

# Association of Transcription-Coupled Repair but Not Global Genome Repair with Ultraviolet-B-Induced Langerhans Cell Depletion and Local Immunosuppression

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**Exposure to ultraviolet-B radiation impairs cellular immune responses. This immunosuppression seems to be associated with Langerhans cell migration. DNA damage appears to play a key role because enhanced nucleotide excision repair, a pathway essential for elimination of ultraviolet-B-induced DNA lesions, strongly counteracts immunosuppression. To determine the effect of DNA repair on ultraviolet-B-induced local immunosuppression and Langerhans cell disappearance, three mouse strains carrying different defects in nucleotide excision repair were compared. XPC mice, which were defective in global genome repair, were as sensitive to ultraviolet-B-induced local suppression of contact hypersensitivity to picryl chloride as their wild-type littermates. CSB mice, defective in transcription-coupled repair, were far more sensitive for immunosuppression**

**as were XPA mice, defective in both transcription-coupled repair and global genome repair. Only a moderate depletion of Langerhans cells was observed in XPC mice and wild-type littermates. Ultraviolet-B-induced Langerhans cell depletion was enhanced in CSB and XPA mice. Hence, the major conclusion is that local immunosuppression is only affected when transcription-coupled DNA repair is impaired. Furthermore, a defect in transcription-coupled repair was linked to enhanced ultraviolet-B-induced Langerhans cell depletion. In combination with earlier experiments, it can be concluded that Langerhans cell disappearance is related to ultraviolet-B-induced local but not to systemic immunosuppression. *Keywords: contact hypersensitivity/nucleotide excision repair/skin/transgenic/knockout. J Invest Dermatol 121:751–756, 2003***

**U**ltraviolet (UV) radiation causes a broad range of effects in the skin. One of these effects, predominantly caused by UV-B (280–315 nm), is the suppression of cellular immunity. This immunosuppression is associated with the disappearance of antigen-presenting cells, the Langerhans cells, from the epidermis. UV radiation can be absorbed by organic molecules such as DNA, leading to the induction of cyclobutane pyrimidine dimers (CPDs) as well as 6–4 pyrimidine–pyrimidone photoproducts. Cells with UV-specific DNA damage, i.e., Langerhans cells, were found in murine lymph nodes (Sontag *et al*, 1995) after exposure of the skin to UV radiation. Furthermore, migrating Langerhans cells were detected in the blister fluid of UV-exposed human skin (Kölgen *et al*, 2002).

*In situ* action spectra suggested that DNA damage is involved in UV-induced immunosuppression of allogeneic reactions in humans (Hurks *et al*, 1997). DNA damage can trigger the production of tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-10 and IL-6, and thereby modulate Langerhans cell activity and local immune responses (Nishigori *et al*, 1996; O'Connor *et al*, 1996; Petit-Frere *et al*, 1998). Treatment of mice with thymidine dinucleotides (pTpT), mimicking DNA fragments that are released when UV-induced DNA damage is repaired (but lacking the dimer conformation), inhibits contact hypersensitivity (CHS) and activates the TNF- $\alpha$  gene (Cruz *et al*, 2000). Experiments on the *Monodelphis domestica*, which has a photoreactivation enzyme that is able to remove CPDs, revealed that DNA damage but also urocanic acid dimerization is involved in the suppression of CHS by UV radiation (Reeve *et al*, 1996). Furthermore, removal of CPDs by treatment with liposomes containing T4 endonuclease V (Applegate *et al*, 1989; Vink *et al*, 1997) resulted in a reversion of UV-induced local as well as systemic immunosuppression. Topical treatment with T4 endonuclease V leads to a protection against UV-induced upregulation of the immunosuppressive cytokine IL-10 (Nishigori *et al*, 1996) and TNF- $\alpha$  (Wolf *et al*, 2000). The products released by UV-damaged cells can be transported by the circulation throughout the whole body, which may contribute to the systemic immunosuppressive effects.

Depending on the type of DNA damage, different DNA repair pathways can be activated (De Boer and Hoeijmakers,

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Abbreviations: CHS, contact hypersensitivity; CPD, cyclobutane pyrimidine dimer(s); CS, Cockayne syndrome; GGR, global genome repair; IL, interleukin; PCl, picryl chloride; NER, nucleotide excision repair; TCR, transcription-coupled repair; TNF, tumor necrosis factor; UV, ultraviolet; XP, xeroderma pigmentosum.

2000). The most important mechanism for the repair of CPDs and 6–4 pyrimidine–pyrimidone photoproducts in placental mammals is nucleotide excision repair (NER). NER consists of two pathways, namely global genome repair (GGR) and transcription-coupled repair (TCR). GGR is involved in helix-distorting DNA damage throughout the entire genome. TCR, on the other hand, preferentially eliminates transcription-blocking damage in actively transcribed genes. A defect in NER can lead to rare photosensitive disorders like xeroderma pigmentosum (XPA-XPG) and Cockayne syndrome (CSA and CSB). The majority of the XP patients, of which XPA with a deficiency in GGR as well as TCR is the most severe subgroup, are extremely sensitive to sunburn and run a more than 1000-fold increased risk of developing skin cancer. XPC patients, who are deficient in GGR alone, have a normal sensitivity to sunburn but are highly cancer-prone. Morison *et al* (1985) showed that XP patients had an impaired development of CHS, depending on the severity of the disease. Patients with CS, who have an selective impairment in TCR, are extremely photosensitive and develop neurological abnormalities. These CS patients, however, do not appear to be predisposed to develop skin cancer.

Studies with NER-deficient mouse mutants, carrying defects in TCR (CSB), GGR (XPC), or both (XPA), have the advantage that confounding genetic background differences can be eliminated. Such studies have provided additional evidence for a significant role of DNA damage in UV-B-induced immunosuppression. Miyauchi-Hashimoto *et al* (1996) and Garssen *et al* (2000) demonstrated that in XPA mice UV-induced local and systemic immunosuppression were increased. Systemic immunosuppression, however, was not enhanced in XPC nor in CSB mice (Garssen *et al*, 2000). Furthermore, Boonstra *et al* (2001) showed that the production of IL-10 and TNF- $\alpha$  was augmented in the lymph nodes of UV-exposed XPA and CSB but not XPC mice.

A decrease in Langerhans cell number could result in a disturbance in immune surveillance of UV-exposed skin. Langerhans cell densities in chronically sunlight-exposed skin of patients with XPA were remarkably reduced. In addition, the recovery of the epidermal Langerhans cell population was delayed in XPA patients compared to healthy controls (Jimbo *et al*, 1992).

The aim of the present study was to determine the influence of GGR and TCR on the UV-induced depletion of Langerhans cells and on UV-induced local immunosuppression. Subsequently, it could be ascertained whether these two phenomena are affected similarly by GGR and TCR, i.e., whether they could be related.

To investigate these different features, experiments on DNA-repair-deficient mice with defects in GGR and TCR (XPA), GGR alone (XPC), or TCR alone (CSB) were performed. After UV irradiation of the skin for five consecutive days, a contact allergen (picryl chloride (PCI)) was applied to the irradiated site. Following antigen challenge with PCI on the ears of the animals, ear swelling was determined as a measure of cellular immunity or conversely of UV-induced local immunosuppression. Similar to this experiment, a separate group of animals was subjected to the same UV exposure regimen. Two days before immunization and on the day of immunization, these mice were euthanized and their ears and lymph nodes from UV-exposed skin were collected. Immunohistochemistry was performed for NLDC145 (a dendritic cell marker) and H3 (a CPD marker) on frozen sections of the ear skin and the lymph nodes, the latter to check for Langerhans cell migration from the epidermis (Sontag *et al*, 1995).

## MATERIALS AND METHODS

**NER-deficient mouse models** XPA, CSB, and XPC mice refer to NER-deficient mice homozygous for the targeted allele in the respective genes (de Vries *et al*, 1995; Cheo *et al*, 1997; van der Horst *et al*, 1997). XPA

deficiency induces a virtual complete defect in TCR as well as GGR. CSB mice have only a total impairment of TCR, whereas XPC mice have a selective inactivation of GGR.

Mixed 129-C57Bl/6 or pure C57Bl/6 littermates of the homozygous knockout mice were used as control animals (the background of all mutant strains used in this study). The genotype of each mouse was determined by PCR. Mice were kept at an ambient temperature of  $25 \pm 1^\circ\text{C}$ . The room was illuminated with yellow fluorescent tubes (Philips TL40W/16) in a 12-h cycle (switched on and off at, respectively, 6.00 a.m. and 6.00 p.m.). These lamps do not emit any measurable UV radiation. No daylight entered the animal facilities. Animals were housed individually in macrolon type I cages for the entire experiment. Standard mouse chow (Hope Farms RMH-B, Woerden, the Netherlands) and tap water were available *ad libitum*.

Formal permission for the animal experiments was granted by an independent ethical committee of the National Institute of Public Health and the Environment, as required by Dutch law.

**Reagents** PCI (Chemotronix, Swannanoa, NC) was used as contact sensitizer. It was recrystallized three times from methanol-H<sub>2</sub>O before use and protected from light during storage at  $4^\circ\text{C}$ .

**UV exposure** The animals were shaven (on the back) 1 d before UV exposure using an electric clipper under light ether anesthesia. The animals were exposed to broadband UV-B radiation from a filtered (Schott WG305 filter) Hanovia Kromayer lamp Model 10S (Slough, UK). This is a hand-held lamp that allows short exposures to limited skin areas by placing the circular port (approximately  $2\text{ cm}^2$ ) in close contact to the skin (Goettsch *et al*, 1999; Sontag *et al*, 1994). The dose rate was  $150\text{ J per m}^2$  per s (280–400 nm), as measured by a Kipp E11 thermopile.

For determination of immunomodulation, the animals were exposed to five consecutive UV doses (one exposure per day; last exposure 4 d before immunization). The NER-deficient mice and their respective wild-type controls were exposed in accordance to their acute UV sensitivity, i.e., to  $150\text{ J per m}^2$  (XPA),  $150$  and  $300\text{ J per m}^2$  (CSB) or to  $300$ ,  $600$ , or  $900\text{ J per m}^2$  (XPC) each day.

**CHS to PCI** The mice were sensitized 4 d after the last day of (sham) irradiation by topical application of  $150\text{ }\mu\text{L}$  of 5% PCI in ethanol:acetone (3:1) to the UV-irradiated shaved back, a standard procedure in our laboratory. Control mice were sham-sensitized by topical application of  $150\text{ }\mu\text{L}$  ethanol:acetone (3:1). Four days after sensitization, both ears of the mice were challenged by topical application of one drop (27-gauge needle) of 0.8% PCI in olive oil. Before, and at 24 h after challenge, duplicate measurements of ear thickness were made using an engineer's micrometer in a blinded fashion. From earlier studies it is known that the maximal CHS response was 24 h after topical ear challenge, also in UV-B pre-exposed animals (Sontag *et al*, 1994). In each experiment, the increase in ear thickness in similarly challenged, nonsensitized, control mice was measured at the same time and subtracted from increments in ear thickness in sensitized test animals (net ear swelling). The CHS response (net ear swelling) in sensitized mice which were not exposed to UV-B radiation (minus the negative background swelling found in non-sensitized mice) was set at 100% to compare the effects of UV between the different mouse strains that were tested.

**Langerhans cell depletion** Groups of mice were irradiated with different UV doses for five consecutive days on their ears as described above. One control group per mouse strain was not irradiated. Forty-eight hours before immunization (2 d after irradiation) or on the day of immunization (4 d after irradiation), the animals were euthanized and the ears and the skin draining lymph nodes were removed. No immunization was performed on these animals. Ears and skin-draining lymph nodes were snap-frozen in liquid nitrogen, embedded in OCT compound (Tissue-Tek, Sakura, Zoeterwoude, the Netherlands), and stored at  $-80^\circ\text{C}$  until further processing. Lymph nodes served to verify that the Langerhans cells actually migrated from the skin.

**Immunohistochemical staining of NLDC145 and pyrimidine dimers** Frozen sections ( $6\text{ }\mu\text{m}$  thickness) of the ears and lymph nodes were mounted onto 3-aminopropyl triethoxysilane- (Sigma, St. Louis, MO) coated slides. A double staining was performed for NLDC145 (a dendritic cell marker; a gift from Prof. Dr. G. Kraal, Department of Cell Biology, Free University, Amsterdam, the Netherlands) and biotinylated H3 (directed against thymidine-containing cyclobutane pyrimidine dimers; a gift from Dr. L. Roza, TNO, Zeist, the Netherlands) as described previously (Sontag *et al*, 1995). The sections were fixed in dry acetone for 10 min, preincubated with blocking reagent (PBS containing

10% normal rabbit serum), and incubated with the primary antibody NLDC145 overnight at 4°C. After incubation for 1 h with biotinylated rabbit anti-rat immunoglobulins (DAKO A/S, Glostrup, Denmark), the sections were subsequently incubated for 30 min with alkaline phosphatase (AP)-conjugated avidin-biotin complex (DAKO A/S). Alkaline phosphatase activity was visualized by incubation in a BCIP/NBT solution of 37.5 µL of 5-bromo-4-chloro-3-indolylphosphate, 50 µL of 4-nitroblue tetrazolium chloride, and 2.5 mg of evamisole per 10 mL of 0.1 M Tris-HCl (pH 9.5)/0.1 M NaCl/0.05 M MgCl<sub>2</sub>. The bluish-black reaction product is water- and ethanol-insoluble and withstands the subsequent harsh treatment. The preparations were fixed in 25% acetic acid (vol/vol) containing 100 µL of 30% H<sub>2</sub>O<sub>2</sub> per 100 mL. After being washed in PBS, the slides were incubated for exactly 2 min in 0.07 N NaOH in 70% ethanol. The slides were rinsed in PBS and incubated for 1 h with biotinylated H3 antibody. Subsequently, the sections were incubated with horseradish peroxidase-conjugated avidin-biotin complex (DAKO A/S) for 30 min. Peroxidase activity was visualized by incubation in 0.1 M acetate buffer (pH 5) containing 20 mg of 3-amino-9-ethylcarbazole (Sigma) and 100 µL of 30% H<sub>2</sub>O<sub>2</sub> per 100 mL, resulting in a red staining.

After each antibody incubation, the slides were rinsed in PBS containing 0.05% Tween 20. Antibody incubations were performed in a humidified chamber at room temperature except for incubation with NLDC145, which was performed at 4°C. Negative staining controls were used in which the primary antibody was omitted.

The sections were evaluated using a light microscope at 250× magnification. The NLDC145 staining of the ears was determined quantitatively by image processing: the percentage of epidermal cross-sectional area that stained for NLDC145 was measured, using a camera mounted onto a light microscope together with the Optimas 6.1 and Microsoft Excel software (the targeted interfollicular epidermal area was demarcated manually).

#### Statistical analysis

**CHS experiments** The CHS responses were statistically evaluated using an independent two-tailed Student's *t* test. A *p* value of <0.05 indicated a significant difference compared to the unexposed sensitized positive control.

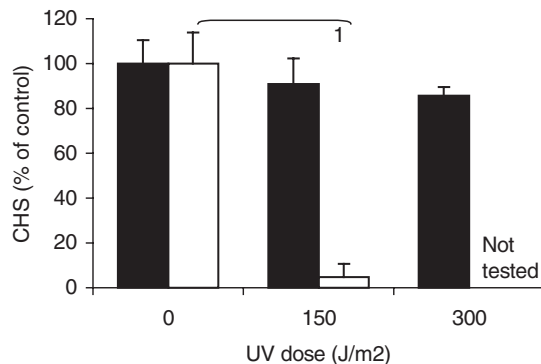
**Immunohistochemical studies** After logarithmic transformation (to correct for the right skewed distribution), an independent two-tailed Student's *t* test was performed to ascertain the significance of the observed differences (significance level *p*<0.05). The percentage of epidermal NLDC145+ area in the cross-section of UV-exposed ears of mutant mice and wild-type littermates were compared to their respective unexposed, control skin. Furthermore, the percentages of epidermal NLDC145+ area in the ears were compared between the mutant mice and their wild-type littermates for the different UV doses.

## RESULTS

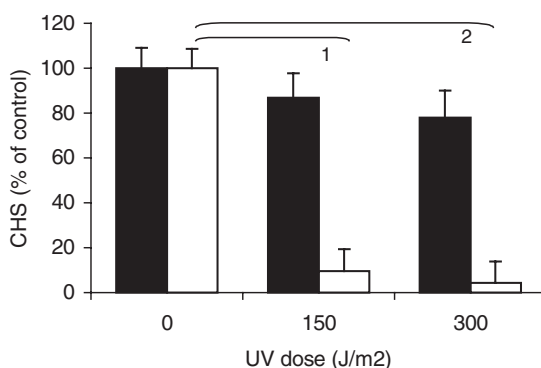
**A defect in NER does not affect CHS to PCI** CHS in XPA, CSB, and XPC mice and their respective wild-type littermates was measured 24 h after ear challenge with PCI in olive oil. Significant CHS (ear swelling) responses (*p*<0.05) to PCI were observed in each strain of mice compared to the background swelling responses found in the nonsensitized control animals from the same strain. The background ear swelling in nonsensitized animals of each strain was always less than 10 µm. Ear swelling in sensitized unirradiated controls was between 50 and 100 µm.

CHS responses in the mutant mice (XPA, CSB, and XPC) were not different from the CHS responses in the respective wild-type littermates. Thus, the various NER defects did not affect the normal T cell-dependent CHS response compared to the repair-competent wild-type littermates (data not shown).

**UV-B-induced suppression of CHS occurs at a lower dose in XPA and CSB but not XPC mice** To compare the suppressive effects of UV exposure, the control response in unirradiated animals was set at 100%. In each study the mutant mice were compared to their wild-type littermates. The findings are depicted in **Figures 1–3**. Each bar represents the percentage of CHS response compared with the control response in the respective unirradiated animals (set at 100%). A UV dose of 150 J



**Figure 1. UV-induced local immunosuppression of CHS in XPA mice (white bars) and wild-type littermates (black bars).** Each bar represents net ear swelling (mean ± SEM). The ear swelling in sensitized unirradiated mice was set at 100%. *p*<0.001 (1).

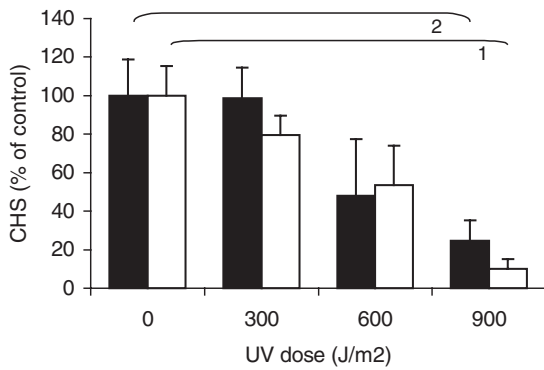


**Figure 2. UV-induced local immunosuppression of CHS in CSB mice (white bars) and wild-type littermates (black bars).** Each bar represents net ear swelling (mean ± SEM). The ear swelling in sensitized unirradiated mice was set at 100%. *p*<0.001 (1 + 2).

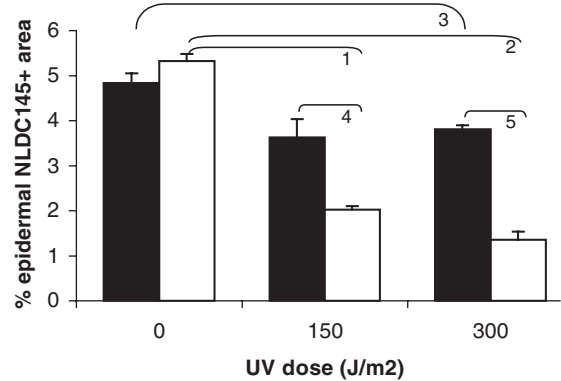
per m<sup>2</sup> was already sufficient for a significant immunosuppression in XPA mice (*p*<0.001) (**Fig 1**) and CSB mice (*p*<0.001) (**Fig 2**). In the respective wild-type littermates, however, CHS was not suppressed after exposure to 150 or 300 J per m<sup>2</sup>. The CHS response of XPC mice was significantly suppressed only after irradiation with 900 J per m<sup>2</sup> (*p*<0.001) (**Fig 3**). A minimal UV dose of 900 J per m<sup>2</sup> was also required for a significant suppression of CHS in the respective wild-type littermates (*p*<0.01).

**UV-B-induced Langerhans cell depletion is enhanced in XPA and CSB but not XPC mice** To examine the influence of GGR and TCR on UV-induced Langerhans cell depletion, we exposed NER-deficient mice and their wild-type littermates to different UV doses and determined the density of Langerhans cells in a cross-section of the (un)exposed skin of the ears. The density of epidermal NLDC145+ Langerhans cells in the unexposed skin of XPA, CSB, and XPC mice did not differ significantly from their wild-type littermates.

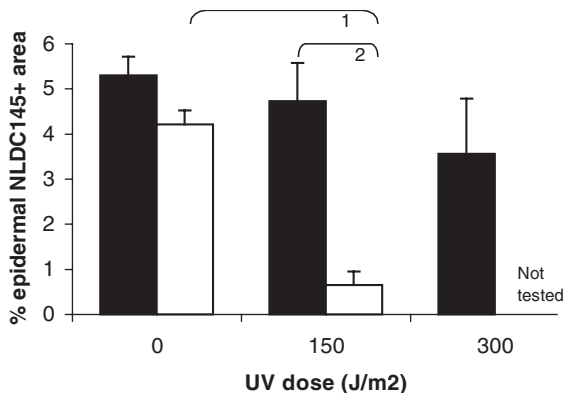
A UV dose of 150 J per m<sup>2</sup> was sufficient for a significant decrease in epidermal Langerhans cell numbers in XPA (*p*=0.011) and CSB mice (*p*<0.001) (**Figs 4, 5**, respectively). Wild-type littermates of CSB mice showed a significant decline in the density of epidermal Langerhans cells after irradiation with 300 J per m<sup>2</sup> (*p*=0.009). A minimal UV dose of 900 J per m<sup>2</sup> was required for a significant decrease in epidermal Langerhans cell density in the skin of wild-type littermates (*p*=0.006), but remarkably at this high dose the density of Langerhans cells in the skin of XPC mice was still not significantly reduced (**Fig 6**).



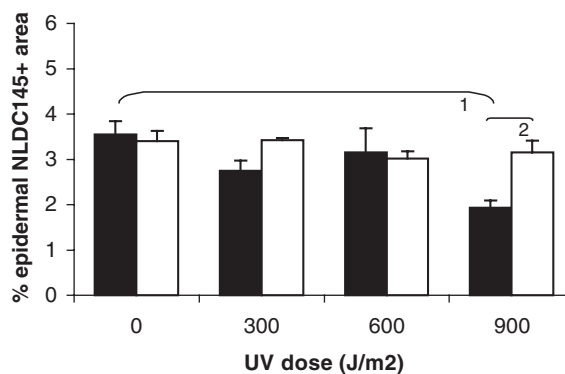
**Figure 3.** UV-induced local immunosuppression of CHS in XPC mice (white bars) and wild-type littermates (black bars). Each bar represents net ear swelling (mean  $\pm$  SEM). The ear swelling in sensitized unirradiated mice was set at 100%.  $p < 0.001$  (1);  $p < 0.01$  (2).



**Figure 5.** Percentage of epidermal NLDC145 + area (mean  $\pm$  SEM) of the (un)exposed ear skin of CSB mice (white bars) and wild-type littermates (black bars).  $p < 0.001$  (1);  $p = 0.001$  (2);  $p = 0.009$  (3);  $p = 0.008$  (4);  $p = 0.017$  (5).



**Figure 4.** Percentage of epidermal NLDC145 + area (mean  $\pm$  SEM) of the (un)exposed ear skin of XPA mice (white bars) and wild-type littermates (black bars).  $p = 0.011$  (1);  $p = 0.012$  (2).



**Figure 6.** Percentage of epidermal NLDC145 + area (mean  $\pm$  SEM) of the (un)exposed ear skin of XPC mice (white bars) and wild-type littermates (black bars).  $p = 0.006$  (1);  $p = 0.015$  (2).

The density of epidermal Langerhans cells was significantly less in the UV-exposed skin (150 and 300 J per  $m^2$ ) of XPA mice ( $p = 0.012$ ) and CSB mice ( $p = 0.008$  and  $p = 0.017$ , respectively) compared to the respective wild-type littermates (Figs 4, 5, respectively). The density of epidermal NLDC145 + Langerhans cells in the UV-B-exposed skin (300 and 600 J per  $m^2$ ) did not differ between XPC mice and their wild-type littermates. The density of epidermal Langerhans cells was even significantly larger in the UV-B-exposed skin (900 J per  $m^2$ ) of XPC mice than in their wild-type littermates ( $p = 0.015$ ) (Fig 6).

Figures 4–6 show data from Langerhans cell depletion of mice ears collected 2 days after irradiation. The differences in Langerhans cell depletion between the different mice strains and between the DNA-repair-deficient mice and their wild-type littermates 4 d after irradiation are comparable to the data of day 2 after irradiation.

Skin draining lymph nodes of the (un)exposed skin of all mouse strains were removed to verify that the Langerhans cells actually migrated from the skin. NLDC145 + cells with CPDs were detected in the draining lymph nodes of the UV-exposed skin, but not in those of the unexposed skin (checked in an select group of samples) (data not shown).

## DISCUSSION

In this study, we investigated whether TCR and/or GGR were involved in UV-induced local immunosuppression using geneti-

cally defined mouse mutants with specific defects in one or both of these DNA repair systems. An immunization with the contact allergen PCI on an irradiated site and subsequent challenge of the ears was used as a tool to determine the susceptibility to UV-induced local immunosuppression. Previously, we found that CHS responses to PCI could be inhibited by low doses of UV-B radiation (Sontag *et al*, 1994). This correlated with decreased levels of interferon- $\gamma$  and IL-12, which are important in the initiation and effector phase of Th1-mediated immunity (Garssen *et al*, 1999). Furthermore, it was shown that a complete defect in NER, as in XPA mice, increased the susceptibility to UV-induced systemic immunosuppression (Miyachi-Hashimoto *et al*, 1996; Garssen *et al*, 2000), whereas interferon- $\gamma$  levels were decreased (Boonstra *et al*, 2001). Systemic immunosuppression was not observed in XPC and CSB mice with a defect in GGR or TCR, respectively (Garssen *et al*, 2000).

In this study, it was demonstrated that UV-induced local immunosuppression was enhanced in XPA and CSB mice but not in XPC mice or wild-type littermates. These observations are in line with and extend the findings by Miyachi-Hashimoto *et al* (1996), who found that local immunosuppression was increased in XPA mice. Importantly, however, the present experiments give evidence that this increased susceptibility to local immunosuppression is not restricted to XPA mice but also applies to CSB mice and not to XPC mice. This implies that UV-B-induced local immunosuppression is influenced by TCR and not GGR, whereas systemic immunosuppression is only affected by a complete NER defect.

The present results further demonstrate that together with an increased local immunosuppression, Langerhans cell depletion was also enhanced in TCR-deficient mice (XPA and CSB) but not in GGR-deficient mice (XPC) in comparison with the respective wild-type littermates. This is in agreement with data from Jimbo *et al* (1992), who demonstrated that UV-induced Langerhans cell depletion was enhanced and Langerhans cell recovery was delayed in XPA patients compared to healthy controls. Boonstra *et al* (2001) showed that TNF- $\alpha$  production was augmented in the draining lymph nodes of the UV-exposed skin of XPA and CSB but not XPC mice. TNF- $\alpha$  is involved in the induction of Langerhans cell migration from the skin to the draining lymph nodes (Cumberbatch *et al*, 1997, 1999). This suggests that the enhanced Langerhans cell depletion, induced by a defect in TCR, is the result of an increased expression of TNF- $\alpha$ . This needs to be confirmed further by determination of TNF- $\alpha$  expression in the skin where Langerhans cells reside and which is the site of UV exposure.

Our data clearly indicate that the immunosuppressive signal stems from interference with transcription caused by the inflicted DNA damage: a lack of repair in the transcribed strand greatly enhances this signaling. Likewise, sunburn and apoptosis (sunburn cells) were found to be related to TCR and not GGR (Van Oosten *et al*, 2000). These responses may share a common initial pathway derived from stalled RNA polymerase. The precise initial signal transduction (probably involving kinases of the ATM superfamily) is unknown, but UV radiation is well known to activate downstream stress kinases like p38 leading to cyclooxygenase-2 expression (Chen *et al*, 2001). Cyclooxygenase-2 is involved in the production of prostaglandin E<sub>2</sub> (contributing to sunburn (Pupe *et al*, 2002)) and triggers the release of IL-4 and IL-10 (Shreedhar *et al*, 1998), which suppress Th1-mediated reactions. A signal transducer from the ATM superfamily sensing UV-induced DNA damage is the FRAP kinase that is involved in the induction of membrane-bound TNF- $\alpha$  (Yarosh *et al*, 2000a) and probably in the release of other immunosuppressive cytokines as topical application of rapamycin (blocking FRAP) protected against UV-induced suppression of CHS (Yarosh *et al*, 2000b). Although p53 activation also appears to be derived from damage in the transcribed DNA strand, the UV-induced immunosuppression does not appear to involve p53 (Jiang *et al*, 2001), and p53 represses TNF- $\alpha$  gene expression (Yarosh *et al*, 2000a). In spite of this emerging knowledge on cellular signaling, it is not yet known how stalled transcription leads to the activation of these signaling pathways.

Dendritic cells with UV-specific DNA damage could be observed in variable numbers in the skin-draining lymph nodes of UV-exposed XPA, CSB, and XPC mice. The presence of dendritic cells with CPDs in the lymph nodes indicated that the observed Langerhans cell depletion in the skin is at least in part caused by migration. Other investigators showed that UV-induced Langerhans cell depletion in mice *in vivo* could be caused by migration (Sontag *et al*, 1995). It was found that UV-irradiated murine Langerhans cells show delayed apoptosis *in vitro* (Tang and Udey, 1992), but *in vivo* barely any apoptotic Langerhans cells were found in the epidermis among the UV-induced apoptotic "sunburn cells" (Okamoto *et al*, 1999; Sontag *et al*, 1995). Van Oosten *et al* (2000) reported that apoptosis was increased in the UV-B-exposed skin of XPA and CSB but not XPC mice. An enhanced apoptosis could not only affect keratinocytes but also Langerhans cells. The experiments by Okamoto *et al* (1999), which showed a lack of Langerhans cells among the UV-induced apoptotic epidermal cells and the observed presence of UV-damaged Langerhans cells in the draining lymph nodes of NER-deficient mice, indicate that UV-induced Langerhans cell depletion in the various mouse strains is most likely mainly caused by migration. This is in line with the results of a recent study from our group on healthy human volunteers (Kölgen *et al*, 2002). It cannot be excluded, however, that the enhanced

Langerhans cell depletion in XPA and CSB mice is (in part) caused by apoptosis.

The present data together with experimental data from other researchers can give more insight into the pathogenesis of XP and CS and the influence of the different NER mechanisms on the skin immune system. In summary, TCR plays a crucial role in sunburn sensitivity (Berg *et al*, 1998; Garssen *et al*, 2000), UV-B-induced Langerhans cell depletion, production of TNF- $\alpha$  in the lymph nodes, UV-B-induced local immunosuppression, and UV-B-induced apoptosis. In contrast, both TCR and GGR play a significant role in systemic immunosuppression and inhibition of interferon- $\gamma$  production by lymph node cells (Boonstra *et al*, 2001). These findings indicate that local and systemic immunosuppression have a different origin. Because XPA and XPC patients are very cancer-prone in contrast to CS patients, GGR seems to be involved in cancer susceptibility, particularly in man.

One should be careful in extrapolation of these observations from mice to man. One of the differences between mice and man is the repair of CPDs. The only CPDs that are removed are eliminated by TCR in mice, while being "overlooked" by GGR. This difference in CPD repair between mice and man is predominantly caused by difference in the expression of p48 in the skin. As a consequence CPDs are barely removed from the non-transcribed strand in mice (Ruven *et al*, 1993; Tang *et al*, 2000; Tan and Chu, 2002). This does not seem to have any consequences, however, for UV survival because this survival is equal for wild-type mice and human fibroblasts (for review see Hanawalt, 2001).

Taken together, this work demonstrates that DNA damage is critical for depletion of epidermal Langerhans cells and local immunosuppression. In particular, the minor fraction of UV-induced lesions in the transcribed strand of active genes appears to be the culprit class of lesions, whereas the bulk of DNA injury in the remainder of the genome is of minor importance. TCR, the DNA repair process that enables recovery of gene expression after DNA damage, is found to be crucial to prevent UV-induced depletion of epidermal Langerhans cells and local immunosuppression. Thus, restoration of gene expression is an important prerequisite for preventing this type of immunomodulation.

In this study, we investigated how defects in TCR and GGR affect UV-induced local immunosuppression and Langerhans cell depletion and whether these phenomena are related. In XPA and CSB, mice the strong immunosuppression after UV irradiation was accompanied by a strong depletion of Langerhans cells from the skin (more than 50%). In XPC mice we have not measured a clear Langerhans cell depletion after irradiation with 900 J per m<sup>2</sup> UV-B (approximately 0.5 MED in these (shaved haired) mice) although they were immunosuppressed at this UV dose. From these data it is clear that immunosuppression can be measured, whereas Langerhans cell depletion is moderate (wild-type mice) or undetectable (XPC mice) but at higher UV doses, and strong immunosuppression the Langerhans cell depletion also becomes stronger and easily detectable. Hence, there does appear to be a correlation between Langerhans cell depletion and immunosuppression. Noonan *et al* (1984) and Morison *et al* (1984) demonstrated that Langerhans cell depletion is not related to systemic immunosuppression. In agreement with these data and in combination with an earlier study by our group (Garssen *et al*, 2000), this study gives evidence for a relationship between UV-B-induced Langerhans cell depletion and local but not systemic immunosuppression.

Future studies should investigate in more detail the impact of the different NER pathways on cytokine production in the skin *in vivo* and the possible correlation with Langerhans cell migration, immunosuppression, sunburn sensitivity, and susceptibility to skin cancer.

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