Effects of therapies on cytokine patterns in psoriasis
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Ultraviolet-B radiation induces a transient appearance of IL-4 positive neutrophils, which support the development of Th2 responses

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Abstract

Ultraviolet-B (UVB) irradiation can cause considerable changes in the composition of cells in the skin and in cutaneous cytokine levels. We found that a single exposure of normal human skin to UVB induced an infiltration of numerous IL-4+ cells. This recruitment was detectable in the papillary dermis already 5 hours after irradiation, reaching a peak at 24 hours and declining gradually thereafter. The IL-4+ cells appeared in the epidermis at 24 hours post-radiation and reached a plateau at days 2 and 3. The number of IL-4+ cells was markedly decreased in both dermis and epidermis at day 4, and at later time-points the IL-4 expression was absent. The IL-4+ cells did not co-express CD3 (T cells), tryptase (mast cells), CD56 (natural killer cells), and CD36 (macrophages). They did co-express CD15 and CD11b, showed a clear association with elastase, and had a multi-lobed nucleus, indicating that UVB-induced infiltrating IL-4+ cells are neutrophils. Blister fluid from irradiated skin, but not from control skin, contained IL-4 protein as well as increased levels of IL-6, IL-8, and TNF-α. In contrast to control cultures derived from non-irradiated skin, a predominant type 2 T cell response was detected in T cells present in primary dermal cell cultures derived from UVB-exposed skin. This type 2-shift was abolished when CD15+ cells (i.e. neutrophils) were depleted from the dermal cell suspension before culturing, suggesting that neutrophils favor type 2 T cell responses in UVB-exposed skin.
Introduction

Exposure to ultraviolet (UV) radiation is inevitable, because 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 features of inflammation that develop and the concurrent alterations in the composition and function of different cell types. The Langerhans cells (LC) in the epidermis are decreased in density and altered in morphology due to photo-toxic effects of UVB. 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. Probably dependent on the amount of DNA damage, these surviving LC undergo either accelerated apoptotic cell death or potentiated maturation. The epidermal T cells are depleted by the deleterious radiation, likely by induction of apoptosis. In the dermis a cellular infiltrate starts to develop upon UVB exposure, beginning few hours after irradiation and peaking at day 2. In the order of entrance, this infiltrate is composed of neutrophils, macrophages and predominantly CD4+ memory T cells. 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, whereas one week post-irradiation a selective influx of CD4+ T cells emerges.

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. The constitutive production of these factors by these cells is rather low, but considerably enhanced by UVB radiation. 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. UVB also induces a strong transient expression of the chemokine psoriasin, first around the dermal capillaries and subsequently in the epidermis. Psoriasin is a specific chemoattractant for CD4+ T cells, and the anatomical 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 skin provides a microenvironment that favors the development of type 2 T cell responses.

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.
situ two days post-irradiation, while reducing the expression of IFN-γ. 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 cells. At day 14 after UVB exposure, as well as in non-irradiated control skin, no IL-4 protein expression could be detected in cryostat sections. The present 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 type(s) expressing this cytokine we performed double-staining immunohistochemistry, using specific antibodies against cell types known to produce IL-4, such as mast cells, granulocytes and natural killer cells. In order to investigate whether the presence of the IL-4+ cells also led to significant concentrations of IL-4 in the irradiated skin we raised blisters and analyzed the fluid for the presence of this cytokine. Further, we tested if the IL-4+ cells could affect the Th1/Th2 balance of the T cell response in dermal cell cultures. In this report we show that UVB radiation induced a transient appearance of IL-4+ neutrophils in normal human skin, having its maximum at day 1 and 2 post-irradiation, and that these cells contributed to the enhanced development of type-2 T cells in dermal cell cultures from UVB-irradiated skin.

**Materials and Methods**

**UVB irradiation of the subjects.** Twelve 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 27 (range 21 - 35) years and none suffered from any skin disease or from light hypersensitivity. One month before the start and during the experiment the volunteers had to refrain from excessive sunlight and were prohibited to use tanning lamps. The minimal erythema dose (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 irradiation was performed with a 1000 W xenon-arc solar simulator lamp (Oriel, Stratford, CT) in combination with a 303 interference filter (Jenaer Glaswerke Schott & Gen., Mainz, Germany) that only transmits the UVB spectrum (280 – 320 nm), as described previously. 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 aluminum foil and stored at -80°C until use. The details of the single- and double-staining procedures are described elsewhere. 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 mAb (clone M1; from Genzyme, Cambridge, MA and from Immunex Corporation, Seattle, WA), 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 an orange-red color by incubation with 3-amino-9-ethylcarbazole (Sigma, St Louis, MO) plus H₂O₂. In the double-staining experiments we used FITC- or alkaline phosphatase (AP)-conjugated primary mAbs to allow simultaneous detection of IL-4 and several cluster of differentiation markers. The binding of the FITC-labeled mAbs was detected by AP-conjugated goat anti-FITC (Dako). 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 to stain mast cells was purchased from Chemicon (Temecula, CA). BB1 mAb to identify basophils was a gift from Dr. A.F. Walls (Southampton General Hospital, England). EG2 mAb to detect eosinophils was purchased from Pharmacia (Uppsala, Sweden) and NP57 (anti-elastase) mAb to recognize neutrophils was from Dako.

**Quantification of cells in cryostat sections.** 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 per time-point was calculated from the mean values of corresponding time-points of all volunteers.

**Suction blisters.** Blisters were raised in duplicate on UVB-exposed sites at 24 h and 48 h after irradiation and on non-irradiated skin at the medial site of the upper arm. We used warmed (37°C) vacuum cups and applied a negative pressure of 200 mmHg. Two 10 mm blisters were raised per vacuum cup and the exudate was collected by a syringe. All blisters were produced in 1 – 2 h at one single time point. The fluid of duplicate blisters was
pooled (approximately 0.35 ml) and centrifuged at 500 g before storage of the supernatant at -20°C. The blister roofs were taken off by scissors and stained overnight at 4°C with FITC-conjugated CD15 after fixation for 10 min in acetone.

**Cytokine analysis.** Measurement of IL-4 protein was performed by a specific solid phase sandwich ELISA (detection limit, 4 pg/ml), using coating Ab, biotin-conjugated detecting Ab and IL-4 standard from CLB (Amsterdam, The Netherlands). Protein levels of IL-6 (detection limit, 20 pg/ml), IL-8 (detection limit, 10 pg/ml), and TNF-α (detection limit, 20 pg/ml) were determined by specific ELISAs from BioSource Europe (Nivelles, Belgium), using the manufacturers’ protocols.

**Preparation of primary dermal cell cultures.** The biopsies were extensively rinsed with PBS and cultured overnight at 4 °C in 0.3 % dispase II (Boehringer Mannheim, Mannheim, Germany). After removal of the epidermis, the dermal tissue was minced by scissors and incubated for 2 h at 37°C in PBS containing 0.2% collagenase D (Boehringer Mannheim), 40 U/ml DNase I (Boehringer Mannheim) and 2 % FCS. The suspension was sieved to remove tissue debris and after centrifugation cells were resuspended in Iscove’s modified Dulbecco’s medium with 5% pooled normal human serum (BioWhittaker, Walkersville, MD) and 50 mg/ml gentamycin (Sigma). The dermal cells were seeded at 10^5 cells in 200 ml per well in a round-bottom 96-well plate (Costar, Cambridge, MA). Because the yield of dermal cells was low (approximately 10^5 cells per biopsy) and contained 10 % or less T cells, the fresh dermal cell suspension was incubated with the polyclonal T-cell stimulus PHA first. In order to promote T cell growth in this primary dermal cell culture, 1 ml/ml phytohemagglutinin ((Difco Laboratories, Detroit, Michigan) and 50 U/ml recombinant human IL-2 (Cetus Corp., Emmeryville, CA) was added to the culture medium. The expanding polyclonal dermal T cells in the primary cultures were transferred to 24-well culture plates and maintained in culture medium with 20 U/ml IL-2. The T cells, expanded within the primary dermal cell cultures, were tested for their cytokine pattern after 2 or 3 weeks.

**Depletion of CD15⁺ cells.** The freshly prepared dermal suspension from UVB-exposed skin was split. One part of the dermal cell suspension was incubated with paramagnetic CD15 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) to label the CD15⁺ cells. According to the manufacturers’ protocol the cells were subsequently loaded into a cell separation column which was placed in a miniMACS magnet (Miltenyi Biotec). The
unlabeled dermal cells were able to run through the column while the CD15+ cells were retained. The collected cells were washed and resuspended in culture medium. The other part of the fresh dermal cells was treated in a similar way but with omission of the magnetic beads. A small number of both cell populations was labeled with FITC-conjugated CD15 to determine by FACS the success of the depletion.

**Analysis of intracellular cytokines.** T cells in primary dermal cell cultures were stimulated with 25 ng/ml PMA (Sigma) plus 1 mg/ml ionomycin (Sigma) for 4 h at 37°C in the presence of 3 mg/ml brefeldin A (Sigma). Cells were washed in FACS medium (PBS with 2 % FCS and 0.1 % azide) and subjected to staining of the cell surface with allophycocyanin-labeled CD4 (Becton Dickinson). Intracellular cytokine staining was performed using phycoerythrin-conjugated anti-IL-4 and FITC-conjugated anti-IFN-γ (Becton Dickinson) according to the manufacturers’ protocol. Labeled isotype controls were from the same company. The triple-stained cells were analyzed with a FACScalibur equipped with CellQuest software (Becton Dickinson).

**Statistic analysis.** The unpaired two-sided Student’s t test was used to evaluate the results, considering values $P < 0.05$ as significant. The data are expressed as mean ± SD.

**Results**

**UVB radiation induces a transient expression of IL-4.** In a recent study we detected a high number of IL-4+ cells in irradiated human skin at day 2 after a single exposure to UVB. The current investigation was aimed to determine the time course of the UVB-induced IL-4 expression. To this end we took biopsies from UVB-exposed skin at different time-points after irradiation and stained cryostat sections with a specific anti-human IL-4 mAb. In non-irradiated control skin an occasional IL-4+ cell was observed in the dermis, whereas the epidermis was devoid of such cells (Figure 1A). Already 5 h after exposure to 4 MED UVB few clearly positively stained cells could be detected in the dermis, which were all localized around the capillaries in the papillary dermis (Figure 1B). Ten h after irradiation the number of IL-4+ cells in the dermis was markedly increased and these cells had a scattered distribution in the dermal region between the vessels and the basal membrane (Figure 1C). The IL-4+ cells were always distinct, not clustered, and strongly positive. At the next time point, day 1, the number of IL-4+ cells had reached a maximum in the dermis (334 ± 86 per 10 mm section, Figure 2), and these cells also started to appear in the epidermis (Figure 1D). From day 2 through day 4 post-irradiation, the number of IL-4+ cells...
infiltration of IL-4+ cells in human skin after UVB irradiation. Skin biopsies were obtained from buttock skin of a healthy volunteer at 5 h, 10 h, or 24 h after a single exposure to UVB, using non-irradiated skin as control (con). IL-4 protein expression was determined in cryostat sections by immunohistochemistry. IL-4+ cells can be recognized as red stained cells. Results are representative of data from 3 different volunteers.

in the dermis declined gradually (Figure 2). In the epidermis the IL-4 expression reached a maximum at day 3 (48 ± 25 per 10 mm section) and clearly decreased at day 4. The IL-4 expression could not be found in the dermis and epidermis at later time points (Figure 2).

The UVB-induced IL-4+ cells are neutrophils. 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. In these experiments we used biopsies taken at day 2 after irradiation, because the IL-4 signal was most prominent at this time-point in both dermis and epidermis. 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, CD56 for natural killer cells, tryptase for mast cells. In line with our previous study, we found that about 2 % of the IL-4+ cells co-expressed CD3 (Figure 3A). Except for an occasional double-positive cell (much less then 1%), the IL-4+ cells were negative for tryptase (Figure 3B) and for CD56 (not shown). These data indicate that the IL-4 was apparently not induced in a resident skin cell.

Next, we extended our panel of mAbs with markers for cells that are known to infiltrate the
Figure 2. Transient expression of IL-4 in UVB-exposed skin. Human skin biopsies were obtained from three different volunteers at indicated time-points following exposure to UVB. Three cryostat sections per time-point were stained for IL-4 and three investigators counted the number of positive cells in the dermis and epidermis; so each bar represents at least 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 (C).

skin after UVB irradiation; i.e. CD11b, CD15, and CD36 for macrophages and/or 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 (Figure 3C). To our surprise, we found that the majority of the IL-4$^+$ cells (Figures 3D and 3E) in the cryostat sections co-expressed CD11b (76.3 % ± 11.9 %) and CD15 (85.7 % ± 7.9 %). In addition, 60.8 % ±

Figure 3. UVB-induced IL-4 expression is associated with expression of CD11b, CD15, and elastase. Skin biopsies were taken at day 2 after irradiation with UVB and cryostat sections were double-stained to detect IL-4 (red color; white arrow) plus the markers CD3 (a), tryptase (b), CD36 (c), CD11b (d), or CD15 (e), which can be recognized by a blue color (black arrow). Take notice of the co-localization (white arrowhead) of IL-4 with CD11b (d) and CD15 (e). Two serial sections from a biopsy taken at 10 h post-irradiation, which were stained for IL-4 (f) and elastase (g) respectively (plus hematoxilin nuclear staining), indicate the co-localization (black arrowhead) of these two markers. Results are representative of data from 3 different volunteers. (=> page 190).
10.3% of the CD11b+ cells and 71.8% ± 9.3% 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.

Because these results pointed out that the UVB-induced IL-4+ cell are presumably granulocytes, we performed single staining of serial sections with mAbs BB-1, EG2, and anti-elastase to discriminate basophils, eosinophils, and neutrophils, respectively. We could not detect basophils or eosinophils in UVB-irradiated skin. Examination of serial sections from UVB-exposed skin revealed that the anti-elastase staining clearly matched the number, distribution, and localization of the IL-4+ cells (Figures 3F and 3G). All these results together indicate that the IL-4+ cells are neutrophils.

**Significant levels of IL-4 in blister fluid upon UVB treatment.** Regarding our finding that UVB irradiation of normal human skin induces the appearance of large numbers of IL-4+ cells, we questioned whether increased levels of this cytokine could be appreciated in irradiated skin as well. To answer this, we produced suction blisters on day 1 and day 2 after UVB exposure, because the recruitment of IL-4+ cells was maximal at these time points (Figure 2). We also determined the levels of pro-inflammatory cytokines IL-6, IL-8, and TNF-α in the blister fluid in order to be able to compare our results to what other investigators have found in their studies. IL-4 could be found at low levels in blister fluid at 24 and 48 h after irradiation in both volunteers (Figure 4, top left), whereas IL-4 protein was not detectable in exudate from non-irradiated skin from the same subject. As compared to blister fluid from non-irradiated skin, the content of IL-6 and IL-8 in the blister fluid was markedly raised at 24 h and remained still high at 48 h after UVB treatment (Figure 4, right top and bottom). UVB exposure also induced high levels of TNF-α at 24 h upon UVB exposure, however the concentration was substantially reduced at 48 h, even reaching levels below control skin (Figure 4, bottom, left).

The blister fluid was centrifuged before storage to remove cells that could be present in this exudate. Although no pellet was perceptible in any sample, we prepared one cytospin per exudate sample of the remainder fluid after removal of the supernatant. The cytospin preparations contained few numbers of cells, which were stained for IL-4 according to the protocol used to stain cryostat sections. Only in the cytopins of blister fluid derived from UVB-exposed skin, some clear IL-4+ cells could be observed (Figure 5A), but because of the limited number of cells we could not perform a reliable quantification. All of the IL-4+ cells possessed a characteristic lobed nucleus providing additional evidence that the IL-
Figure 4. IL-4 protein present in blister fluid from UVB-exposed skin. Suction blister fluid was obtained from two subjects (indicated by open and closed circles, respectively) 24 h or 48 h after treatment with UVB. Control blister fluid (values at time-point 0 h), was obtained from non-irradiated skin of the same subjects. The fluid was centrifuged to remove cells and was analyzed for the presence of IL-4, IL-6, IL-8, and TNF-α by specific ELISAs.

4+CD15+CD11b+ cells indeed are neutrophils. The blister roofs were stained with FITC-labeled CD15 showing the distribution of these cells in the epidermis from irradiated skin from a different angle (Figure 5B). No CD15+ cells were present in the epidermis of non-irradiated control skin (data not shown).

In order to study the IL-4+ cells in more detail we attempted to purify these cells, using a separation technique, which was successful to isolate neutrophils from peripheral blood by means of CD15 mAb conjugated magnetic microbeads. The small number of dermal cells that could be isolated from the skin biopsies, however, hampered these experiments.

Figure 5. IL-4 expression in dermal CD15+ cells with a multi-lobed nucleus. Cytospins of cells from blister fluid (a) and from a suspension of dermal cells (c and d) both derived from UVB-exposed skin were stained for the presence of IL-4 (red color). Take notice of the multi-lobed nucleus in the positive cells. In one experiment the cells were also stained for the expression of CD15 (blue color), indicating that the IL-4+ cells co-express CD15 (c). The blue nuclear staining in the two other samples (a and d) was provoked by hematoxilin. An epidermal sheet (blister roof) was obtained from UVB-treated skin at day 2 post-irradiation and was stained with FITC-conjugated CD15, showing the distribution of infiltrating CD15+ neutrophils (b). ( page 190).
The yield of CD15+ cells was very low (less than $10^3$), and because the viability and purity of these cells were bad (less than 50 %) they were not suitable for further tests (data not shown). On the other hand, application of this magnetic cell separation technique appeared to be useful to prepare a dermal cell suspension devoid of CD15+ cells (see below).

**Depletion of CD15+ cells from the dermal cell suspension from UVB-exposed skin abolishes the favored development of Th2 responses.** Now we have demonstrated that UVB exposure induces not only the appearance of IL-4+ cells but also significant levels of IL-4 in the skin, we wondered whether these infiltrating CD15+CD11b+ cells could affect the Th1/Th2 balance in irradiated skin, because IL-4 is a strong Th2-polarizing cytokine. Earlier studies indicated that the determination of the Th1/Th2 status *in situ* was not possible, but that primary dermal cell cultures are suitable to monitor the Th1/Th2 status of dermal T cells. This approach enabled us to demonstrate that dermal T cells from *in vivo* irradiated skin are skewed towards Th2, as compared to control skin. In cytospin preparations of fresh dermal cell suspensions derived from UVB-exposed skin we were able to detect the presence of IL-4+CD15+ cells (Figure 5C), indicating that this suspension can be useful to study the role of the CD15+ cells in Th1/Th2 development. The presence of a typical multi-lobed nucleus in these cells again indicates that the IL-4+ cells are neutrophils (Figures 5C and 5D). Fresh dermal cell suspensions from non-irradiated control skin did not contain CD15+ cells (data not shown).

To test whether the CD15+ cells may affect the development of Th1/Th2 responses of dermal T cells, we compared the Th1/Th2 status in non-separated and in CD15-depleted primary dermal cell cultures, both originating from the same batch of dermal cells derived from UVB-exposed skin. The dermal cell suspension from irradiated skin contained 2.7% ± 0.9% CD15+ cells (Figure 6A). Depletion of these cells with magnetic CD15 MicroBeads was very efficient because no CD15+ cells could be detected in the dermal suspension.

![Figure 6](image-url) **Figure 6.** Depletion of CD15+ cells from dermal cell suspensions. A single cell suspension was prepared from the dermis of irradiated normal human skin 2 day after UVB exposure. The dermal cell suspension contained a small population of CD15+ neutrophils, as indicated by a rectangle (a). This population of neutrophils was successfully removed by the application of CD15-coupled magnetic beads (b).
Table 1 Depletion of CD15+ cells in cultures from UVB-irradiated skin reduces Th2 skewing ^

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^ A single cell suspension was prepared of dermal tissue derived from non-irradiated skin (control) or from UVB-exposed skin, 2 day after irradiation. Part of the dermal cells from UVB-treated skin was incubated with CD15 Microbeads to eliminate the CD15+ cells (UVB CD15 depleted), whereas the remainder part was mock treated (UVB). Primary dermal cell cultures were maintained for 2 or 3 weeks allowing polyclonal expansion of T cells. Intracellular IL-4 and IFN-γ expression in the dermal T cells was determined according to the protocol developed by Becton Dickinson, see Methods for details. Results of 3 separate experiments using 3 different volunteers (#1 - #3) are summarized in this table.

b The data are expressed as percentage IFN-γ or IL-4 cells of the electronically gated CD4+ T cells. The Th1 cells (IFN-γ positive and IL-4 negative) appear in the lower-right quadrant of the FL1-FL2 dot plot, as illustrated in Figure 7 depicting the results of volunteer #3. The Th2 cells (IL-4 positive and IFN-γ negative) are in the upper-left quadrant.

after this treatment (Figure 6B). The CD4+ T cells in the primary dermal cell cultures from control skin and from irradiated skin, either untouched or CD15-depleted, were analyzed for the intracellular expression of IL-4 and IFN-γ by FACS. T cells in primary dermal cell cultures from UVB-exposed skin showed a marked increased expression of IL-4, as compared...
to T cells from non-irradiated control skin (Figure 7A and 7B; Table I), confirming our earlier observations. However, when the CD15<sup>+</sup> cells were depleted prior to the onset of the primary dermal cell cultures this raised IL-4 expression was abolished (Figure 7c; Table I), indicating that the CD15<sup>+</sup> cells participate in the Th2 skewing effect of UVB radiation. Figure 7c shows that the percentage of IL-4 / IFN-γ double positive T cells was increased in the CD15-depleted dermal cell culture. However, in cultures of the other two volunteers no increase of these double positive cells was found.

Discussion

It is well known that UVB radiation causes considerable changes in the cell distribution and local cytokine levels in skin. In this study we showed that a single exposure to UVB induced a temporary influx of numerous IL-4<sup>+</sup> cells in normal human skin. These cells, having a scattered distribution, appeared in the dermis already within few h after irradiation (peak at day 1) and subsequently in the epidermis (from day 1, reaching a plateau at days 2 and 3). Double-staining experiments ruled out that the IL-4 expression was induced in resident cutaneous cells, such as T cells, mast cells or natural killer cells, all known to possess the capacity to synthesize IL-4<sup>19</sup>. For several reasons we believe that the UVB-induced IL-4<sup>+</sup> cells are neutrophils: 1) Neutrophils are able to produce IL-4<sup>20</sup>. 2) The IL-4<sup>+</sup> cells co-express CD11b and CD15, but not CD36. 3) They have a multi-lobed nucleus. 4) IL-4 expression is associated with the neutrophil marker elastase, as indicated by serial section staining and distribution pattern. 5) Neutrophils are well known to infiltrate human and murine skin after exposure to an erythemogenic dose of UVB or UVA<sup>8, 9, 21, 22</sup>. 6) The time course of infiltration of the IL-4<sup>+</sup> cells matches perfectly the infiltration kinetics of polymorphonuclear leukocytes upon UV irradiation, as reported by others<sup>8, 9, 23</sup>. Although macrophages also express CD11b and infiltrate UVB-irradiated skin, they are improbable candidates for the IL-4<sup>+</sup> cells because of the presence of CD36 and the absence of CD15. In connection to this, it was recently shown that the majority of the CD11b<sup>+</sup> cells, which infiltrate UVB-exposed skin, lack the macrophage marker CD68<sup>24</sup>. Basophils and eosinophils also have a multi-lobed nucleus and express CD11b and CD15, but they are not known to infiltrate UVB-exposed normal human skin<sup>25</sup>, and by means of specific mAbs we demonstrated that these two cell types are absent in irradiated skin. It has been reported that UVB radiation can cause a considerable decrease in the expression of CD11b on neutrophils isolated from peripheral blood<sup>26</sup>. Nevertheless, this marker is clearly detectable on infiltrating neutrophils in UVB-treated skin as demonstrated by immunohistochemistry. A low but significant level of IL-4 was detected in blister fluid obtained from irradiated skin.
It is tempting to assume that this UVB-induced IL-4 is derived from the numerous IL-4+ cells appearing in irradiated skin. Attempts to purify the infiltrating CD15+ cells and to demonstrate IL-4 production in these cells in vitro failed unfortunately, because of limited dermal cell numbers. We can not exclude the possibility that IL-4 in the blister fluid originated from other cutaneous cells, for instance from mast cells, which are known to degranulate upon UVB irradiation. Irrespective which cell type is the actual source of the UVB-induced IL-4, our results clearly indicate that IL-4 can be added to the list of cytokines, induced or up-regulated by UVB and giving rise to a substantially altered cytokine micromilieu in irradiated skin. In addition to IL-4, we demonstrated an induction or increase in the levels of pro-inflammatory molecules TNF-α, IL-6, and IL-8 in the suction-bluister fluid of UVB-exposed skin. The concentrations and time course of these cytokines are in line with previous studies. The high concentration of IL-8, a strong chemoattractant of neutrophils, correlates with the recruitment of high numbers of these cells in irradiated skin.

Due to its pleiotropic property, IL-4 can concomitantly affect many different cell types in the irradiated skin site. Amongst others, IL-4 can down-regulate UVB-induced E-selectin expression on endothelial cells, limiting the influx of inflammatory cells. The phagocytic activity of infiltrated neutrophils and macrophages is enhanced by IL-4, facilitating the removal of UVB-induced damage. IL-4 can delay apoptosis and stimulate cytokine production in neutrophils. Repopulation of the epidermis by LC may be delayed by IL-4, because this cytokine can inhibit the migration of these cells through the down-regulation of TNF-α receptor II expression. UVB radiation can induce serum IL-4 (in an unknown source) in a dose-dependent fashion in mice and this IL-4 seems to be responsible for the subsequent induction of serum IL-10, a cytokine with immunosuppressive properties. Injection of blocking anti-IL-4 can abolish UVB-induced immunosuppression indicating that IL-4 plays an important role in the development of this immunosuppression. In addition, in IL-4 gene knockout mice the delayed-type hypersensitivity response is not suppressed by UVB exposure. Because IL-4 is a strong Th2-polarizing cytokine, the presence of IL-4 in UVB-exposed skin may favor the development of type 2 T-cell responses in this tissue, while type 1 T-cell responses are concomitantly inhibited. In this view our results, together with the findings of many others, support the hypothesis that UVB radiation activates a cytokine cascade and disrupts the function of antigen-presenting cells, causing the selective inhibition of type 1 T cells while allowing type 2 T cell activation to proceed. All these processes together contribute to an immunosuppressive state of the irradiated skin.

In connection to immunosuppression in UVB-exposed skin, much attention has been paid to the function of infiltrating macrophages, which can activate CD4+ autologous suppressor
T cells\textsuperscript{48}. The T cells responding to these macrophages have a typical IL-2R\(\alpha\) negative phenotype and the proliferation of these T cells appears to be dependent on IL-4 but not on IL-2, as measured by \textit{in vitro} assays\textsuperscript{49-51}. Further, in comparison to control epidermal cell suspensions (containing LC), epidermal cells from UVB-irradiated skin (containing macrophages) stimulate higher numbers of allogeneic peripheral blood CD4\(^+\) T cells to produce IL-4, as measured in primary and secondary cultures by an ELISA spot assay\textsuperscript{52}. In line with this reported Th2 shift, we demonstrated by means of intracellular FACS analysis a much higher IL-4 production in responding autologous CD4\(^+\) T cells in primary dermal cell cultures derived from UVB-irradiated skin than in the T cell population from control skin. Depletion of the CD15\(^+\) neutrophils from the \textit{in vivo}-irradiated dermal cell suspension before the onset of the primary culture abrogated this Th2 shift. This indicates that infiltrating neutrophils, though no antigen-presenting cells themselves, can augment somehow the IL-4 production of the responding T cells, which are activated by antigen-presenting cells present in UVB-exposed skin. Future studies are necessary to clarify whether neutrophil-derived IL-4 may play a role in this modified T cell response or to explore the possibility that the infiltrating neutrophils affect the T cell cytokine production via another mechanism.
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