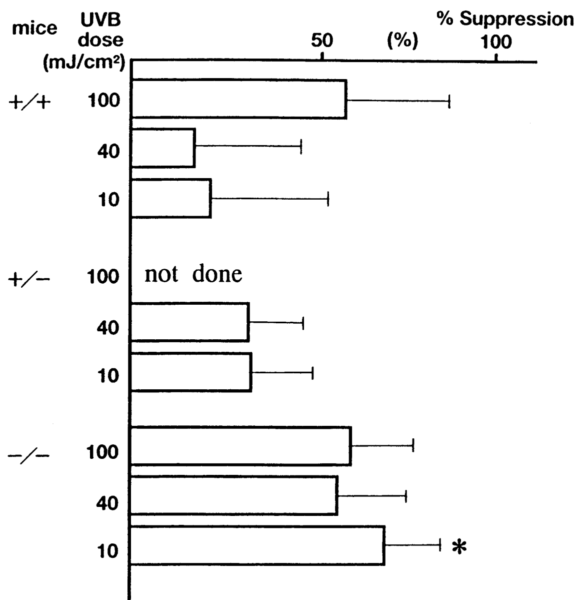

Figure 4. Local immunosuppression was increased by UVB irradiation in XPA-deficient mice. Groups of mice were exposed to UVB at a dose of 10, 40, or 100 mJ per cm^2. Sensitization was attempted 1 d after irradiation by applying 1% DNFB to the irradiated skin. Five days later, the mice were challenged with 0.2% DNFB. Data are expressed as the mean of percentage suppression (±SD). $^*p < 0.05$ *vs* 10 mJ per cm^2 UVB-exposed (+/+) and (–/–) mice group.

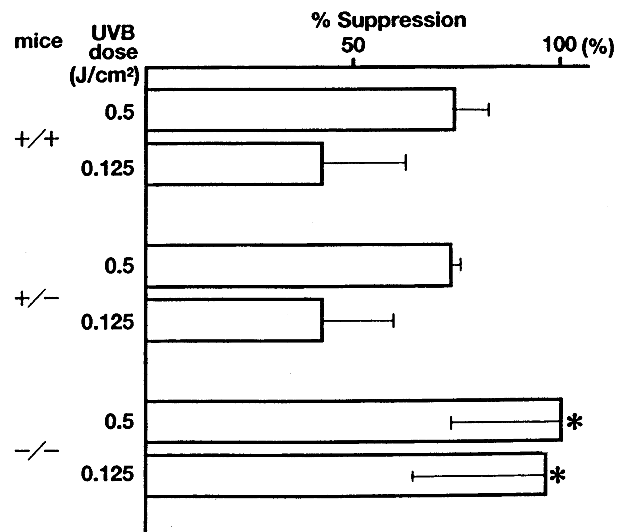

Figure 5. Systemic immunosuppression was increased by UVB radiation in XPA-deficient mice. Dorsal skin of groups of mice was exposed to UVB at a dose of 0.125 or 0.5 J per cm^2. Sensitization was attempted 5 d after irradiation by 1% DNFB to the unirradiated abdominal skin. Five days later, the mice were challenged epicutaneously with 0.2% DNFB. Data are expressed as mean of percentage suppression (±SD). $^*p < 0.0005$ *vs* the (+/+) and (–/–) mice group received the same dose of UVB.

challenge was $12.17 \times 10^{-2} \pm 2.9$ mm in the (–/–) mice, $10.2 \times 10^{-2} \pm 2.4$ mm in the (+/+) mice, and $8.6 \times 10^{-2} \pm 3.9$ mm in the (+/–) mice. There are reports that XP patients have defects in cell-mediated immunity, including impaired cutaneous responses to recall antigens (Dupuy and Lafforet, 1974; Wysenbeek *et al*, 1986) and a contact sensitizer (Dupuy and Lafforet, 1974; Morison *et al*, 1985). In these studies using patients, the effect of sunlight exposures cannot be completely neglected. In this study, the (–/–) mice developed CHS to DNFB similarly to the control (+/+) and (+/–) mice without UV irradiation.

UVB-induced local and systemic immunosuppression were enhanced in the (–/–) mice Because the Langerhans cell, an antigen-presenting cell, was severely damaged by UVB radiation, as observed above, the effect of UVB radiation on CHS in the model mice was investigated.

Sensitization with DNFB on skin that had been exposed to 100 mJ per cm^2 UVB resulted in a significantly decreased CHS response in both (+/+) and (–/–) mice (59.1% suppression and 54.1% suppression, respectively) (**Fig 4**). In the (–/–) mice, almost the same degree of suppression was induced by lower doses of UVB radiation such as 40 mJ per cm^2 and 10 mJ per cm^2 (69.4% and 56.3% suppression, respectively), whereas less suppression was induced in the (+/+) mice (18.6% and 21.7% suppression, respectively) and in the (+/–) mice (30.9% and 18.4% suppression, respectively).

Exposure to 0.5 J per cm^2 UVB irradiation 5 d before sensitization with DNFB on nonirradiated skin induced stronger suppression of CHS in the (–/–) mice than in the (+/+) or (+/–) mice (> 100% *vs* 76.5%, or 76.7%) (**Fig 5**). Although UVB radiation at a lower dose (0.125 J per cm^2) produced only little suppression in (+/+) or (+/–) mice (43.1% or 41.9% suppression, respectively), it produced pronounced suppression in (–/–) mice (92.0% suppression) (Miyauchi-Hashimoto *et al*, 1996).

Inhibitory effect of indomethacin on UV-induced immunosuppression It has been shown that PG have an immunosuppressive effect (Snijdewint *et al*, 1993; Shreedhar *et al*, 1998). To examine whether PG play a role in UV-induced immunosuppression, we investigate the effect of 1% indomethacin on UVB-induced local immunosuppression (Kuwamoto *et al*, 2000). Topical application of 1% indomethacin on irradiated skin immediately after irradiation abrogated the suppressive activity of UV in both (+/+) and (–/–) mice. Epicutaneous application of acetone after irradiation did not abrogate local immunosuppression. Compared with the positive control (no UV irradiation and no indomethacin), sensitization with DNFB on abdominal skin that had been exposed to 50 mJ per cm^2 UVB resulted in suppressed CHS response in both (+/+) and (–/–) mice (by 52.9% and 74.1% suppression, respectively). These results suggest that UV-induced PG might cause immunosuppressive activity of UV.

Striking increase of PG production after UVB irradiation in (–/–) mice Because indomethacin inhibited UV-induced acute inflammation and immunosuppression, we measured the amounts of PGD_2, PGE_2, and PGF_2 in mouse ears at various times after 250 mJ per cm^2 UVB irradiation, by enzyme immunoassay (**Fig 6**) (Kuwamoto *et al*, 2000).

The amounts of PGD_2, PGE_2, and PGF_2 in (–/–) mouse skin significantly increased at 48 and 72 h after irradiation. Among three PG, PGE_2 most markedly increased, to levels 4–10-fold higher than those of PGD_2 or PGF_2. Furthermore, the amount of PGE_2 in (–/–) mice was approximately 8- and 15-fold higher than that in (+/+) mice at 48 and 72 h after irradiation. The amount of PGE_2 in the skin of UV-irradiated (–/–) mice was not detected by treatment of 1% indomethacin at 24 and 48 h after irradiation. Serum amounts of PG have not been determined.

Expression of mRNA for COX-2 was remarkably increased after UVB irradiation Increased production of PG in the ears of irradiated mice suggested that UVB exposure induced synthesis of COX. To analyze the expression of COX-1, COX-2, and $cPLA_2$ genes, mRNA was isolated from the mouse ears after 250 mJ per cm^2 UVB irradiation, and subjected to RT-PCR analysis.

The expression of COX-2 mRNA in (–/–) mice increased from 24 h after UV irradiation in a time-dependent manner (**Fig 7**).

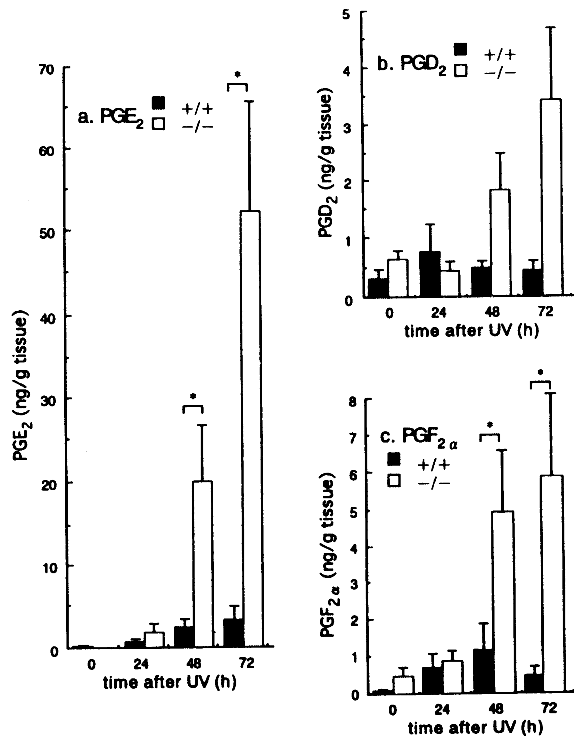

Figure 6. Production of PGD_2, PGE_2, and PGF_2 in ears of (+/+) and (–/–) mice after UVB irradiation. Both ears of each mouse were removed and directly dropped into liquid nitrogen at various intervals after 250 mJ per cm^2 of UVB irradiation. The amount of PGE_2 (*a*), PGD2 (*b*), and PGF_2 (*c*) in ears was determined by enzyme immune assay (EIA). *p < 0.05.

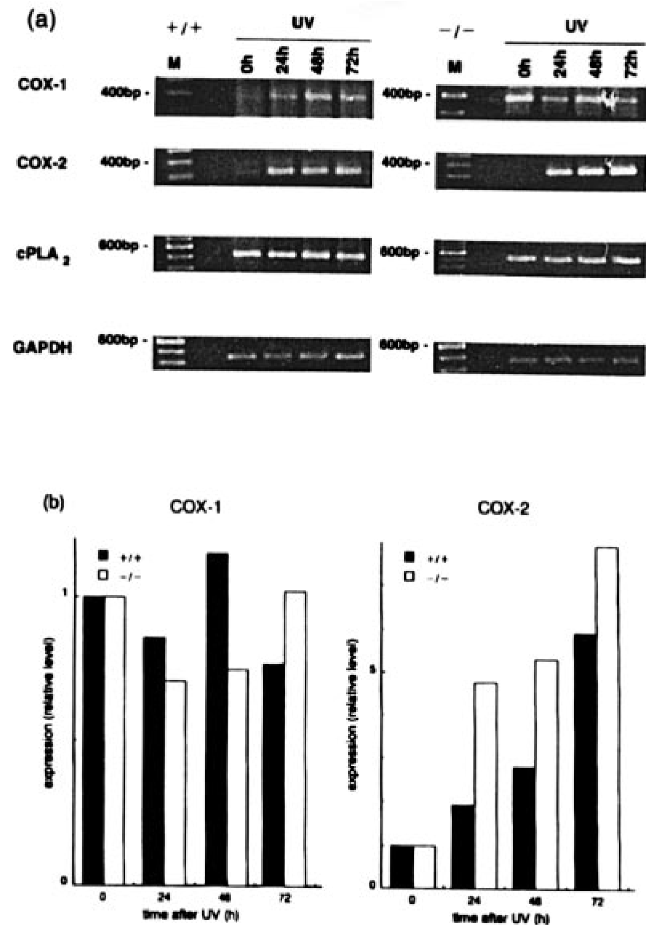

Figure 7. Expression of mRNA for COX-1, COX-2, $cPLA_2$, and GAPDH after UVB radiation. The levels of mRNA for COX-1, COX-2, $cPLA_2$, and GAPDH in the ears of UVB-irradiated mice determined by RT-PCR. (*a*) PCR products were separated on 2% agarose gels and stained with ethidium bromide. M, markers; 100 bp ladder. (*b*) Graphed bars represent the relative level of COX-1 and COX-2 PCR bands against GAPDH analyzes with a densitometer.

COX-1 and $cPLA_2$ mRNA were expressed nearly constantly and were not affected by the irradiation in either (+/+) or (–/–) mice.

The molecular mechanisms of the severe inflammation and immunosuppression found in XPA-deficient mice after UV exposure have not been clarified. This experiment indicated that the mechanisms would be due to increased expression of mRNA for COX-2 and subsequent overproduction of PGE_2 after UV exposure.

Peripheral lymphocyte and natural killer cell counts decreased after UVB radiation in XPA mice It has been previously shown that UV radiation can affect the population and function of peripheral blood cells (Morison *et al*, 1979; Hersey *et al*, 1983). The effect of UVB irradiation on the blood cell counts was examined in (–/–) and (+/+) mice (Miyauchi-Hashimoto *et al*, 1999). One group of mice was irradiated with a single exposure of 500 mJ per cm^2 UVB. The second group was exposed to 500 mJ per cm^2 UVB, three times on three consecutive days. The third group received 500 mJ per cm^2 UVB daily for five consecutive days. Peripheral blood was collected 24 h after the last irradiation. In the (+/+) control mice group, the WBC, lymphocyte, and natural killer cell counts were not altered by any of the exposures. In the (–/–) XPA mice group, the number of WBC was not affected by UVB exposures. The lymphocyte count, however, was significantly decreased ($p < 0.05$) after three or five daily exposures to 500 mJ per cm^2 UVB, and the natural killer cell count was more significantly decreased ($p < 0.01$ or $p < 0.005$) after three or five exposures, respectively.

Next, time course changes in the number of WBC, lymphocytes, and natural killer cells in peripheral blood were studied after five daily exposures to 500 mJ per cm^2 UVB. In (+/+) mice, the WBC, lymphocyte, and natural killer cell counts reached a minimum at 3 d after the last exposure and returned to preirradiation levels by day 5, although these changes were not significant. In (–/–) mice, the decrease in cell counts was more prominent and longer lasting than in wild-type mice. The number of WBC showed a significant decrease ($p < 0.05$) only at 5 d after irradiation in (–/–) mice. The counts of lymphocytes and natural killer cells in (–/–) mice decreased 24 h after the last irradiation reached a minimum 3–5 d after irradiation, and recovered more slowly than in control mice. By day 15, they returned to preirradiated levels.

UVB irradiation inhibited natural killer cell activity in XPA mice We examined the effects of UV radiation on natural killer cell function, which has an important role in tumor surveillance (Miyauchi-Hashimoto *et al*, 1999). As spontaneous natural killer cell activity was low in both strains, assays were also performed after a standard *in vivo* natural killer cell induction with poly I:C. Three groups from each strain were irradiated with 500 mJ per cm^2 UVB as single, three, or five daily exposures, respectively. The spontaneous and poly I:C-inducible natural killer activity 24 h after a single exposure of 500 mJ per cm^2 UVB in (+/+) and (–/–) XPA mice were comparable with those in the unirradiated mice of each strain (data not shown). In (+/+) mice, the poly I:C indocibility of natural killer cell activity was also not impaired after three and five daily exposures with 500 mJ per cm^2 UVB (**Fig 8**). The poly I:C-inducible natural killer activity in (–/–) XPA mice,

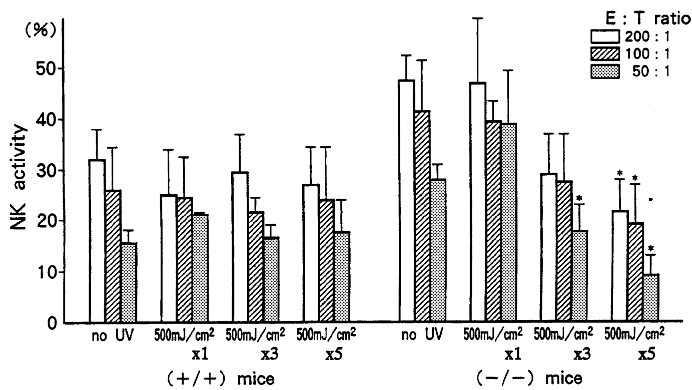

Figure 8. UVB irradiation impaired natural killer activity in XPA-deficient mice. At 24 h after three and five daily exposures to 500 mJ per cm^2 UVB, the natural killer activity in XPA-deficient mice decreased to 20%–30% and 10%–20%, respectively, but was not decreased in wild-type mice. Data are expressed as the percentage lysis of target cells (±SD) in an natural killer cytotoxicity assay. *p < 0.025; **p < 0.005; ***p < 0.0005, compared with the value at the same E/T ratio in the nonirradiated group.

however, decreased to 20%–30% and 10%–20% after three and five daily exposures to 500 mJ per cm^2 UVB, respectively. These natural killer cell activity levels were equivalent to 60% and 30% of the preirradiated levels, respectively. The decline of natural killer activity in XPA mice was not due to the decrease of natural killer cell number, because the absolute number of splenic natural killer cells was not changed 1 and 3 d after the five daily exposures to 500 mJ per cm^2 UVB. The depression of natural killer activity in (–/–) mice recovered to a normal level at 10 and 15 d after the last exposure of three and five daily exposures, respectively.

The mechanism for suppression of natural killer activity by UV radiation is not known. It is possible that UV radiation exerts a direct effect on natural killer cells circulating through the dermal capillaries. Alternatively, soluble mediators may be released from the epidermal or dermal cells on UV irradiation that subsequently enter the circulation and indirectly affect natural killer cell function. Cytokines, such as transforming growth factor- and IL-10, and PGE_2, have been shown to inhibit natural killer cell activity. PGE_2 suppresses natural killer activity through the suppression of IFN- and IL-2 induction by Th1 cells (Koren and Leung, 1982). IL-10 also suppresses natural killer activity through the suppression of IFN- and IL-2 from Th1 cells, macrophages, and natural killer cells (Liu *et al*, 1994).

CONCLUSION

The xeroderma pigmentosum model mouse is a useful experimental animal not only to investigate photosensitivity in the disorder, but also to study photoimmunology, because photobiologic reactions are greatly intensified in this mouse. Although various findings have been reported in patients with XP, they may be caused by unintentional exposures to the natural sunlight rather than by constitutive abnormalities. In contrast, animal experiments can be done under completely nonexposed conditions or after exposures to desired UV doses and wavelengths. The high incidence of skin cancers in XP patients may be mainly due to a defect in the repair of UV-damaged DNA of cutaneous cells. It is now well known that UV radiation has immunosuppressive effects as well as mutagenic properties, both of which contribute to the sunlight-induced skin cancers (Kripke, 1984; Ullrich, 1995). Our experiments using XPA gene-deficient mice indicated that the enhanced UV-induced immunosuppression and impairment of natural killer function can also be involved in cancer development in XP. The extremely severe sunburn reaction in XP patients is possibly due to the increased expression of COX-2 mRNA after UV irradiation and the subsequent production of high amounts of PGE_2.

This work was supported by grants from the Scientific Research Fund of the Ministry of Education (Grant-in-Aid for Scientific Research no. 07770712, 09770666, 09670901, and 11770488), from the Ministry of Health and Welfare, Japan, and from the SHISEIDO Grant for Skin Aging Research (SRG # 198043).

REFERENCES

Cleaver JE: Defective repair replication of DNA in xeroderma pigmentosum. *Nature* 218:652–656, 1968

Dupuy JM, Lafforet D: A defect of cellular immunity in xeroderma pigmentosum. *Clin Immunol Immunopathol* 3:52–58, 1974

Hersey P, Haran G, Hasic E, *et al:* Alteration of T cell subsets and induction of suppressor T-cell activity in normal subject after exposure to sunlight. *J Immunol* 131:171–174, 1983

Koren HS, Leung KH: Modulation of human NK cells by interferon and prostaglandin E2. *Mol Immunol* 19:1341–1346, 1982

Kripke ML: Immunological unresponsiveness induced by ultraviolet radiation. *Immunol Rev* 80:87–102, 1984

Kuwamoto K, Miyauchi-Hashimoto H, Tanaka K, Eguchi N, Inui T, Urade Y, Horio T: Possible involvement of enhanced prostaglandin E_2 production in the photosensitivity in xeroderma pigmentosum group A model mice. *J Invest Dermatol* 114:241–246, 2000

Liu Y, Wei SHY, Ho ASY, *et al:* Expression cloning and characterization of human IL-10 receptor. *J Immunol* 152:1821–1829, 1994

Miyauchi-Hashimoto H, Tanaka K, Horio T: Enhanced inflammation and immunosuppression by ultraviolet radiation in xeroderma pigmentosum group A (XPA) model mice. *J Invest Dermatol* 107:343–348, 1996

Miyauchi-Hashimoto H, Okamoto H, Tanaka K, Horio T: Ultraviolet radiation-induced suppression of natural killer cell activity is enhanced in xeroderma pigmentosum group A (XPA) model mice. *J Invest Dermatol* 112:965–970, 1999

Morison WL, Parrish JA, Block KJ, *et al:* In vivo effect of UVB on lymphocyte function. *Br J Dermatol* 101:513–519, 1979

Morison WL, Bucana C, Hashem N, Kripke ML, Cleaver JE, German JL: Impaired immune function in patients with xeroderma pigmentosum. *Cancer Res* 45:3929–3931, 1985

Nakane H, Takeuchi S, Yuba S, *et al:* High incidence of ultraviolet-B- or chemical-carcinogen-induced skin tumours in mice lacking the xeroderma pigmentosum group A gene. *Nature* 377:165–168, 1995

Robbins JH, Kraemer KH, Lutzner MA, Festoff BW, Coon HG: Xeroderma pigmentosum. an inherited disease with sun sensitivity, multiple cutaneous neoplasmas, and abnormal DNA repair. *Ann Intern Med* 80:221–248, 1974

Shreedhar V, Giese T, Sung VW, Ullrich SH: A cytokine cascade including prostaglandin E_2, IL-4, and IL-10 is responsible for UV-induced systemic immune suppression. *J Immunol* 160:3738–3789, 1998

Snijdewint FGM, Kalinski P, Wierenga EA, Bos JD, Kapsenberg ML: Prostaglandin E_2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J Immunol* 150:5321–5329, 1993

Ullrich SE: The role of epidermal cytokines in the generation of cutaneous immune reactions and ultraviolet radiation-induced immune suppression. *Photochem Photobiol* 62:389–401, 1995

Wysenbeek AJ, Weiss H, Duczyminer-Kahana M, Grunwald MH, Pick AI: Immunologic alterations in xeroderma pigmentosum patients. *Cancer* 58:219–221, 1986