Effects of ultraviolet radiation on cutaneous T cells

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Chapter 6

Ultraviolet-B radiation induces the expression of interleukin-4 in a cutaneous CD11b+CD15+ cell subset in normal human skin: a preliminary report

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Abstract
There is ample evidence that UVB irradiation causes considerable changes in the cutaneous cell distribution and local cytokine levels in the normal human skin. We show in this report that high numbers of IL-4+ cells appeared in the dermis, and to a lesser extent in the epidermis, within 24 h upon a single exposure to a physiological dose of UVB. In the next 24 h the number of IL-4+ cells in the epidermis raised and reached a peak, while in the dermis the numbers remained high. At day 4 the number of IL-4+ cells was markedly decreased and at later time-points the IL-4 expression was not detectable. Immunohistochemical double-staining experiments revealed that all the cutaneous cells that are known to be able to express IL-4 (i.e. T cells, mast cells, and natural killer cells) were not triggered by UVB to express IL-4. The expression of this cytokine seemed to be associated with CD11b+CD15+CD36+ cells, possibly neutrophils, which are known to infiltrate UVB-exposed skin. However, the results so far do not provide conclusive evidence that the IL-4 is actually produced by these cells. Additional experiments are necessary to exclude the possibility that the IL-4 we detect is taken up by IL-4 receptors or phagocytosis and thus derived from other cells.
Chapter 6

Introduction

Exposure to UV radiation is inevitable, since it is part of the sunlight that reaches the earth surface. UV radiation, especially UVB, has considerable impact on the natural homeostasis within the skin. Amongst others, the skin immune system is affected, as illustrated by the features of inflammation that develop and the concurrent alterations in the composition and function of different cell types (1). The LC in the epidermis are decreased in density and altered in morphology due to the phototoxic effects of UVB (2). Some of the LC can survive and are still able to migrate and to stimulate T cells, despite the presence of UVB-induced DNA damage (3). Probably depended on the amount of DNA damage, these surviving LC undergo either accelerated apoptotic cell death or potentiated maturation (4). The epidermal T cells are depleted by the deleterious radiation (5), likely by induction of apoptosis (6,7). In the dermis a cellular infiltrate starts to develop upon UVB exposure, beginning few hours after irradiation and peaking a day 2. In the order of entrance, this infiltrate is composed of neutrophils, macrophages (8) and predominantly CD4+ memory T cells (5). During the next few days, these infiltrated cells tend to migrate into the epidermis; first the neutrophils appear, later followed by the macrophages. Three days after UVB irradiation LC start to migrate from the hair follicles to repopulate the epidermis (9), whereas one week post-irradiation a selective influx of CD4+ T cells emerges (5).

In addition to the dynamics of these different cell populations in time, UVB radiation also causes a temporal change in the cutaneous cytokine micro milieu. Keratinocytes are believed to be major sources of all kinds of factors, such as cytokines, chemokines, growth factors and many others (10). The constitutive production of these factors by these cells is rather low, but considerably enhanced by UVB radiation (11). UVB potently induces the release of pro-inflammatory mediators IL-1, IL-6, IL-8, TNF-α, and PGE2 from keratinocytes, likely responsible for the onset of the inflammation and the induction of the chemotaxis of the neutrophils and macrophages into the skin. The infiltrating macrophages have been shown to produce huge amounts of IL-10 (12). UVB also induces a strong transient expression of the chemokine psoriasin, first around the dermal capillaries and subsequently in the epidermis (Chapter 4). Psoriasin is a specific chemoattractant for CD4+ T cells, and the anatomically location of UVB-induced psoriasin expression nicely correlates with the influx of the CD4+ T cells into the irradiated skin site at all time points. As a result of all the changes in the composition and function of the different cutaneous cells and their cytokine-production patterns, the UVB-exposed
UVB-induced influx of CD11b⁺CD15⁺IL-4⁺ cells

Skin provides a micro environment that favors the development of type 2 T cell responses (Chapter 5).

In this respect it was interesting to find by RT-PCR and immunohistochemistry that UVB radiation induced a strong expression of IL-4 mRNA and protein in normal human skin in situ two days post-irradiation, while reducing the expression of IFN-γ (Chapter 5). The majority of the IL-4⁺ cells were found in the papillary dermis and to a lesser extent in the epidermis, and they had a scattered distribution. Double-staining with CD3 antibody indicated that only 2% of the IL-4⁺ cells could be identified as T cell. At day 14 after UVB exposure, as well as in unirradiated control skin, IL-4 protein expression was not found. This study was set up to determine the kinetics of UVB-induced IL-4 expression, using skin biopsies obtained at different time-points after irradiation. To identify the actual cell source of this cytokine we performed double-staining immunohistochemistry, using specific antibodies against cell types known to produce IL-4, such as T cells, mast cells and natural killer cells. In this report we show that UVB induced cutaneous CD11b⁺CD15⁺ cells to express IL-4 and that this expression was maximal at day 1 and 2 post-irradiation.

Materials and Methods

UVB irradiation of the subjects. Four adult Caucasian volunteers participated in this study after informed consent according to the guidelines of the Medical Ethical Committee of the hospital. Their mean age was 26 (range 21-31) years and none suffered neither from any skin disease nor from light hypersensitivity. One month before the start and during the experiment the volunteers had to refrain from excessive sunlight exposure and were prohibited to use tanning lamps. The MED for each donor was determined on the left buttock one week before the experiment by irradiating separate small areas of skin with increasing doses of UVB and reading the results 24 h later. The lowest dose inducing erythema was taken as 1 MED. The irradiations were performed with a 1000 W xenon-arc solar simulator lamp (Oriel, Stratford, CT) in combination with a 303 nm interference filter (Schott Glaswerke, Mainz, Germany), as described previously (5). Single doses of 4 MED were given to separate sites of the right buttock at various time points before taking biopsies. The biopsies were taken under local anesthesia and were immediately frozen in liquid nitrogen and stored at -80°C.

Immunohistochemistry. Series of 6-mm cryostat sections were cut and after drying
overnight they were separately wrapped in aluminium foil and stored at -80°C until use. The details of the single- and double-staining procedures are described elsewhere (5). In brief, the cryostat sections were thawed, unwrapped and fixed in acetone for 10 min at 4°C. The sections were incubated overnight with mouse anti-human IL-4 antibody (clone M1; Genzyme, Cambridge, MA), followed by an incubation for 30 min with biotin-conjugated goat anti-mouse (Dako, Glostrup, Denmark), and another incubation for 30 min with horseradish peroxidase-conjugated streptavidin (Dako). The peroxidase activity was visualized as a orange-red color by incubation with AEC (Sigma, St Louis, MO) plus H₂O₂. In the double-staining experiments we used FITC- or AP-conjugated primary antibodies to allow simultaneous detection of IL-4 and several cluster of differentiation markers. The binding of the FITC-labeled antibodies was detected by AP-conjugated goat anti-FITC immunoglobulin (Dako) and the AP activity was visualized as a blue color by incubation with naphthol-AS-MX-phosphate (Sigma) plus fast blue BB (Sigma). The following FITC-labeled antibodies were used: CD3 (Becton Dickinson, Mountain View, CA), CD11b (Immunotech, Marseille, France), CD15 (Dako), CD36 (Immunotech), CD56 (Becton Dickinson). The AP-conjugated anti-tryptase was purchased from Chemicon (Temecula, CA).

Enumeration and statistic analysis. The identification labels of all object glasses were covered and the sequence of the glasses was mixed before counting to enable blind quantification by three different investigators. Only clearly stained cell bodies were counted in three different sections per time-point per volunteer. The value of each individual section was adjusted to 10-mm horizontal section values by dividing with the horizontal width multiplied by 10. The total mean of cell numbers of each time-point was calculated from the mean values of corresponding time-points of each volunteer. The unpaired two-sided Student's t test was used to evaluate the results, considering value p < 0.05 as statistically significant.

Results

UVB radiation induces a transient expression of IL-4. In our previous study (Chapter 5) we detected by immunohistochemistry a clear expression of IL-4 in UVB-exposed human skin 2 days after irradiation. Because UVB-exposed skin 14 days post-irradiation and unirradiated control skin did not contain IL-4 cells, we may conclude that UVB can induce a transient expression of IL-4 in normal human skin. This investigation was aimed to determine the course
of the UVB-induced IL-4 expression. To this end we took skin biopsies from UVB-exposed buttock skin at different time-points after irradiation and stained cryostat sections with an anti-human IL-4 specific antibody, which is suitable for immunohistochemistry. In unirradiated control skin an occasional IL-4+ cell was observed in the dermis, whereas the epidermis was devoid of such cells (Fig. 1). Already 1 day after UVB exposure, several hundreds (307 ± 98 per 10 mm section) of IL-4+ cells were present in the dermis, and they also started to appear in the epidermis.

**Figure 1. Transient expression of IL-4 in human skin after UVB irradiation.** Skin biopsies were obtained from buttock skin of 4 volunteers at various times after UVB exposure with 4 MED, using unirradiated skin as control (c). Three cryostat sections per time point were stained for IL-4 and the number of positive cells in the dermis and epidermis was counted by three investigators, so each bar represents the mean of 27 determinations with the SD. The asterisk indicates the time-points at which the number of IL-4+ cells was significantly different from that found in control skin.

At day 2 post-irradiation, the number of IL-4+ cells in the dermis was comparable to the numbers of day 1, but in the epidermis the IL-4 expression had reached a maximum (45 ± 23 per 10 mm section). The IL-4+ cells in the dermis and epidermis had a scattered distribution (Fig. 2A). The number of IL-4+ cells was clearly decreased at day 4 in the dermis and epidermis. The IL-4 expression could not be found at later time-points (Fig. 1).

The UVB-induced IL-4+ cells co-express CD11b and CD15. In order to determine which cell-type in the normal human skin was triggered by UVB to express IL-4, we performed double-staining experiments. We used the biopsies taken at day 2 after irradiation for these experiments, because the IL-4 signal was most prominent in both dermis and epidermis at this time-point. The skin sections were stained with anti-IL-4 plus one antibody specific for cell-types that are known to produce IL-4; i.e. CD3 as a marker for T cells, tryptase for mast cells, CD56 for natural killer
cells. In line with our previous study, we found that about 2% of the IL-4+ cells co-expressed CD3 (Fig. 2A). Except for an occasional double-positive cell (much less than 1%), the IL-4+ cells were negative for tryptase (Fig. 2B) and for CD56 (not shown). These data indicate that the IL-4 was apparently not induced in a resident skin cell.

Because the approach mentioned above did not resolve the issue about the identity of the IL-4+ cells, we extended our panel of antibodies with markers for cells that are known to infiltrate the skin after UVB irradiation; i.e. CD11b, CD15, and CD36 for macrophages/granulocytes. Although many CD36+ cells were found in the UVB-exposed skin samples (mainly in the dermis), they did clearly not co-express IL-4 (Fig 2C). Much to our surprise, we found that the majority (75% ± 11%) of the IL-4+ cells in the cryostat sections co-expressed CD11b and CD15 (Fig 2D, 2E). In addition, 60% ± 10% of the CD11b+ cells and 61% ± 13% of the CD15+ cells were also positive for IL-4. The CD11b and CD15 expressing cells had a scattered distribution, like the IL-4 expressing cells. The presence of IL-4 within CD11b+ and CD15+ was confirmed in cytothin preparations of dermal cell suspensions derived from UVB-exposed skin (Fig 2F). These data indicate that UVB-induced IL-4 co-localized with infiltrating granulocytes.

**Discussion**

In this study we show that exposure of normal human skin to a physiological dose of UVB induced the transient appearance of numerous IL-4+ cells in the dermis (peak at 24 h and 48 h after irradiation) and in the epidermis (peak at 48 h), having a scattered distribution. Double-staining experiments ruled out that the IL-4 expression was induced in local cutaneous T cells, mast cells or natural killer cells, which are known to possess the capacity to synthesize IL-4 (13). It was quite astounding to discover that the UVB-induced IL-4 expression was associated with infiltrating CD11b+CD15+ cells, because neutrophils (CD11b+CD15+) and macrophages (CD11b+), which do infiltrate UVB-exposed skin, are not known to express IL-4 (13,14). On the other hand, basophils and eosinophils can produce IL-4 (13) and do express CD11b and CD15, but they are not known to infiltrate UVB-exposed normal human skin (15). By means of specific antibodies we can check the possible but unlikely presence of the latter two cell types in irradiated skin. The absence of CD36 and the presence of CD15 on the IL-4+ cells make the macrophage an improbable candidate. Taken all together, our results thus far suggest that the IL-4 expression we detect in UVB-exposed skin is associated with neutrophils, which infiltrate the skin after UVB
UVB-induced influx of CD11b*CD15*IL-4* cells
Figure 2. IL-4 present in UVB-induced infiltrating CD11b\textsuperscript{+}CD15\textsuperscript{+} cells. Skin biopsies were taken at day 2 post-irradiation with 4 MED of UVB and cryostat sections were double-stained to detect IL-4 plus the markers (a) CD3, (b) tryptase, (c) CD36, (d) CD11b, or (e) CD15. The presence of IL-4 can be recognized by a red color (black arrowhead) and the mentioned cell-markers by a blue color (open arrowhead), whereas an asterisk indicates a double-positive cell. In cytospin preparations of dermal cell suspensions derived from UVB-exposed skin (f) double-positive cells for IL-4 and CD15 were present.
UVB-induced influx of CD11b$^{+}$CD15$^{+}$IL-4$^{+}$ cells

irradiation. Additional studies with neutrophil-specific markers, such as elastase, should be performed to confirm this.

Our observation that IL-4 expression is associated with UVB-induced neutrophil-like cells does not necessarily mean that these cells are the actual IL-4 source. In other words, the neutrophil-like cells may be unable to synthesize IL-4, but did acquire this expression through binding and/or uptake of environmental IL-4 by the IL-4 receptor or by phagocytosis. Indeed neutrophils have been shown to exhibit these receptors (16) and thus may have picked up exogenous IL-4. In forthcoming experiments we will isolate the CD11b$^{+}$CD15$^{+}$ cells from the dermal cell suspension derived from UVB-exposed skin, and determine by RT-PCR and ELISA if these cells can express mRNA for IL-4 and can secrete IL-4 protein upon a short-term culture in vitro.

The anti-IL-4 antibody we used in this study should be suitable for immunohistochemistry according to the data sheet of the manufacturer. In a previous study in which we discovered the UVB-induced IL-4 expression (Chapter 5), we also used another anti-IL-4 antibody that was selected out of a large panel of anti-IL-4 antibodies for the application in immunohistochemistry. The latter anti-IL-4 antibody confirmed the results obtained with the former. Although both anti-IL-4 antibodies are described as useful tools for the staining of IL-4 in cryostat sections, we cannot exclude the possibility that the antibodies recognize a cross-reactive epitope on an irrelevant molecule in the CD11b$^{+}$CD15$^{+}$ infiltrating cells. The succeeding studies as suggested above will resolve this issue.

Several reports on in vivo studies with mice indicate that IL-4 plays an important role in the development of UVB-induced immunosuppression, as convincingly evidenced by the findings that injection of blocking anti-IL-4 can abolish this immunosuppression (17) and that in IL-4 gene knockout mice the delayed-type hypersensitivity response is not suppressed by UVB exposure (18). UVB radiation can induce serum IL-4 in a dose-dependent fashion in mice and this IL-4 seems to be responsible for the subsequent induction of serum IL-10 (19), a cytokine with immunosuppressive properties. Thus far the cellular source of the UVB-induced IL-4 in mice is unknown. Due to its pleiotropic property, IL-4 may be involved in UVB-induced immunosuppression in several ways as described in the discussion of Chapter 5 of this thesis. One important effect of the presence of IL-4 in the cutaneous tissue is that the development of type 2 T-cell responses in skin are promoted, while the type 1 T-cell responses are concomitantly
inhibited, nicely fitting in the model of UVB-induced immunosuppression (20).

In summary, we observed the transient presence of numerous IL-4$^+$ cells in the dermis and epidermis of UVB-exposed normal human skin. This expression was not induced in resident skin cells, but rather associated with the UVB-induced infiltrating CD11b$^+$CD15$^+$CD36$^-$ cells, possibly neutrophils.

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UVB-induced influx of CD11b+CD15+IL-4+ cells

References


