Effects of therapies on cytokine patterns in psoriasis

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Neutrophils infiltrating UVB-irradiated normal skin display high IL-10 expression

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Abstract
Exposure to an erythemal dose of ultraviolet B (UVB) is known to induce interleukin (IL)-10 expression in human skin. It is generally believed that this IL-10 is predominantly expressed by CD11b+HLA-DR+ macrophages that infiltrate the UVB-exposed skin. This cytokine is supposed to contribute to the immunosuppressive effects of UVB by blocking cell-mediated immune responses. We recently demonstrated that neutrophils, which also invade UVB-irradiated skin, express CD11b and HLA-DR as well. In addition, we showed that the presence of these neutrophils affects T cell responses in primary T cell cultures derived from UVB-exposed skin. Because neutrophils invade UVB-exposed skin and express CD11b and HLA-DR, like macrophages, we questioned whether neutrophils represent another source of IL-10. Skin biopsies were obtained from 4 healthy volunteers before and 2 days after exposure to 4 minimal erythema doses of UVB. Cryostat sections from these biopsies were double-stained with IL-10 and markers CD11b, HLA-DR, CD36 or neutrophil elastase. As expected IL-10 could be detected in CD11b+HLA-DR+CD36+ macrophages in the epidermis and dermis of UVB-exposed skin. Surprisingly, the majority of the abundant IL-10 expression was found in CD11b+HLA-DR+elastase+ neutrophils. Cytospin preparations from dermal cell suspensions confirmed the IL-10 expression by neutrophils displaying their characteristic multilobular nuclei. Neutrophils in UVB-exposed skin express IL-10 and should be recognized as active co-players in the creation of a UVB-induced immunosuppressive microenvironment.
Introduction

Although ultraviolet B (UVB) can be harmful as inducer of skin tumours, advantage can be taken of this radiation under controlled conditions as effective treatment of several immune-mediated skin diseases. Many different research groups have studied the changes in cytokine expression in human skin after UVB exposure to explain these features. A delicate balance of type 1 and type 2 immune responses provided by expression of type 1 [e.g. interferon (IFN) -\(\gamma\)] and type 2 [e.g. interleukin (IL)-4] cytokines, respectively, exist in the steady state. This balance was found to be disturbed in the UVB-irradiated skin in favour of type 2 cytokine expression, which could generate a defect in the defence against intracellular microorganisms and tumour cells. It is known that shortly after UVB exposure neutrophils, macrophages and T cells enter the skin, while Langerhans cells leave the skin. All these trafficking cells are able to produce different cytokines. A cytokine of major interest is IL-10, because it is induced in normal human skin upon UVB irradiation and is known as a cytokine with immunosuppressive effects by decreasing antigen presentation and Th1 cell proliferation. The major IL-10 producing cells were shown to be CD11b\(^+\) macrophages that infiltrate the skin after UVB exposure.

In a recent study, we found that a very large number of the CD11b\(^+\) cells infiltrating the skin early after UVB exposure are neutrophils. This result prompted us to determine whether these CD11b\(^+\) neutrophils, in addition to the CD11b\(^+\) macrophages, could contribute to the UVB-induced IL-10 expression in human skin.

Materials and methods

Four healthy volunteers (3 females and 1 male) received a single local exposure of 4 minimal erythema doses of broad band-UVB from a 1000 W xenon-arc lamp (Oriel, Stratford, CT) equipped with a 303-nm interference filter. Two days post-irradiation, skin biopsies were taken and either snap frozen for immunohistochemical staining or processed to get dermal cell suspensions as follows. Upon overnight 0.3 % dispase (Boehringer Mannheim, Mannheim, Germany)-treatment, the epidermis was removed and the dermis was incubated with collagenase (Boehringer Mannheim) and DNase (Boehringer Mannheim) in phosphate-buffered saline (PBS) for 2 h at 37 °C. Cytospin preparations were prepared from single dermal cells suspended in PBS with 2 % fetal calf serum. Antibodies used were anti-IL-10 (R&D Systems, Minneapolis, MN), FITC-conjugated anti-CD36 (Beckman Coulter, Fullerton, CA), peroxidase-conjugated anti-elastase (Dako, Glostrup, Denmark), FITC-conjugated anti-HLA-DR (Becton Dickinson, San Jose, CA) and FITC-conjugated anti-CD11b
(Beckman Coulter). Immunohistochemical staining was performed as described before ⁹. Shortly, acetone-fixed 4 mm sections or cytospin preparations were sequentially incubated with 10 % normal goat serum (Dako), anti-IL-10, goat anti-mouse (Dako) and APAAP complex (Dako) for 1 h at room temperature. For double staining, the preparations were further incubated sequentially with 10 % normal mouse serum, FITC-conjugated primary antibody, rabbit anti-FITC (Dako) and peroxidase-conjugated goat anti-rabbit (Dako). Colour development was achieved with naphtol-AS-MX-phosphate (Sigma-Aldrich, St. Louis, MO) for blue, 3-amino-9-ethyl carbazole (Sigma-Aldrich) or fast red substrate system (Dako) for red. Nuclear staining was performed with haematoxylin. Cell numbers were counted horizontally in the whole length of each section, including epidermis and 1 mm of upper dermis and were corrected to numbers per mm section-length.

**Results**

IL-10 was abundantly expressed in UVB-exposed human skin, but not in irradiated skin, both in the dermal cellular infiltrate and in the epidermis (Figure 1). Double staining revealed that 95 ± 1 % of the epidermal and dermal IL-10 positive cells co-expressed CD11b (Figure 2A). Using CD36 as marker for activated macrophages, we found that 33 ± 12 % of the IL-10 expressing cells were CD36 positive macrophages especially in the upper dermis (Figure 2B). However, because CD11b is also expressed by neutrophils, we performed a double staining with IL-10 and neutrophil elastase and observed that 73 ± 11 % of the IL-10 expressing cells showed reactivity with elastase antibody. These cells were abundant in epidermis and at the vicinity of the dermoepidermal junction (Figure 2C). Earlier studies on UVB-irradiated skin revealed that IL-10 expressing cells were positive for HLA-DR showing that these cells were able to present antigen ¹⁰. Supporting this finding, we observed that approximately 56 ± 4 % of the IL-10 expressing cells in the irradiated skin were HLA-DR positive as well (Figure 2D). Sixty one ± 9 % of these HLA-DR positive cells was double stained with CD36 showing that most of these cells were macrophages (Figure 2E). Interestingly, double staining with HLA-DR and elastase showed that 39 ± 6 % of the HLA-DR⁺ cells were neutrophils (Figure 2F). To be sure that CD36 and elastase were not expressed by the very same cell type, we performed a double staining with CD36 and elastase and proved that these markers were mutually exclusive (Figure 2G). In cytospin preparations of dermal cells derived from UVB-irradiated skin, we noticed clear IL-10 positive cells with typical multilobular nuclei that are characteristic for neutrophils (Figure 2H).
Unirradiated

Irradiated

Figure 1. Expression of IL-10 (fast blue staining) in unirradiated normal skin (left) and 1 day after 4 MED UVB irradiation (right), original magnification x 100. No IL-10 positive cells were detected in normal skin. After UVB exposure, a scattered pattern of IL-10 expression was present in the dermis and epidermis (page 191).

Figure 2. a-g. Double staining of UVB-exposed skin. a-d, Expression of IL-10 (blue) was detected in cells (arrows) displaying the markers (red) a, CD11b; b, CD36; c, neutrophil elastase; d, HLA-DR. HLA-DR (blue) positive cells co-expressed e, CD36 and f, neutrophil elastase in red (arrows). g, Expression of CD36 (blue) or neutrophil elastase (red) was mutually exclusive. h, Neutrophils (arrow heads) in dermal cell suspensions from UVB-irradiated skin expressed IL-10 (red). Nuclear staining was performed with haematoxylin. Original magnifications were a-f x 400, g x 200, and h, x 1000 (page 192).

Discussion

The suppression of cell-mediated immunity caused by UVB exposure is found to be, at least partially, related to an increase in the IL-10 expression. IL-10 downregulates IFN-γ expression and antigen presentation, thereby having a great negative impact on the conduction of cell-mediated immune surveillance as well as on the delayed type hypersensitivity. This prominent role of IL-10 is clearly demonstrated in UVB-irradiated
mice, in which IL-10-blocking resulted in the abolishment of UVB-induced suppression of delayed type hypersensitivity. As concerns IL-10 expression in UVB-irradiated skin, human keratinocytes were reported to express this cytokine after UVB exposure. However, our finding that keratinocytes in situ did not show IL-10 staining contradicts this and confirms our earlier observation that IL-10 mRNA and protein is not expressed in human keratinocytes before and after UVB exposure. CD11b+HLA-DR+ macrophages invading the skin after UVB exposure were described as the most potent source of IL-10. In the current study we clearly showed that neutrophils, which have similar features as these macrophages (i.e. UVB-induced infiltration and expression of both CD11b and HLA-DR), appeared to be the major cell type with abundant IL-10 expression in irradiated skin. Neutrophils migrating into the UVB-exposed normal and psoriatic skin can also express IL-4. In addition, in vitro studies indicate that neutrophils in dermal cell suspensions derived from UVB-exposed skin are able to favour type 2 T cell responses and concomitantly inhibit type 1 T cell responses. All together, these results suggest that neutrophils can contribute to the immunosuppressive changes in UVB-exposed skin by expression of IL-4 and IL-10. Immunosuppression after UVB exposure probably requires a cooperative action of resident and invading cells, which create a microenvironment where exaggerated immune responses causing excessive tissue damage are avoided.
References


