Effects of ultraviolet radiation on cutaneous T cells

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Chapter 3
UVB radiation preferentially induces recruitment of memory CD4+ T cells in normal human skin: long-term effect after a single exposure

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Abstract
Acute, low-doses of ultraviolet-B radiation affect the immune competent cells of the skin immune system. In this study, we examined the time-dependent changes of the cutaneous T cell population in normal human volunteers following a single local exposure to UV. Solar-simulated UV radiation caused an initial decrease in intraepidermal T cells numbers, even leading to T cell depletion at day 4, whereupon a considerable infiltration of T cells in the epidermis occurred which peaked at day 14. In the dermis the number of T cells was markedly increased at day 2 (peak) and 4 after irradiation, and subsequently declined to the non-irradiated control values at day 10. Double-staining with several T cell markers showed that the T cells, infiltrating the (epi)dermis upon UV exposure, were almost exclusively CD4+ CD45RO+ T cells, expressing an α/β type T cell receptor, but lacking the activation markers HLA-DR, VLA-1 and IL-2R. Application of UVB radiation resulted in similar dynamics of T cells, indicating that the UVB wavelengths within the solar-simulated UV radiation were responsible for the selective influx of CD4+ T cells. In conjunction with UVB-induced alterations in the type and function of antigen-presenting cells (i.e. Langerhans cells and macrophages), the changes of the cutaneous T cell population may also contribute to UVB-induced immunosuppression at skin level in man.
Chapter 3

Introduction

Low-dose UVB radiation can impair cutaneous immunity in humans as illustrated by the reduction in an effective sensitisation and the promotion of tolerance to antigens epicutaneously applied onto UVB-exposed skin (1, 2). The exact mechanisms of this low-dose UVB-induced local immunosuppression have not yet been elucidated (3). Upon irradiation the Langerhans cells disappear from the epidermis in a dose-dependent manner (4) and their capacity as antigen-presenting cells to stimulate T cells is reduced (5). Concurrently, the epidermis is infiltrated by a population of macrophages (6), which possess the ability to stimulate CD4⁺ suppressor-inducer T cells (7). This reversal of the antigen-presenting cell population into the UVB-irradiated site has been proposed as one of the cellular mechanisms of UVB-induced immunosuppression.

In a recent report we demonstrated that in normal human skin the T cell population is also altered by low-doses of UV treatment (8). This preliminary study showed that a single exposure to physiological erythema-inducing doses of solar-simulated UV radiation caused a reduction or even depletion of intraepidermal T cells at day 1 and 2 after irradiation, whereas at day 7 postirradiation remarkably high numbers of CD4⁺, but not CD8⁺ T cells, were found in the epidermis. In the dermis, a selective infiltration of CD4⁺ T cells was observed during the first few days after exposure. Although these findings clearly demonstrate a preferential recruitment of CD4⁺ T cells upon UV exposure, the number of time points studied were limited. Consequently, it was not known at what time-point the influx of CD4⁺ T cells reaches its maximum and when the T cells numbers recede to preirradiation values. Further, it is also not clear whether this UV-induced cutaneotropic CD4⁺ T cell represents a specific subtype of T cell, such as naive or memory, α/β T cell receptor (TCR) or γ/δ TCR, or whether these T cells are activated or not. As concerns the CD8⁺ T cells we may have missed a possible influx because we have looked at limited array of time points.

Therefore, the present investigation was undertaken to determine in more detail the kinetics of the CD4⁺ and CD8⁺ T cells, as well as the phenotype of the T lymphocytes that infiltrate the human skin after a single exposure to solar-simulated UV radiation. We observed that UV radiation caused a selective influx of CD4⁺, α/β TCR⁺, non-activated, memory T cells in the skin that reached a maximum in the dermis at day 2 and in the epidermis at day 14. In addition, we found that the UVB region within the solar simulated radiation source was responsible for this phenomenon.
Materials and Methods

*Subjects.* Ten healthy volunteers (9 male, 1 female) participated in this investigation. All subjects were caucasian with skin type II or III. The age ranged from 18 - 48 years (mean 26.3). None suffered from any skin disease nor from light sensitivity and they were on no chronic or intermittent medication. All participants gave informed consent.

*UV irradiation and biopsies.* A 1000 W xenon-arc lamp (Oriel, Stratford, CT) was used as the UV-source for the induction of skin erythema. In order to reduce heat (infrared radiation) the light beam was passed through a waterfilter, reflected by a dichroic (cold) mirror and filtered by a UG 11 filter (Jenaer Glaswerke Schott & Gen., Mainz, Germany). A 3 mm WG 305 filter (Jenaer Glaswerke Schott & Gen.) was used to adjust the short wave side of the xenon-arc emission for the solar irradiance at sea level. Alternatively, narrow band UVB irradiations were performed with a 303 nm interference filter (Jenaer Glaswerke Schott & Gen.), the WG 305 and UG11 filters were removed.

Several weeks before the experiment, the individual MED was determined. Single doses of 1 or 4 MED were then given to multiple sites on the opposite buttocks at various time periods before the biopsies. Control biopsies were taken from unirradiated skin. Three millimeter punch biopsies were obtained under 2% local anesthesia. All biopsies were oriented in O.C.T. compound (Tissue Teck), immediately frozen in liquid nitrogen and stored at -70°C until use.

*Immunohistochemistry.* Immunohistochemical single-staining was performed by using two different protocols. The first protocol was based on a two-step indirect peroxidase technique as described previously (8). Briefly, the cryostat sections (6 mm) were incubated with primary monoclonal antibody mouse anti-human CD3 (Becton Dickinson, Mountain View, CA), followed by an incubation with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako, Glostrup, Denmark). To detect TCR(s) a second protocol was applied using the streptavidin-biotin-complex (streptABC) method. The sections were incubated with primary monoclonal antibodies mouse anti-human TCR δ1 chain (T Cell Diagnostics, Woburn, MA) or mouse anti-human TCR β chain (T Cell Diagnostics), followed by an incubation with biotinylated goat anti-mouse IgG, then treated with the peroxidase-streptABC solution (Dako). In both protocols before counterstaining with haematoxilin, the horseradish peroxidase activity was detected with H₂O₂ as substrate and AEC (Sigma, St Louis, MO) as chromogen.
Immunohistochemical double-staining was performed using different, previous described protocols based on combination of a polyclonal rabbit and a monoclonal mouse primary antibodies (9). The subsequent steps at room temperature were performed: (1) an incubation of 60 min with a cocktail of rabbit anti-human CD3 (Dako) plus one of each of the mouse antibodies anti-human CD4, CD8 (Becton Dickinson), CD45RA, CD45RO, CD25, HLA-DR (Dako), CD49a (Serotec, Oxford, England); (2) an incubation of 30 min with a cocktail of biotin-conjugated goat anti-mouse (Dako) plus either alkaline phosphatase-conjugated goat anti-rabbit (Dako) (protocol 1) or horseradish peroxidase-conjugated goat anti-rabbit (Dako) (protocol 2) and (3) an incubation of 30 min with either horseradish peroxidase-conjugated streptavidin (Dako) (protocol 1) or alkaline phosphatase-conjugated streptavidin (Dako) (protocol 2). (4) Alkaline phosphatase activity was detected as blue color, using naphthol-AS-MX-phosphate (Sigma) as a substrate and fast blue BB (Sigma) as azo dye. (5) Peroxidase activity was detected as an orange-red color, using the chromogen AEC. Double-stained cells could be appreciated by their purple color.

Lymphocyte enumeration and statistical analysis. Enumeration and subdivision of the skin in different compartments was performed as described before (10). Briefly, the skin was divided into two compartments: epidermis and dermis. The identifications on the object glasses were covered before counting to enable blind quantification. The number of single or double clearly stained cell bodies of three up to six different serial sections per irradiation dose per time point were counted. The values of each biopsy specimen were adjusted to 10 mm horizontal section values by dividing with the horizontal width multiplied by 10. The mean value of each biopsy was then used for the determination of total mean of lymphocyte numbers. The unpaired Student's t-test was used for statistical evaluation taking P< 0.05 (two-tailed) as the level of significance.

Results

Prolonged disturbance of the cutaneous T cell population upon a single exposure to solar-simulator UV radiation. Analysis of single-stained skin sections revealed that both doses of UV radiation provoked a significant reduction of the number of intraepidermal CD3+ cells at day 2 and day 4 (Fig. 1a). This initial reduction in T-cell numbers was followed by a significant increase at day 10 and was maximal after 14 days (4 MED). Although, the T-cell kinetics after 1 MED treatment showed a similar tendency, the increase of T cell numbers at
day 10 and 14 was not significant as compared to pre-irradiation values, but was significant when compared to day 2 and 4 values. The number of T cells returned to pre-irradiation values at day 21, showing that a single exposure to a physiological erythema-inducing dose of simulated-solar radiation can cause a long term effect (about 3 weeks) on intraepidermal T lymphocytes. A different response of the CD3⁺ cell population was found in the dermis (Fig. 1b).

Figure 1. Solar-simulated ultraviolet radiation induces prolonged disturbance of the cutaneous T cell population. Kinetics of the CD3⁺ (Fig. 1A and 1B), CD3⁺CD4⁺ (Fig. 1C and 1D) and CD3⁺CD8⁺ (Fig. 1E and 1F) T cell populations in the epidermis and dermis following a single exposure to 1 (open bar) or 4 (black bar) MED of solar-simulated UV radiation. Exposures to UV were performed at various time periods (-28,-21,-14,-10,-4,-2 days) before collecting biopsies. Unirradiated skin served as control (gray bar). Each bar represents the mean value of 9 determinations (i.e. triple countings in 3 volunteers, n=3). Data are expressed as a number of T cells per 10 mm horizontal section + SD (* indicates p<0.05).
The number of dermal T cells (predominantly perivascular) was considerably increased 2 days after irradiation (both applied doses). The number was still significantly higher at day 4 and returned to pre-irradiation values at day 10 (4 MED). The time-course of the CD3\(^+\) cells in the epidermis and dermis showed a dose-dependent tendency. Statistic analysis revealed that this tendency was only significant for the dermal T-cell numbers at day 2 and 4 but not for the epidermal T cell numbers.

**CD3\(^+\) cells migrating into the (epi)dermis upon solar-simulated ultraviolet irradiation coexpressed CD4 but not CD8.** Examination of the double-stained sections revealed that the (epi)dermal CD3\(^+\)CD4\(^+\) cells (Fig. 1c and 1d) showed similar kinetics as the single-stained CD3\(^+\) cells in response to solar-simulated radiation. The number of intraepidermal CD3\(^+\)CD4\(^+\) cells was decreased at day 2, reached the minimum at day 4 for both the applied doses, subsequently followed by an increase which was maximal after 14 days and receded to pre-irradiation values at day 21 (Fig. 1c). The dermal CD3\(^+\)CD4\(^+\) cells were significantly increased at day 2 and 4 (both applied doses), and reached the preirradiation values at day 10 (Fig. 1d). By contrast, the intraepidermal CD3\(^+\)CD8\(^+\) cells only returned to preirradiation numbers at day 14 after initial depletion (Fig. 1e). The dermal CD3\(^+\)CD8\(^+\) cells were not significantly altered in number at all (Fig. 1f). The double-staining data showed a dose-dependent tendency, but this was however not significant.

**UVB wavelengths within the solar-simulated UV radiation are responsible for the changes of the cutaneous T cells.** The solar-simulated radiation from our xenon-arc lamp setup mainly consisted of UVA, UVB and visible light. We assumed that the UVB wavelengths within our solar-simulated UV radiation spectrum were responsible for the changes of the cutaneous T cells after exposure. Like the solar-simulated radiation, UVB radiation significantly reduced the intraepidermal T cells at day 2 (Fig. 2a), and the numbers of dermal CD3\(^+\) and CD3\(^+\)CD4\(^+\), but not CD3\(^+\)CD8\(^+\) cells were significantly increased at day 2 in the dermis (Fig. 2b). The numbers of intraepidermal single-stained CD3\(^+\) and double-positive CD3\(^+\)CD4\(^+\) cells at day 14 were significantly higher as compared to non-irradiated control values (Fig. 2a), and the number of intraepidermal double-stained CD3\(^+\)CD8\(^+\) cells only receded to pre-irradiation values. These results indicate that indeed the UVB wavelengths within the solar-simulated radiation caused the observed modulation of the cutaneous T cell population.
UVB-induced selective influx of memory CD4⁺ T cells

**Figure 2. Differential effect of UVB on cutaneous CD4⁺ and CD8⁺ T cell subpopulations.** Quantitative analysis of T cell subpopulations in the epidermis (Fig. 2A) and dermis (Fig. 2B) after single exposure of 4 MED of UVB. Exposures to UVB were performed at 2 time points (-14,-2 days) before collecting biopsies. Unexposed skin served as control. Each bar represents the mean value of 12 determinations (i.e. 6 countings in 2 volunteers, n=2) of the number of CD3⁺ (gray bar), CD3⁺CD4⁺ (black bar), CD3⁺CD8⁺ (open bar) T cells. Data are expressed as a number of T cells per 10 mm horizontal section + SD (* indicates p<0.05).

_T cells infiltrating the UV irradiated skin are predominantly helper/inducer, TCR αβ⁺, resting memory T cells._ At day 2 the number of double-stained epidermal T cells was reduced for all markers (Fig. 3A, 3C and 3E), which is in line with above findings. The dermis showed a predominant influx of memory T cells (CD3⁺CD45RO⁺) and a small but significant increase in naive T cells (CD3⁺CD45RA⁺) (Fig. 3B). The majority of the infiltrating T cells expressed α/β TCR (Fig. 3F) and did not express markers of recent activation, like HLA-DR and IL-2R (CD25), nor of late activation, such as VLA-1(CD49a) (Fig. 3D). The UV-induced intraepidermal T cell at day 14 expressed a similar phenotype; i.e. they were predominantly non-activated, TCR α/β⁺, memory T cells (Fig. 3C, 3E and 3A respectively).
Figure 3. Solar-simulated ultraviolet radiation preferentially induces influx of memory, non-activated, TCR αβ+ T cells in normal human skin. Quantitative analysis of T cell subpopulations in the epidermis (Fig. 3A, 3C and 3E) and dermis (Fig. 3B, 3D, 3F) after single exposure of 4 MED of solar-simulated UV radiation. Exposures were performed at 2 time points (-14, -2 days) before collecting biopsies. Unexposed skin served as control. Each bar represents the mean value of 24 determinations (i.e. triple countings in 8 volunteers, n=8). Data are expressed as a number of T cells per 10 mm horizontal section ± SD (* indicates p<0.05).
Discussion

In this study we demonstrated that a single exposure to UV radiation leads to a long-term change in the number and composition of the local T cell population of normal human skin. We found that the numbers of intraepidermal T cells, which can be detected in non-lesional, clinically normal human skin (10,11), were significantly reduced in the first few days after UV irradiation. A similar result was found in mice: dendritic epidermal T cells, which are resident epithelial TCR γδ-expressing cells found in normal mouse skin, have been depleted from the epidermis by UVB irradiation (12,13). Because human epidermal T lymphocytes have been postulated to provide immunosurveillance against the development of cutaneous neoplasms and persistent infections with intracellular pathogens (14), the disappearance of epidermal T lymphocytes upon single exposure to UV radiation may be considered as a permissive factor for UVB-induced skin tumor and skin infection development.

The dermis showed a perivascular CD4+ T cell-dominated infiltrate during the first few days after UV irradiation, likely due to influx of T lymphocytes from the blood stream. This is in line with the finding that sun-exposed subjects had a significant decrease in circulating CD4+ cells in the days after exposure to sunlight (15). Apparently, UV-exposure induces an increased homing of CD4+ lymphocytes into the skin, possibly by altering the expression of adhesion molecules. Indeed, E-selectin, thought to be a mediator of lymphocyte trafficking into inflamed skin is induced on endothelial cells at sites of UVB exposed skin (16,17). We found that the initial depletion of intraepidermal T cells is followed by an infiltration of CD4+ T cells into the epidermis. Because these T cells expressed the same phenotype of those infiltrating the dermis we reasoned that this might be due to the fact that T cells travel from the perivascular infiltrates, through the dermis and the basal membrane, to the epidermis, which is in line with the finding that the immigration of T cells into the epidermis started later than the influx of T cells into the dermis.

The function of CD4+ T lymphocytes infiltrating the UVB-irradiated sites is unclear. One might speculate that they are involved in the recognition of neo-antigens induced by UV-exposure. T cells entering the dermis from the microvasculature in UV-exposed skin in vivo, may reasonably expected to encounter infiltrated UV-induced macrophages or UV-exposed Langerhans cells. Taking in mind that the "dermal perivascular unit" is a site of immunological reactivity (18) antigen presentation to T cells could occur directly in UV-
exposed skin without the necessity for T-cell recruitment from a regional lymph node. Presentation of antigens to memory or naive T cells under this circumstances could result in activation of suppressor pathways. The lack of expression of IL-2R on T cells could be in line with the finding that the UV-induced macrophages stimulate CD4+ T cells in a novel form of T cell activation characterized by a deficient IL-2Rα expression (19). Studies conducted in humans after hapten application to UVB-exposed skin have revealed that UVB-irradiated skin acquires sustained immunosuppressive properties (20) and fails to develop the primary allergic reaction (21). The time course of increase in CD4+ epidermal T cell, rising between day 7 and 21, may play a role in the inability of human UV-exposed skin to express immunity. This hypothesis is in line with the fact that T cells of the CD4+ subtype have been proposed to mediate the local low-dose UVB-induced immunosuppression (3). At this moment, immunophenotyping cannot explain the exact mechanism(s) by which these T cells cause immunosuppression.

In conclusion, our results indicate that UVB radiation has long term effects on the local T cell population within human skin by depleting the majority of epidermal T cells and promoting a selective influx of non-activated, memory, CD4+ T cells. We believe that these T cells play a important role in the UVB-induced local immunomodulation. Further studies are required to determine their cytokine profiles and their functional properties in order to gain a better understanding of the role of T cells in local low-dose UVB-induced immunomodulation.

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References


