Transcription-Coupled and Global Genome Repair Differentially Influence UV-B-Induced Acute Skin Effects and Systemic Immunosuppression[1]

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Exposure to UV-B radiation impairs immune responses in mammals by inhibiting especially Th1-mediated contact hypersensitivity and delayed-type hypersensitivity. Immunomodulation is not restricted to the exposed skin, but is also observed at distant sites, indicating the existence of mediating factors such as products from exposed skin cells or photoactivated factors present in the superficial layers. DNA damage appears to play a key role, because enhanced nucleotide excision repair (NER) strongly counteracts immunosuppression. To determine the effects of the type and genomic location of UV-induced DNA damage on immunosuppression and acute skin reactions (edema and erythema) four congenic mouse strains carrying different defects in NER were compared: *CSB* and *XPC* mice lacking transcription-coupled or global genome NER, respectively, as well as *XPA* and *TTD/XPD* mice carrying complete or partial defects in both NER subpathways, respectively. The major conclusions are that 1) transcription-coupled DNA repair is the dominant determinant in protection against acute skin effects; 2) systemic immunomodulation is only affected when both NER subpathways are compromised; and 3) sunburn is not related to UV-B-induced immunosuppression. *The Journal of Immunology*, 2000, 164: 6199–6205.

Besides the beneficial effects of UV exposure, such as vitamin D production, cosmetic tanning, and adaptation to solar UV radiation, UV exposure can also have adverse consequences on human health, notably sunburn, skin cancer, and ocular damage. Over the last 2 decades it has become evident that UV-B exposure (280–320 nm) also impairs specific and nonspecific immune responses (1). Several studies have shown that UV-B-induced immunomodulation plays at least a partial role in photocarcinogenesis (2). In addition, UV-B exposure has been demonstrated to impair resistance to bacterial, viral, parasitic, and fungal infections. Importantly, the effects of UV are not restricted to skin-associated infections, but also to systemic (non-skin-associated) infections (3–9). Because UV-B is not able to penetrate much beyond the upper cell layers of the epidermis, UV-B-induced immunosuppression is probably mediated by these exposed cells, their products, or photoactivated factors present in the superficial layers. UV irradiation can directly induce acute effects in the skin, such as membrane damage resulting in activation of the transcription factor NF-κB (10), activation of Src tyrosine kinases (11), production of H_2O_2 (12), urocanic acid isomerization (13), and neuropeptide release (14). Some of these are shown to be involved in UV-B-induced immunomodulation, even at loci distant from the UV-B-exposed skin (i.e., systemic immunosuppression). These include urocanic acid isomerization (13) and neuropeptide release (14).

In addition, DNA damage appears to play a crucial role in UV-induced immunomodulation, locally as well as systemically. Exposure to UV-B radiation, which induces cyclobutane pyrimidine dimers (CPDs)[3] as well as pyrimidine 6-4 pyrimidone photoproducts in DNA, suppresses cellular (i.e., T cell-dependent) immune responses even when initiated at UV-unexposed sites. Cells with DNA damage can migrate from the skin to other sites in the body. The products released by exposed epidermal cells can be transported through the body by the circulation, which may contribute to systemic immunosuppressive effects (15). Kripke and co-workers demonstrated that DNA damage is at least partially involved in local as well as systemic UV-induced immunomodulation. Direct photoreactivation of CPDs (16, 17) and enhanced excision repair of CPDs by T4N5 liposomes provided direct evidence that CPDs induce the suppression of contact hypersensitivity (CHS) locally as well as systemically. In addition, systemic suppression of delayed-type hypersensitivity (DTH) to *Candida albicans* was also mediated at least partially by CPDs (18, 19). Additional evidence for a significant role of DNA damage in UV-B-induced immunosuppression was provided by Miyauchi-Hashimoto et al. (20), who

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[3] Abbreviations used in this paper: CPD, cyclobutane pyrimidine dimer; CHS, contact hypersensitivity; DTH, delayed-type hypersensitivity; NER, nucleotide excision repair; XP, xeroderma pigmentosum; CS, Cockayne syndrome; TTD, trichothiodystrophy; PCl, picryl chloride; MED, minimal erythema/edema dose; UDS, unscheduled DNA synthesis.

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Table I. *NER mutant mouse strains*[a]

Mutant Strain	Global Genome Repair (GGR)	Transcription Coupled Repair (TCR)
XPA	−	−
CSB	+	−
XPC	−	+
TTD/XPD	+/−	+/−

[a] −, Deficient; +, normal; +/−, intermediate.

demonstrated that in nucleotide excision repair (NER)-deficient *XPA* mice local and systemic immunosuppression were increased.

Depending on the primary DNA lesions, one or more DNA repair pathways become active. Examples are base excision repair, recombinational repair, mismatch repair, and NER (21, 22). CPDs and 6-4 photoproducts are important substrates for the NER pathway. Both lesions are formed between adjacent pyrimidines, and represent the major DNA damage induced by UV-B. NER also removes a wide range of chemical adducts, and intrastrand DNA cross-links in a complex "cut and paste" reaction mechanism involving ~30 proteins (23). Two distinct subpathways can be discerned. Global genome NER eliminates lesions anywhere in the genome in a lesion- and location-dependent manner. For lesions such as CPDs, for which global genome NER is quite slow, a second subpathway, designated transcription-coupled NER, has evolved that preferentially eliminates damage that blocks ongoing transcription. Both systems use common proteins as well as subpathway-specific factors, and the processes operate in an independent fashion.

In this study the sensitivity of four transgenic mouse models was studied for UV-induced systemic immunomodulation (DTH and CHS) and acute skin effects (i.e., edema). The NER-deficient mouse mutants carried defects in the *XPA*, *XPC*, *CSB*, and *XPD/TTD* genes (Table I). Conventional gene targeting of the mouse *XPA* gene yielded a model for the UV-sensitive, cancer-prone prototype DNA repair syndrome xeroderma pigmentosum (XP). XPA deficiency induces a complete NER defect (24). *XPC* mice, on the other hand, have a selective defect in global genome NER (25–27), whereas *CSB* mice, mimicking the UV-sensitive neurodevelopmental condition Cockayne syndrome (CS), carry a specific impairment of transcription-coupled repair. This mouse model was obtained by mimicking a truncating *CSB* null allele found in a CS group B patient (28). The fourth model mimics an *XPD* point mutation of a trichothiodystrophy (TTD) patient that exhibits most CS features as well as characteristic brittle hair and nails. The NER defect includes both transcription-coupled as well as global genome NER, but is partial (29, 30).

Materials and Methods

NER-deficient mouse models

XPA, *CSB*, *XPC*, and *TTD* mice refer to NER-deficient mice homozygous for the targeted allele in the respective genes (Table I) (24, 27–30).

Because in previous studies neither DNA repair defects nor an obvious (UV-related) phenotype were found, heterozygous mice were not included in the present experiments. 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 ± 1°C. The room was illuminated with yellow fluorescent tubes (Philips TL40W/16; Philips, Eindhoven, The Netherlands) in a 12-h cycle (switched on and off at, respectively, 0600 and 1800 h). These lamps do not emit any measurable UV radiation. No daylight entered the animal facilities. Animals were housed individually in Macrolon type I cages (Tecniplast, Gazzada, Italy) 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

Picryl chloride (PCl; Chemotronix, Swannanoa, NC) was used as the contact sensitizer. It was recrystallized three times from methanol/H_2O before use and protected from light during storage at 4°C.

Listeria monocytogenes *bacteria*

The strain of *L. monocytogenes* was isolated in 1973 from the cerebral spinal fluid of an adult (human) male suffering from *Listeria meningitis* (L242/73 type 4b). These bacteria lose their virulence after a few weeks of culture. For this reason the culture was restimulated with an egg passage before use in animal infection studies (31). Activated *Listeria* cells were prepared by taking one colony scraped from a sheep blood agar plate, and diluted in 8 ml of bovine broth. The suspension was incubated overnight at 37°C. After incubation bacteria were collected by centrifugation for 5 min at 1200 × *g* at 4°C. The pellet was resuspended in 8 ml of PBS and vortexed. The solution contained ~5×10^8 *Listeria*/ml, as measured by CFU on sheep blood agar plates. From this solution the desired infection dilution was prepared. For inactivation the bacteria were heat-treated (10 min at 100°C). Thereafter, the sample was tested for inactivation using overnight culture on sheep blood agar plates. Heat-killed *L. monocytogenes* suspensions were used for ear challenge tests (DTH).

UV exposure

The animals were shaven (on the back) 1 day 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, Tiel, The Netherlands) Hanovia Kromayer Lamp (model 10S, Slough, U.K.). This is a hand-held lamp that allows short exposures to limited skin areas by placing the circular port (~2 cm^2) in close contact to the skin (32, 33). The dose rate was 150 $J/m^2/s$ (280–400 nm), as measured by a Kipp E11 thermopile (Middleburg, The Netherlands).

For determination of acute effects the animals received only a single dose, and for determination of immunomodulation the animals were exposed to five consecutive UV doses (one exposure per day, last exposure 4 days before immunization or infection).

Quantification of acute UV effects

The mice were exposed on the shaven dorsal (back) skin in the early morning (between 0800–0900 h) and were critically diagnosed for edema and erythema 24 h later by a biotechnician without knowledge of the treatment. In other words all results were scored in a blinded fashion. Eight UV doses were tested (from 1–32 s; i.e., 150-4800 J/m^2), and at least three animals were used per UV dose. The acute effects were categorized into four classes: −, no detectable macroscopic effect; +, slight, but detectable, edema/erythema; ++, moderate edema/erythema; and +++, severe edema/erythema and crust formation. Besides macroscopic evaluation of edema and erythema, the increase in skin thickness was determined as a value of acute UV effects in some experiments. In these cases the ears of mice were exposed to the Kromayer UV source. Ear thickness was measured before and 24 h after Kromayer exposure using an engineer's micrometer (model 193–10, Mitutoyo, Veenendaal, The Netherlands) in a blinded fashion. The lowest dose that was able to induce a significant ($p < 0.05$ compared with sham exposure) ear swelling response was the minimal erythema/edema dose (MED) for that mouse strain.

CHS to PCl

The mice were skin-sensitized 4 days after the last day of (sham) irradiation by topical application of 150 μl of 5% PCl in ethanol/acetone (3/1) to the non-UV-irradiated shaved abdomen, chest, and four feet. Control mice were sham-sensitized by topical application of 150 μl of 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% PCl 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 occurred 24 h after topical ear challenge even in UV-B-pre-exposed animals (33). 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).

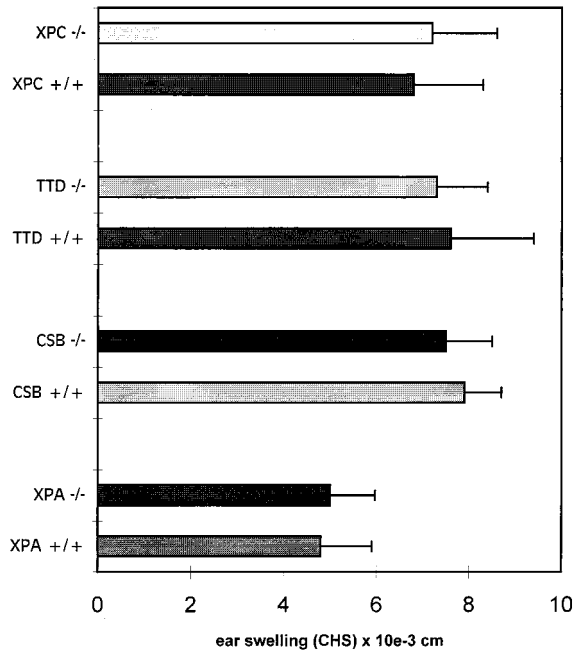

FIGURE 1. Net (Ag-specific) ear swelling 24 h after challenge of the ear pinnea with one drop of 0.8% PCl in olive oil. The background swelling, induced by PCl application to the ears in nonsensitized animals ($<1 \times 10^{-3}$ cm), was subtracted from the swelling in PCl skin-sensitized animals. All strains of mice demonstrated a significant ($p < 0.05$) CHS response compared with the background response in nonsensitized controls of the same strain. Each group contained at least six mice.

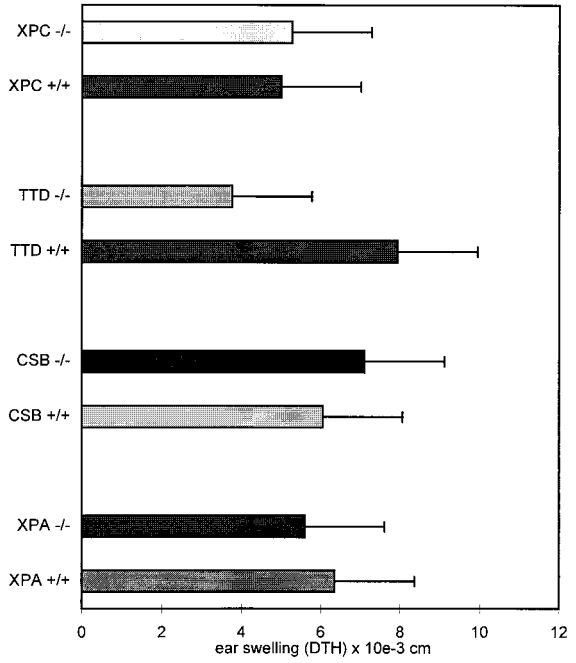

FIGURE 2. Net (Ag-specific) ear swelling 24 h after challenge of the ears with heat-killed *Listeria* particles. The background swelling, induced by the s.c. injection of heat-killed *Listeria* into the ears of noninfected animals ($<1 \times 10^{-3}$ cm), was subtracted from the swelling in *Listeria*-infected animals. All strains of mice demonstrated a significant ($p < 0.05$) DTH response compared with the background response in noninfected controls of the same strain. Each group consisted of at least six mice.

DTH to L. monocytogenes *bacteria*

The animals were infected s.c. (tail base) with $\sim 2 \times 10^4$ activated *L. monocytogenes* 4 days after the last day of (sham) irradiation. Six days after the infection the animals in each group were injected s.c. in the ear pinnea with 10 μl (10^7) of heat-killed *Listeria* particles under light ether anesthesia. Before and at 24 h after *Listeria* ear challenge duplicate measurements of ear thickness were made using an engineer's micrometer as outlined above (net ear swelling). In earlier studies it was demonstrated that the maximal DTH response was found 24 h after s.c. ear challenge.

Statistics

Levels of significance were calculated using two-tailed Student's *t* test; $p < 0.05$ was taken as a significant difference between groups. For determination of the MED, at least three animals per dose and at least eight doses (1-, 2-, 4-, 6-, 8-, 10-, 16-, and 32-s Kromayer exposure) were tested. For photoimmunology studies each group consisted of at least six mice, and each experiment was repeated at least twice.

Results

CHS to PCl in XPA, CSB, TTD, *and* XPC *and their wild-type littermates*

CHS in *XPA*, *CSB*, *TTD*, and *XPC* mice and their respective wild-type littermates was measured 24 h after ear challenge with PCl in olive oil. Significant CHS (ear swelling) responses ($p < 0.05$) to picrylchloride were observed in each strain of mouse compared with the background swelling responses found in the nonsensitized control animals from the same strain. Each bar in Fig. 1 represents the net Ag-specific ear swelling response. The background ear swelling in nonsensitized animals of each strain was always $<1 \times 10^{-3}$ cm.

In the mutant mice (*XPA*, *XPC*, *TTD*, *CSB*) the CHS responses 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 with CHS in the repair-competent wild-type littermates.

DTH to L. monocytogenes *in* XPA, CSB, TTD, *and* XPC *mice and their wild-type littermates*

DTH in the NER-deficient mutant mice and their respective wild-type littermates was measured 24 h after ear challenge with heat-killed *Listeria* bacteria. Fig. 2 shows the results. Each bar represents the net Ag-specific ear swelling response. The background ear swelling in noninfected animals of each strain was always $<1 \times 10^{-3}$ cm. We detected significant DTH responses to heat-killed *Listeria* particles in each strain of mouse compared with the noninfected control animals from the same strain ($p < 0.05$). In all mutant mouse strains the DTH responses were not significantly different from the DTH responses measured in control wild-type littermates. However, a nonsignificant trend was observed in *TTD* mice, which showed a lower DTH response to *Listeria* compared with their normal littermates. In summary, the *XPA*, *XPC*, *TTD*, and *CSB* mutant mice were not statistically significantly affected with respect to the T cell-dependent immune response to *L. monocytogenes* bacteria.

Acute skin effects induced by a single UV exposure

Acute macroscopic UV skin effects were studied $\sim$24 h after a single UV spot exposure. The MED for all wild-type littermates was 1500 J/m^2. The MED for the *CSB* and *XPA* mouse models was $<$150 J/m^2, consistent with the high UV sensitivity of the corresponding human patients. The MED for the *TTD* mouse model was 1200 J/m^2, thus slightly, but significantly, less than that in the wild-type littermates. Remarkably, the MED for the *XPC* mouse model was similar to that for the repair-proficient littermates, i.e.,

Table II. *Acute skin effects induced by a single UV exposure*[a]

UV dose (280–400 nm)	Mouse Strain							
	XPA, +/+	XPA, −/−	XPC, +/+	XPC, −/−	CSB, +/+	CSB, −/−	TTD, +/+	TTD, −/−
150 J/m^2	−	+	−	−	−	+	−	−
300 J/m^2	−	+++	−	−	−	++	−	−
600 J/m^2	−	+++	−	−	−	+++	−	−
900 J/m^2	−	+++	−	−	−	+++	−	−
1200 J/m^2	−	NT	−	−	−	+++	−	+
1500 J/m^2	+	NT	+	+	+	NT	+	++
2400 J/m^2	+++	NT	++	++	+++	NT	+++	+++
4800 J/m^2	+++	NT	+++	+++	+++	NT	+++	+++

[a] Acute skin effects 24 h after one single Kromayer spot exposure on the shaven dorsal skin in J/m^2 (280–400 nm) (+/+, wild type; −/−, mutant mice). NT, not tested (ethically not allowed); −, no macroscopic effects detectable; +, slight but detectable edema reaction; ++, moderate edema reaction; +++, severe edema reaction and crust formation. There was no difference detectable between the three animals per dose.

1500 J/m^2. The detected effects are summarized in Table II for each mouse strain.

UV-B-induced suppression of CHS

To compare suppressive effects distant from the site of UV exposure on the shaven back, the control response in non-UV-exposed animals was set at 100%. In each study the mutant mice were compared with their wild-type littermates. The findings are depicted in Fig. 3. Each bar represents the percent CHS compared with the control response in non-UV-exposed animals, which was set at 100%. For CHS responses the minimal UV dose necessary to induce a statistically significant suppression ($p < 0.05$) was 6 s for all wild-type control littermates (i.e., 900 J/m^2; 280–400 nm). The minimal dose necessary to suppress CHS in *XPA* mice was 1 s or possibly even less (150 J/m^2; 280–400 nm). In *CSB* and *XPC* mice the minimal dose required to suppress CHS was 6 s, thus in the same range as in their wild-type littermates. Finally, *TTD* mice had to be exposed to 4 s (600 J/m^2; 280–400 nm) to induce a significant suppression of CHS, which is slightly but significantly less than in their normal littermates.

UV-B-induced suppression of DTH to L. monocytogenes

In parallel to the influence on CHS we determined the suppressive effects of distant UV exposure on DTH. To this aim the control response in non-UV-exposed animals was set at 100%, and mutant mice were compared with the control wild-type littermates. The data are compiled in Fig. 4. Each bar represents the percent ear swelling compared with the control response in non-UV-exposed animals (set at 100%). For DTH responses to *L. monocytogenes* the minimal UV dose that was necessary to significantly suppress this immune response ($p < 0.05$) was 4 s for the *XPA* wild-type mice and 6 s for the *CSB* wild-type, *TTD* wild-type, and *XPC* wild-type mice. The minimal UV dose necessary to induce a statistically significant suppression ($p < 0.05$) was l s (150 J/m^2) or less for *XPA* mutants and 6 s (900 J/m^2) for *CSB*, *TTD*, and *XPC* mice.

Discussion

In our analysis CHS and DTH assays were used as tools to examine the role of DNA damage and repair in UV-induced immunomodulation. It is well known that this photoimmunomodulation may have deleterious consequences for resistance to infections and rejection of certain tumors, but may also be advantageous with respect to certain autoimmune and/or hyperimmune diseases (1, 34). In our CHS studies PCl was used as the Ag. Previously, we showed that low doses of UV-B could inhibit the CHS response to PCl correlating with decreased levels of IFN-γ and IL-12 (35, 36). Both cytokines play crucial roles in the initiation and effector phase of Th1-mediated immunity (35). We demonstrate here that even when animals are exposed to suberythemal doses distant from the sensitization locus, UV-B can easily inhibit CHS to PCl. In the

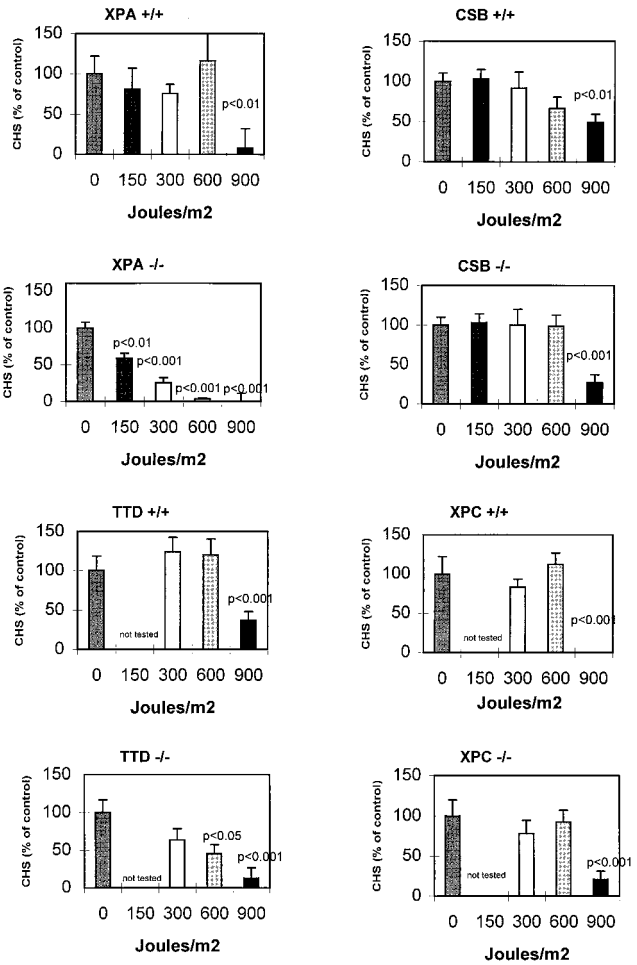

FIGURE 3. UV-B-induced systemic suppression of CHS to PCl in NER-deficient mice and their control wild-type littermates. Each bar represents the net ear swelling. The net ear swelling in sensitized non-UV-exposed animals was set at 100%. Each group (bar) contains at least six mice. The *p* values reflect comparison with the UV-unexposed group (0 s of Kromayer exposure).

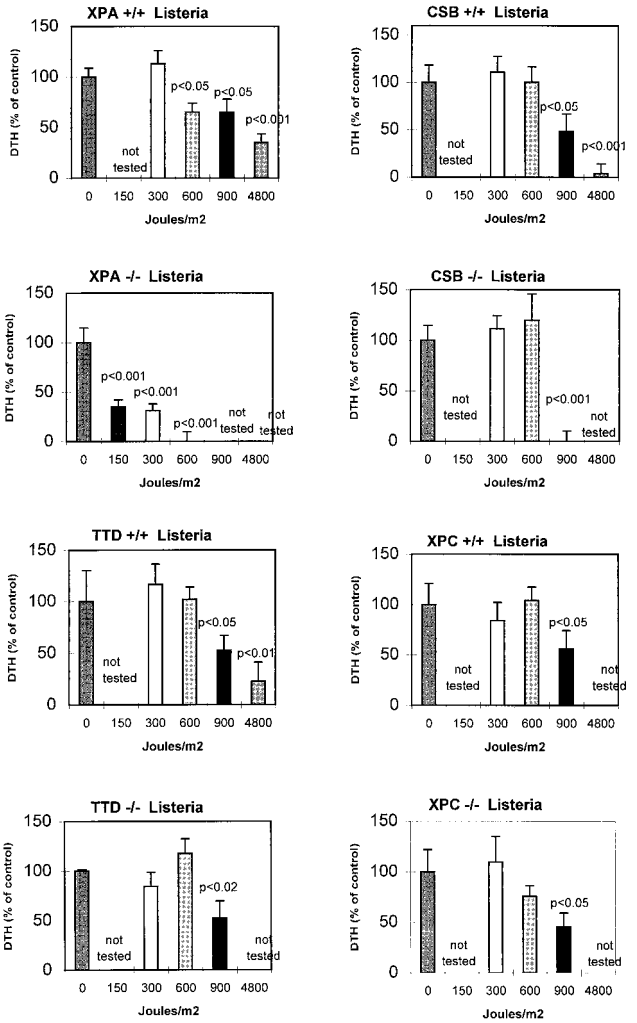

FIGURE 4. UV-B-induced systemic suppression of DTH to *L. monocytogenes* in NER-deficient mice compared with wild-type littermates. Each bar represents the net ear swelling. The net ear swelling in infected non-UV-exposed animals was set at 100%. Each group contains at least six mice. The *p* values reflect comparison with the UV-unexposed group (0 s of Kromayer exposure).

DTH studies *L. monocytogenes* was employed as an infective agent. Suberythemal, distant UV exposure can inhibit specific cellular immunity to *L. monocytogenes*. Lymphocyte proliferation assays as well as DTH responses to *Listeria* particles in a rat infection model were significantly inhibited after suberythemal UV exposure (6). An increased *Listeria* load in the spleen and liver was observed, which indicates that the cellular immune parameters lymphocyte proliferation and DTH correlated well with impaired resistance. In the current study the effect on DTH to *Listeria* described in Wistar Unilever rats appeared to be reproducible in mice. Mice were used because no mutant NER-deficient rat strains were available.

The implications of defective NER in humans are apparent from three autosomal, recessive syndromes: XP, CS, and the sunlight-sensitive form of TTD (37). Seven complementation groups have been described in NER (XP-A to XP-G), two in CS (CS-A and CS-B), and three in TTD (XP-B, XP-D, and TTD-A). All XP-related genes are involved in both global genome as well as transcription-coupled repair with the exception of *XPC*, which acts in global genome NER only (27). Hypersensitivity to sunlight is associated with skin cancer predisposition in the case of XP, but not in patients with CS and TTD. Many studies indicate that the immune system in XP patients is impaired, with lower DTH and CHS, decreased CD4/CD8 ratios, impaired mitogen responsiveness and production of IFN-γ, reduced NK cell activity, and delayed recovery of Langerhans cell depletion by UV (38–45). These findings suggest that not only mutagenesis in skin cells but also impaired immune surveillance or increased susceptibility to UV-induced immunomodulation may contribute to the observed skin cancer susceptibility in humans with XP. Immunological deficits have also been noted for CS and TTD.

CPDs and 6-4 photoproducts are the main DNA lesions induced by UV. Both are substrates for the NER machinery. CPDs are very efficiently repaired in the transcribed strand of active genes by transcription-coupled repair, but repair of this lesion by global genome NER in the remainder of the genome is much slower and less efficient. It is thought that especially CPDs play a critical role in UV-induced immunomodulation (16, 18, 46). The less abundant 6-4 photoproducts are removed very rapidly and genome-wide by global genome NER. When this NER mode is not operative, repair in the transcribed sequences is taken over by the transcription-coupled repair subpathway. Some important differences in NER activity exist between rodents and man. In particular, CPDs (but not 6-4 photoproducts) are hardly removed from nontranscribed sequences in mice. However, this difference does not appear to have severe consequences, because UV survival of wild-type mouse and human fibroblasts is similar. Moreover, repair parameters in mouse fibroblasts from repair-deficient mice, such as unscheduled DNA synthesis (UDS), recovery of RNA synthesis after UV exposure, and sensitivity to UV light, correlate very well with those of human patients fibroblasts.

In this study four mouse strains carrying different NER defects were investigated with respect to their sensitivity for UV-B regarding acute cutaneous and immunological effects (Table I, 2). For instance, UDS is $<$5% in *XPA* mice, 25% in *TTD* mice, 30% in *XPC* mice, and $>$95% in *CSB* mice of the UDS found in wild-type mice (30, 47) (see Table III). In addition, RNA synthesis recovery is $<$5% in *XPA* and *CSB* mice, $\sim$20% in *TTD* mice, and $>$95% in *XPC* mice compared with the 100% recovery found in control wild-type littermates (30).

Acute skin effects due to a single UV-B exposure revealed that *XPA* and *CSB* mice were at least 10-fold more sensitive than their wild-type littermates. Miyauchi-Hashimoto et al. (20) reported that *XPA* mice were hypersensitive to UV-B with respect to acute skin effects such as edema, effects on Langerhans cells, and induction of sunburn cells, in accordance with our study. Our findings indicate that transcription-coupled repair, rather than global genome repair, is responsible for the protection against acute skin effects. *XPA* mice are affected in both NER subpathways, but *CSB* mutants are only deficient in transcription-coupled repair. Berg et al. demonstrated that acute UV effects, macroscopically characterized by erythema and edema of the skin, are caused by lesions in actively transcribed DNA and thus serve as a parameter for transcription-coupled NER (48). A significant, but only mild, increase in sensitivity for acute UV skin effects is observed in *TTD* mice. This might be due to the fact that transcription-coupled repair is only partially disturbed in *TTD* mice (30). *XPC* mice, on the other hand, exhibit wild-type UV sensitivity, confirming the idea that erythema and edema reactions induced by UV-B are primarily determined by transcription-coupled repair and not significantly by global genome NER. For CSB-deficient mice UV sensitivity might be more exaggerated compared with that in human CS, because global genome NER in the mouse does not eliminate CDPs from transcribed sequences. In human CS this NER subpathway may still remove a significant proportion of these transcription-blocking lesions. The

Table III. *UV sensitivity and NER*[a]

Strain	UDS (%)[b]	RNA rec.[b]% of WT	MED (J/m^2)	MID^{chs} (J/m^2)	MID^{dth} (J/m^2)	Susceptibility[b] to Photocarcinogenesis
XPA$^{+/+}$	100	100	1500	900	600	−
XPA$^{-/-}$	<5	<5	(<) 150	(<) 150	(<) 150	+++
CSB$^{+/+}$	100	100	1500	900	900	−
CSB$^{-/-}$	100	<5	(<) 150	900	900	+
TTD$^{+/+}$	100	100	1500	900	900	−
TTD$^{-/-}$	25	20	1200	600	900	++
XPC$^{+/+}$	100	100	1500	900	900	−
XPC$^{-/-}$	30	>95	1500	900	900	+++

[a] MED, minimal edema dose; MID^{chs}, minimal immunosuppressive UV-B dose for CHS to picrylchloride; MID^{dth}, minimal immunosuppressive UV-B dose for DTH to *L. monocytogenes*; and UDS, UV-induced unscheduled DNA synthesis.

[b] The values for photocarcinogenesis susceptibility (last column) were adapted from Refs. 24, 25, 28, and 30, and for UDS from Ref. 47. RNA synthesis recovery values are from Ref. 30.

wild-type MED found for *XPC*-deficient mice fits very well with the results of a study by Kondo et al. (49), who described XP-C patients with normal MED. Extrapolating our observations from mice to humans, it should be taken into account that the absence of CPD removal by murine global genome NER makes a wild-type mouse in this regard more like a human *XPC* mutant. As such, the phototypical difference between wild-type and *XPC* mice is much smaller compared with that in humans. However, *XPC* mice are still extremely cancer-prone, exhibiting almost the same sensitivity as *XPA* mice. The strong dependence of acute UV-B effects on a functioning transcription-coupled repair process is in accordance with the idea that blocked transcription constitutes a potent trigger of p53 activation, resulting in apoptosis (50, 51).

Remarkably, strains with completely or partially active global genome repair (*CSB* or *TTD*) are not significantly or are only marginally sensitive with respect to UV-induced systemic immunomodulation. However, *XPC* mice, with a total defect in this subpathway, fail to display significant sensitivity in this regard, whereas the total NER-deficient *XPA* animals were very sensitive. This suggests that the trigger for inducing immunomodulation is strongly reduced when NER is still partially active in either global genome or transcription-coupled repair or partially in both. Alternatively, the activity of each of the NER pathways may have a separate link with immune surveillance. The fact that *XPC* mice do not show altered CHS and DTH, whereas it is a very cancer-prone form of XP (see Table III), suggests that the onset of cancer is in this case not dependent on compromising the immune system. On the other hand, our finding of a near normal immune response in CS and TTD can contribute to the low cancer susceptibility noted with these conditions, particularly in man. Another important conclusion of our work is that acute UV effects, such as erythema or edema, are not predictive for immunosuppression. This implies that different molecular mechanisms underlie these phenomena. Probably, the strong induction of apoptosis (sunburn) by UV is not a major mechanism triggering the immune response.

A follow-up study might examine the role of TNF-α and/or IL-10 in photoimmunosuppression, induced at least partially by UV-induced DNA damage, in the four different NER-deficient mouse strains. One of the most important issues for future research are the roles of the different types of UV-induced DNA damage in the induction of immunosuppression, although studies by Kripke et al. indicate that CPDs in particular are crucial (16, 18). We found a slight sensitivity of TTD mice for CHS. This suggests that 6-4 photoproducts are also involved, because the partial NER-deficient *TTD* mice differ from *CSB* as well as *XPC* mice in the rate of removal of this UV lesion in both the transcribed compartment and the remainder of the genome. To gain more insight into the precise roles of different types of UV damage, we are generating new transgenic mouse models expressing CPD- and/or 6-4 photoproduct-specific photoreactivating enzymes. These should be instrumental for assessing the relative contributions of both lesions in the induction of photoimmunosuppression.

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