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IL-18 Reduces Ultraviolet Radiation-Induced DNA Damage and Thereby Affects Photoimmunosuppression

Agatha Schwarz,* Akira Maeda,* Sonja Ständer,† Harry van Steeg,‡ and Thomas Schwarz2*

UV-induced DNA damage has been recognized as the major molecular trigger for photoimmunosuppression. IL-12 prevents UV-induced immunosuppression via its recently discovered capacity to reduce DNA damage presumably via induction of DNA repair. Because IL-18 shares some biological activities with IL-12 we studied the effect of IL-18 on UV-induced DNA damage and immunosuppression. IL-18 reduced UV-induced apoptosis of keratinocytes and supported long-term cell survival on UV exposure. Injection of IL-18 into mice that were exposed to UV radiation significantly lowered the number of apoptotic keratinocytes. Accordingly, radiation immunohistochemistry revealed reduced amounts of DNA damage in epidermal cells upon injection of IL-18. These effects were not observed in DNA repair-deficient (XpaKO) mice, indicating that IL-18 like IL-12 reduces DNA damage via DNA repair. UV-mediated suppression of the induction of contact hypersensitivity, which is known to be primarily triggered by DNA damage, was prevented upon injection of IL-18 before UV exposure in wild-type but not in XpaKO mice. In contrast to IL-12, IL-18 was not able either in wild-type or in XpaKO mice to break UV-induced immunotolerance that is mediated via regulatory T cells rather than in a DNA damage-dependent fashion. This result indicates that IL-12 is still unique in its capacity to restore immune responses because of its effect on regulatory T cells. Together, these data identify IL-18 as a further cytokine that exhibits the capacity to affect DNA repair. Though being primarily a proinflammatory cytokine through this capacity, IL-18 can also foster an immune response that is suppressed by UV radiation. The Journal of Immunology, 2006, 176: 2896–2901.

Ultraviolet radiation represents one of the most important environmental factors affecting humans. Besides its well-known advantages and its indispensable effects on human life, UV radiation, in particular the middle wavelength (UVB, range 290–320 nm), can be a hazard to human health by inducing cancer, premature skin aging, immunosuppression, inflammation, and cell death (1–5). Accordingly, the incidence of UV-induced skin cancer is rapidly rising, accounting for >40% of all human cancers in the United States (6). UV-induced DNA damage is one of the major molecular events when a cell is hit by UV radiation. These photolesions have been recognized not only to be the primary basis for malignant transformation but also to be essentially involved in UV-induced signal transduction (7). DNA damage is the crucial molecular trigger for a variety of UV effects, e.g., immunosuppression and apoptotic cell death (8, 9). The vast majority of UVB-induced DNA lesions are removed by the nucleotide excision repair (NER), the essential endogenous DNA repair system (10). The importance and efficacy of the NER is best illustrated by the disease xeroderma pigmentosum (XP). Due to genetic mutations in the various NER genes, XP patients do not exhibit a functional NER and suffer from a dramatically increased risk of developing UV-induced skin cancer at an early age (11). Accordingly, mice in which the different genes of the NER complex have been knocked out (KO) develop skin tumors upon chronic UV exposure earlier and at higher frequency when compared with wild-type (WT) mice (12). In turn, accelerated or enhanced removal of UV-induced DNA damage, e.g., via the topical application of the bacterial DNA repair enzyme T4 endonuclease V, impaired the development of skin tumors in mice exposed to chronic UV radiation (13). Likewise, topical T4 endonuclease V was shown to lower the risk of developing actinic keratoses, the prestage of skin cancer, in XP patients (14).

Until recently it was thought that the genes coding for NER components are constitutively expressed and not subjected to any regulation. However, Eller et al. (15) first demonstrated that administration of DNA oligonucleotides induces DNA repair via a p53-dependent mechanism. Accordingly, a recent in vivo study indicated that topical pretreatment with DNA oligonucleotides enhanced the rate of DNA photoproduct removal, decreased UV-induced mutations, and reduced photocarcinogenesis in UV-irradiated mice (16). We recently observed that the immunostimulatory cytokine IL-12 is able to reduce the amount of DNA damage both in vitro and in vivo (17). This result was not due to a filtering effect of IL-12 but rather related to NER because this unique effect of IL-12 was not observed in NER-deficient (XpaKO) mice. IL-12 is also able to antagonize UV-induced immunosuppression (18–20). This activity also appears to be related to the effect of IL-12 on DNA repair because IL-12 prevented both UV-induced suppression of the induction of contact hypersensitivity (CHS) and the depletion of Langerhans cells, the primary APC of the skin, in WT mice but not in DNA repair-deficient mice (21). Thus, these findings identified a new mechanism by which IL-12 can restore immune responses and also demonstrated a link between DNA repair and the prevention of UV-induced immunosuppression by IL-12.

IL-18 is a proinflammatory cytokine that exhibits the unique capacity to induce Th1 or Th2 polarization, depending on the immunologic context (22). Although not related to IL-12 structurally,
IL-18 shares some biological effects with IL-12, e.g., the induction of IFN-γ (23, 24). Because modulation of DNA repair by cytokines may have important practical implications, we were interested to study whether the effect on DNA repair on UV-induced immunosuppression is unique for IL-12 or can also be mediated by other cytokines. In this study, we show that IL-18 like IL-12 suppresses UV-induced apoptosis by reducing UV-mediated DNA damage. This effect appears to be related to NER because it was not observed in XpaKO mice. In addition, through NER activity, IL-18 was able to prevent UV-induced immunosuppression. However, unlike IL-12, IL-18 could not break UV-induced immunotolerance, a phenomenon that still remains unique for IL-12. This result indicates that IL-18, although being primarily a proinflammatory cytokine, through the described capacity to affect DNA repair can restore an immune response that is suppressed by UV radiation.

Materials and Methods

Animals and cells

The epidermoid carcinoma cell line KB (American Type Culture Collection, Rockville, MD) was cultured in RPMI 1640 containing 10% FCS and 1% glutamine. Normal human keratinocytes (Promo Cell) were cultivated in Keratinocyte Growth Medium 2 (Promo Cell). UV irradiation was performed as previously described (17) using a bank of six fluorescent bulbs (TL12; Philips), which emit most of their energy within the UVB range (290–320 nm) with an emission peak at 315 nm. Subconfluent cells (2 × 10^4/ml) were exposed to UV in PBS.

C57BL/6 mice were purchased from Harlan-Winkelmann. XpaKO mice were generated at the National Institute of Public Health and the Environment (Bilthoven, The Netherlands) (12). Animal care was provided by expert personnel in compliance with the relevant laws and institutional guidelines. Recombinant human and murine IL-18 was obtained from BioSource International, IL-18 was diluted in sterile endotoxin-free saline and 2000 ng (i.p.) and 300 ng (intracutaneously), respectively, were injected per mouse.

Detection of cell death

For the detection of DNA fragmentation, a cell death detection ELISA (Boehringer Mannheim) was used. The enrichment of mononucleosomes and oligonucleosomes released into the cytoplasm of cells is detected by biotinylated antihistidine- and peroxidase-coupled anti-DNA Ab. OD is shown as the mean ± SD of triplicate experiments.

To determine colony-forming efficiency, normal human keratinocytes were supplemented with either IL-18 (150 ng/ml) or an equal amount of diluent for 2 h before UV exposure. After irradiation (250 J/m^2), cells were trypsinized, replated at different densities, and supplemented with complete medium containing IL-18 or diluent. The cultures were monitored daily, and when colonies were easily visible, cells were stained with crystal violet.

Evaluation of sunburn cell formation

Biopsy samples were taken from the UV-exposed areas of the skin, fixed in formaldehyde, and embedded in paraffin. Tissue sections (5 μm) were H&E stained, and the number of sunburn cells, defined as apoptotic cells within the epidermis exhibiting a shrunken eosinophilic cytoplasm and a condensed nucleus, was counted throughout the epidermis of sections per sample using a 1 × 1-cm grid inserted in a conventional microscope. Sunburn cells per 6 mm in length of epidermis were counted. At least five fields of each sample were evaluated and the number of sunburn cells (mean ± SD) calculated. The Student t test was used to test the significance of the differences.

Immunohistochemical staining for dimers

C57BL/6 mice were exposed to UV radiation (500 J/m^2). Three hours before irradiation, IL-18 (300 ng/ml) was injected intracutaneously into the UV-exposed skin area. Sixteen hours after UV exposure, skin biopsy samples were taken from these areas, fixed in 7% buffered paraformaldehyde, dehydrated, and embedded in paraffin. After deparaffinization with xylol and ethanol at decreasing concentrations, sections were immersed in an aqueous solution containing 0.1 M citric acid/0.1 M sodium citrate and subsequently microwave-treated for 15 min to unmask antigenic epitopes. Endogenous peroxidase activity was blocked by incubation with 0.1% H2O2 and 0.6% sodium azide in PBS for 20 min. After rinsing with PBS, unspecific binding sites were blocked with 2% BSA for 30 min at room temperature followed by incubation with the primary Ab diluted in 1% BSA overnight. A mAb directed against thymine dimers (Kamiya Biomedicals) was used at a dilution of 1/2000. Staining was achieved by an indirect immunoperoxidase technique using the following reagents: PBS, peroxidase-conjugated anti-mouse Ab (undiluted, Dako envision; DakoCytomation), 0.01% H2O2, and 3-amino-9-ethylcarbazole (Sigma-Aldrich). Negative controls comprised omission of the first Ab.

UV-induced immunosuppression

C57BL/6 mice were sensitized by painting 50 μl of 2,4-dinitro-1-fluorobenzene (DNFB; Sigma-Aldrich) solution (0.5% in acetone to olive oil, 4:1) on the shaved back on day 0. On day 5, 20 μl of 0.3% DNFB were applied to the left ear. Ear swelling was quantified with a spring-loaded micrometer 24 h later. CHS was determined as the amount of swelling of the hapten-challenged ear compared with the thickness of the vehicle-treated ear and expressed in centimeters (mean ± SD × 10^-3). Resensitization was performed on abdominal skin 14 days after the first sensitization. Second challenge was performed on the ear right 5 days after second sensitization.

The shaved back was exposed to UV daily for 4 consecutive days. WT mice received 1000 J/m^2 per exposure. Because XpaKO mice are more UV susceptible (25), they received only 400 J/m^2 per exposure to achieve the same level of immunosuppression (21). After the last UV exposure (24 h), DNFB was carefully painted on the UV-irradiated skin area.

Statistical analysis

Each experiment was performed at least twice. Each group of mice consisted of at least five mice. Data were analyzed by Student’s t test, and differences were considered significant at p < 0.05.

Results

IL-18 inhibits UV-induced apoptosis

To determine the effect of IL-18 on UV-induced DNA damage, we tested how IL-18 affects UV-induced apoptosis. DNA damage is the major determinant on UV exposure whether a cell undergoes apoptosis or not; thus apoptosis can be used as a readout system for the severity of DNA damage. Exposure of the epithelial cell line KB to UV resulted in apoptosis, which was significantly reduced when cells were preincubated with IL-18 2 h before irradiation (Fig. 1A). Similar findings were obtained with normal human keratinocytes (Fig. 1B). To exclude that IL-18 only delays apoptosis but does not rescue cells, the colony-forming efficiency of normal human keratinocytes after UV irradiation was performed. Preconfluent dishes of cells were either left unirradiated or exposed to UV in the absence or presence of IL-18. After exposure to UV, IL-18 partially rescued more colonies compared with the UV only group (Fig. 1C). This finding indicates that IL-18 enables long-term survival of UV-exposed keratinocytes.

IL-18 reduces UV-induced DNA damage and sunburn cell formation in vivo

Because the severity of DNA damage is one of the major determinants of cell apoptosis upon UV exposure (8), the impact of IL-18 on the amounts of UV-specific DNA lesions was determined. UV induces two major types of DNA lesions, 6-4 photoproducts and cyclobutane pyrimidine dimers (CPD), with the latter being the predominant lesions (26). Southern blot analysis using an Ab directed against CPD revealed that in DNA samples extracted 3.5 and 6 h after UVB exposure, respectively, UV-induced DNA damage was significantly reduced upon IL-18 treatment (data not shown). In samples extracted 10 min after UV, equal amounts of CPD were observed irrespective of whether the cells were pretreated with IL-18 or not. This observation excludes the possibility that the reduction of DNA damage by IL-18 is due to a filtering effect.

To elucidate the in vivo relevance of these findings, the shaved backs of C57BL6 mice were exposed to UV radiation. One group
reflected by increase of absorbance (OD).

Two hours before UV exposure, recombinant human IL-18 (150 ng/ml) was added (UV/H11001). Rate of apoptosis was assessed by determining nucleosomal DNA fragmentation using an apoptosis determination kit. Rate of apoptosis is increased by UV exposure (250 J/m²). KB cells (A) or normal human keratinocytes (B) were exposed to UV radiation (250 J/m²). Two hours before UV exposure, recombinant human IL-18 (150 ng/ml) was added (UV + IL-18). Control cells (Co.) were left untreated. After 16 h irradiation, apoptosis was examined by determining nucleosomal DNA fragmentation using an apoptosis determination kit. Rate of apoptosis is reflected by increase of absorbance (OD); **, p < 0.005 vs UV. C. Normal human keratinocytes were supplemented with either 150 ng/ml IL-18 or an equal amount of diluent for 2 h before and after UV irradiation (250 J/m²). Cells were replated, and colonies were stained after 21 days. Data show one representative experiment of two independently performed.

FIGURE 1. IL-18 inhibits UV-induced apoptosis. KB cells (A) or normal human keratinocytes (B) were exposed to UV radiation (250 J/m²). Two hours before UV exposure, recombinant human IL-18 (150 ng/ml) was added (UV + IL-18). Control cells (Co.) were left untreated. After 16 h irradiation, apoptosis was examined by determining nucleosomal DNA fragmentation using an apoptosis determination kit. Rate of apoptosis is reflected by increase of absorbance (OD); **, p < 0.005 vs UV. C. Normal human keratinocytes were supplemented with either 150 ng/ml IL-18 or an equal amount of diluent for 2 h before and after UV irradiation (250 J/m²). Cells were replated, and colonies were stained after 21 days. Data show one representative experiment of two independently performed.

received IL-18 intracutaneously 3 h before UV exposure. At 16 h later biopsy samples were taken and the presence of CPD was assessed by immunohistochemistry using the Ab directed against CPD. Although in UV-exposed samples CPD were detected in almost any keratinocyte, the amounts of DNA lesions were remarkably reduced in animals that had received IL-18 before UV exposure (Fig. 2). In contrast, in biopsy specimens obtained immediately after UV exposure, no differences in the staining pattern were observed between the UV-irradiated mice that were injected with IL-18 or saline. The fact that the amounts of UV-induced DNA damage were the same in the IL-18-treated and untreated animals immediately after UV exposure, but reduced by IL-18 at later time points both in vitro and in vivo, implies that IL-18 might remove UV-induced DNA lesions by inducing DNA repair, most likely NER.

IL-18 does not reduce sunburn cell formation in XpaKO mice

To finally prove that IL-18 reduces UV-induced DNA damage via NER, XpaKO mice were used, which due to their mutation in the XPA gene are severely deficient in NER (12). Accordingly, XpaKO mice revealed a higher number of sunburn cells than C57BL/6 WT mice exposed to the same UV dose, confirming previous findings (25). Although IL-18 significantly reduced sunburn cells in UV-irradiated WT mice, it had no significant effect on sunburn cells in XpaKO animals (Fig. 3A). To exclude the possibility that so much DNA damage was induced in the XpaKO mice that they were unable to repair DNA damage, in the next experiment a lower UV dose was applied, which induces an equivalent number of sunburn cells. Again, IL-18 significantly reduced the number of sunburn cells in WT mice but not in XpaKO mice upon application of equitoxic and thus comparable UV doses (Fig. 3B). Because the effect of IL-18 is lost in XpaKO mice, this indicates that IL-18 like IL-12 affects NER and thereby reduces UV-induced DNA damage.

IL-18 prevents UV-induced suppression of the induction of CHS

UV-induced DNA damage is the major molecular trigger for photoimmunosuppression because reduction of DNA damage is associated with a mitigation of the compromise to the immune system by UV exposure (9, 27). In addition, it was recently shown that the well-known antagonism of UV-induced immunosuppression by IL-12 is at least partially due to its capacity to reduce UV-mediated DNA damage (21). Therefore, we assumed that the same should apply for IL-18. Thus, C57BL/6 mice were exposed to UV and sensitized after the last exposure by topical application of DNFB onto the UV-exposed skin. Application of DNFB to UV-exposed skin failed to induce sensitization (Fig. 4A). However, i.p. injection of IL-18 3 h before DNFB application restored sensitization in UV-exposed mice, as demonstrated by a vigorous CHS response upon ear challenge.

Application of hapten onto UV-exposed skin not only inhibits the induction of CHS but also induces hapten-specific tolerance (28). Accordingly, mice that had had the hapten applied to UV-exposed skin did not respond with an ear swelling response upon sensitization with DNFB, indicating that mice have been tolerized to DNFB (Fig. 4B). IL-12 is known to break established tolerance (18–21). Although the mechanism by which IL-12 breaks tolerance is still unclear, it is certainly independent of the effect of IL-12 on DNA damage (21). In contrast to IL-12, CHS responses following DNFB challenge were not restored following injection of IL-18 3 h before DNFB application in UV-tolerized mice (Fig. 4B). This result indicates that IL-12 and IL-18 only share the same effect on preventing UV-induced immunosuppression, whereas IL-18, in contrast to IL-12, is not able to break established tolerance. Because UV-induced immunosuppression can be prevented by reducing DNA damage (9, 27), it is obvious that IL-18 like IL-12 appears to prevent immunosuppression via its effect on DNA repair.

IL-18 does not prevent UV-induced suppression of the induction of CHS in XpaKO mice

To consolidate this assumption, again XpaKO mice were used because in the case of involvement of DNA repair IL-18 should not be able to prevent UV-induced immunosuppression in DNA repair-deficient mice. In contrast to C57BL/6 WT mice (Fig. 4A), UV-exposed XpaKO mice remained unresponsive to DNFB despite the injection of IL-18 (Fig. 5A), indicating that indeed the preventive effect of IL-18 on UV-induced immunosuppression...
may depend on functional NER. Because IL-18 was not able to
break tolerance in WT mice, it was not surprising to observe the
same for \textit{Xpa} KO mice (Fig. 5B).

**Discussion**

UV radiation is the major causative agent for skin cancer because
of its potent ability to induce DNA lesions, which if not removed
can give rise to mutations and subsequently to skin cancer (1).
However, the vast majority of UV-induced DNA lesions are re-
moved by NER. Besides NER, induction of apoptosis represents
an additional protective mechanism because it eliminates cells that
still harbor considerable amounts of DNA damage because of in-
sufficient repair or as a consequence of a too high UV dose (1).
Therefore, dysregulation of UV-induced apoptosis will affect the
photocarcinogenic risk. Several ways exist to inhibit UV-induced
apoptosis, e.g., by overexpressing heat shock (29) or antiapoptotic
proteins (30, 31), by disrupting p53 function (32), or by blocking
death receptor activation (33, 34). Because these approaches allow
the survival of cells carrying DNA damage, they may give rise to
mutations and skin cancer. In turn, augmentation of UV-induced
apoptosis may reduce the risk of photocarcinogenesis. We could
recently show that this effect may apply for the proinflammatory
cytokine IL-1 that inhibits death ligand-mediated apoptosis but
enhances UV-induced cell death (35, 36). This observation was the
first that cytokines can modulate UV-induced apoptosis. Along this
line it was also observed that the immunostimulatory cytokine
IL-12 reduces UV-induced apoptosis (17). However, this effect
was related to a significant reduction of UV-induced DNA dam-
age, presumably via the induction of NER because this reduction
was not observed in NER-deficient \textit{Xpa} KO mice. This demonstration
was the first to show that a cytokine may modulate NER. Because
induction of NER results in reduction of DNA damage it was sug-
gested that modulation of the NER by a cytokine might represent a
new strategy to prevent formation of skin cancer and could supple-
ment other strategies, e.g., the application of exogenous DNA repair
enzymes (13, 14, 37).

Therefore, we were interested in whether this effect on DNA
damage is unique for IL-12 or whether the effect on DNA damage
can also be mediated by other cytokines. In this study, we dem-
onstrate that the same applies for the proinflammatory cytokine
IL-18. Like IL-12, IL-18 reduced UV-induced apoptosis (17). However, this effect
was related to a significant reduction of UV-induced DNA dam-
age, presumably via the induction of NER because this reduction
was not observed in NER-deficient \textit{Xpa} KO mice. This demonstration
was the first to show that a cytokine may modulate NER. Because
induction of NER results in reduction of DNA damage it was sug-
gested that modulation of the NER by a cytokine might represent a
new strategy to prevent formation of skin cancer and could supple-
ment other strategies, e.g., the application of exogenous DNA repair
enzymes (13, 14, 37).

**FIGURE 2.** IL-18 reduces UVB-induced DNA damage in vivo. C57BL/6 mice were irradiated with UV (500 J/m²) on the shaved back. At 3 h before UV exposure, 300 ng of IL-18 (B and D) or equal volumes (100 µl) of saline (A and C) were injected intracutaneously. After 10 min (A and B) and 16 h (C and D), respectively, biopsy specimens were taken and subjected to immunohistochemical staining using an Ab against CPD.

**FIGURE 3.** IL-18 reduces sunburn cell formation in WT but not in \textit{Xpa} KO mice. A, C57/BL6 (WT) and \textit{Xpa} KO mice were irradiated with UV (750 J/m²) on the shaved back. At 3 h before UV exposure IL-18 (300 ng) was injected intracutaneously. Biopsy samples were taken 16 h later and sunburn cells counted per 6-mm length of epidermis. B, To deliver equi-
toxic doses, \textit{Xpa} KO mice were exposed only to 350 J/m² and the effect of IL-18 on sunburn cell formation compared with WT mice, which were exposed to 750 J/m². *, *p < 0.005 vs UV.
cystokine (39, 40) and thus topical application might induce inflammatory reactions. Accordingly, development of atopic dermatitis in transgenic mice overexpressing caspase-1, the enzyme that cleaves IL-18 into its active form, is critically dependent on IL-18 (41). IL-18 is secreted by a variety of cells including macrophages, dendritic cells, and epithelial cells (23). In particular, both human and murine keratinocytes have been demonstrated to be a source of IL-18 (42, 43). Thus, it is fair to speculate whether constitutive secretion of IL-18 in the skin might represent an endogenous mechanism reducing the risk of photocarcinogenesis. It remains to be determined whether mice deficient in the expression of IL-18 exhibit an increased risk to develop skin tumors upon chronic UV exposure. It is also not yet known whether IL-18-deficient mice are more susceptible to UV-induced immunosuppression or exhibit a higher number of sunburn cells as it has been demonstrated for IL-12-deficient mice (17).

UV-induced DNA damage is not only a crucial step in carcinogenesis but is also essentially involved in UV-induced signal transduction. This applies primarily for UV-induced immunosuppres-

**FIGURE 4.** IL-18 prevents UV-induced suppression of the induction of CHS but does not break UV-induced tolerance. *A*, C57BL/6 were treated daily with UV (1000 J/m²) on the back for 4 days and sensitized 24 h after the last exposure through UV-exposed skin. Five days later, mice were challenged on the left ear, and ear swelling was measured 24 h later. One group received 2000 ng of IL-18 3 h before sensitization. *B*, Mice were treated daily with UV (1000 J/m²) on 4 days on the back and sensitized 24 h after the last exposure through UV-exposed skin. After 14 days of the first sensitization, mice were resensitized with DNFB applied onto the abdomen (UV, UV+IL-18). One group (UV+IL-18) received IL-18 (2000 ng) 3 h before resensitization. Five days later, mice were challenged on the right ear, and ear swelling was measured 24 h later. Positive control (Pos. Co.) mice were sensitized (A) and resensitized (B), respectively, and challenged and negative control (Neg. Co.) animals were only challenged. Ear swelling is expressed as the difference in centimeters (mean ± SD × 10⁻²) between the thickness of the challenged ear and that of the vehicle-treated ear. *, p < 0.005 vs positive control; **, p < 0.001 vs UV; ***, p < 0.0005 vs positive control; ****, p < 0.0001 vs positive control.

**FIGURE 5.** IL-18 does not prevent UV-induced immunosuppression in XpaKO mice. *A*, XpaKO mice were treated daily with UV (400 J/m²) on the back for 4 days and sensitized 24 h after the last exposure through UV-exposed skin (UV, UV+IL-18). Five days later, mice were challenged on the left ear and ear swelling was measured 24 h later. One group (UV+IL-18) received 2000 ng of IL-18 3 h before sensitization. *B*, XpaKO mice were treated daily with UV (400 J/m²) for 4 days on the back and sensitized 24 h after the last exposure through UV-exposed skin. After 14 days from the first sensitization, mice were resensitized with DNFB applied onto the abdomen (UV, UV+IL-18). Five days later, mice were challenged on the right ear and ear swelling was measured 24 h later. One group (UV+IL-18) received 2000 ng of IL-18 3 h before resensitization. Positive control (Pos. Co.) mice were sensitized (A) and resensitized (B), respectively, and challenged and negative control (Neg. Co.) animals were only challenged. Ear swelling is expressed as the difference in centimeters (mean ± SD × 10⁻²) between the thickness of the challenged ear and that of the vehicle-treated ear. *, p < 0.001 vs positive control; **, p < 0.005 vs positive control; ***, p < 0.0001 vs positive control.
and antagonizing the activity of regulatory T cells by IL-12 re-
 mains to be determined, it is certainly independent of DNA dam-
age and DNA repair, respectively, because IL-12 exerted this ac-
tivity also in repair-deficient XpcKO mice (21). In contrast, IL-18
was not able to break UV-induced tolerance. Thus, breaking of
established immunotolerance still remains a unique feature of IL-
12. Through this and other activities IL-12 represents a potent
immunostimulatory cytokine. In contrast, IL-18 resembles a potent
proinflammatory cytokine. However, through the capacity de-
scribed in this study to affect DNA repair, IL-18 like IL-12 can
restore an immune response that is suppressed by UV radiation.
In addition, these data further support the notion that DNA damage
is the major molecular trigger for UV-induced immunosuppression
that can be prevented by reducing UV-induced DNA lesions.
Application of IL-18 may represent one of these routes.

Disclosures
The authors have no financial conflict of interest.

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