Effects of therapies on cytokine patterns in psoriasis
Piskin, G.

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Chapter 5

IL-4 expression by neutrophils in psoriasis lesional skin upon high dose UVB exposure

Piskin G, Tursen U, Bos JD, Teunissen MBM

Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Abstract
Upon a single high dose of UVB irradiation of psoriatic lesional skin, IFN-γ expression is decreased, whereas, IL-4 expression is enhanced. A similar type 1 to type 2 shift was found in dermal T cells derived from irradiated lesional skin as compared to unexposed lesional psoriatic skin. We found recently that the IL-4 protein detected in situ upon UVB exposure of normal skin was not associated with T cells, but with infiltrating neutrophils. To determine which cell types express IL-4 in psoriatic skin after UVB irradiation. Skin biopsies were obtained from healthy controls and psoriasis patients before and after local UVB exposure. Double immunohistochemical stainings were performed to determine the identity of IL-4 expressing cells. In the irradiated skin of both healthy controls and patients, IL-4 positive cells co-expressed elastase and CD15, but not CD3. IL-4 expressing cells found in psoriatic skin after a single high dose UVB exposure appeared to be neutrophils.
Introduction

Ultraviolet B (UVB)-radiation is a widely used therapy for psoriasis for more than three decades. It has been shown that UVB-induced apoptosis of T cells could be an important mechanism in the healing of psoriatic skin lesions after this therapy\(^1\). However, a considerable number of T cells in the dermis still survive after UVB exposure\(^1,2\). Because T cells are involved in the pathogenesis of psoriasis\(^3\), it is important to determine the properties of these remaining T cells.

In an earlier study, we demonstrated that an influx of CD4+ memory T cells into normal human skin takes place after a single high dose UVB exposure\(^4\). The T cell population present in UVB-irradiated skin expresses neither activation markers HLA-DR and CD25, nor cytokines IFN-γ and IL-4. In order to elucidate their type 1/type 2 nature, these recruited T cells were stimulated in vitro and found to exhibit reduced expression of IFN- and increased expression of interleukin-4 (IL-4), as compared to dermal T cells from unirradiated skin\(^5\). This switch from type 1 to type 2 cytokine expression may, at least partially, contribute to the UVB-induced immunosuppression\(^5,7\). Neutrophils also invade the skin after UVB exposure, but at a much earlier time point than T cells\(^2,7,8\). We showed that these infiltrating neutrophils express IL-4. When these neutrophils were depleted from primary dermal cell suspensions derived from UVB exposed skin, the type 1 to type 2 shift in T cells was abolished\(^7\). These results suggest that the neutrophils are functionally involved in the type 2 cytokine skewing in UVB-exposed skin.

In another study\(^2\), we observed that a single high dose of UVB exposure of psoriatic plaques results in an increased expression of IL-4 in situ. In the same study, we also found that the dermal T cells isolated from the UVB-exposed lesional skin expressed increased IL-4 and decreased IFN-γ upon UVB exposure as compared to unexposed skin showing that the type 1/ type 2 skewing upon UVB radiation holds true in psoriatic skin as well\(^2\). In the present study, we wanted to investigate which cell type in psoriasis lesion does express IL-4 upon a single high dose UVB exposure as in normal skin: The two probable candidates would be either the T cell, which was found to exhibit upregulated IL-4 expression in vitro, or the neutrophil, which was demonstrated to express IL-4 while infiltrating UVB irradiated normal human skin. With this aim we performed immunohistochemical double stainings.
Methods

Three untreated psoriasis patients with chronic plaque type psoriasis (1 female, 2 male; 47 – 52 years old, mean 49) and 3 healthy controls (2 female, 1 male; 20 - 23 years old, mean 22) were included in this study. Study protocol was approved by the local ethical committee. Local UVB irradiation was applied with a 1000 W xenon arc lamp (Oriel, Stratford, CT) equipped with a 303 nm interference filter (Jenaer Glaswerke, Schott & General, Germany). After determination of the individual minimal erythema dose (MED), 4 MED of UVB was introduced locally to the gluteal area of healthy controls and chronic plaque lesions on the same area of psoriasis patients. At 48 h following irradiation, 4 mm punch biopsies were taken from unirradiated and irradiated areas and the biopsies were immediately frozen in liquid nitrogen. The details of the immunohistochemical double staining procedure was reported elsewhere 7. Primary antibodies used for the stainings of 5 μm sections in the present study were anti-IL-4 (Immunex, Seattle, WA), FITC-labelled anti-CD3 to stain T cells (BD Biosciences, Mountain View, CA), FITC-labelled CD15 to detect polymorphonuclear cells (DAKO, Glostrup, Denmark) and peroxidase-labelled anti-elastase to identify neutrophils (DAKO).

Results

The recent availability of enzyme labeled anti-elastase antibody made it possible to obtain direct proof that the strong IL-4 expression found in normal human skin upon irradiation was exhibited by elastase+ neutrophils (Figure 1A, 1B). This result confirms our earlier circumstantial evidence that IL-4 expression was associated with elastase+ cells, using single staining of serial sections7. In contrast to unirradiated normal human skin, in which IL-4 expression is absent, the expression of IL-4 was occasionally detected in some biopsies

Figure 1. Double staining of normal human skin with IL-4 (blue) and elastase (red), a, before UVB irradiation b, 2 days after UVB irradiation (page 191).
from the untreated psoriatic skin (Figure 2A). Similar to irradiated normal skin, but less pronounced, expression of IL-4 was induced or increased in psoriatic lesions upon UVB exposure (Figure 2B). Double staining with CD3 revealed around 1% double positive cells in the sections from irradiated skin indicating that the IL-4 expression was not confined to T cells (Figure 2C). Almost all of the IL-4+ cells in unirradiated and irradiated psoriatic skin co-expressed CD15 and elastase (Fig 2A, 2B, 2D). This results confirmed that the UVB-induced expression of IL-4 was confined to neutrophils.

**Discussion**

By means of double staining we demonstrated in this study that the IL-4+ cells which infiltrate the lesional psoriatic skin upon a single high dose of UVB exposure are neutrophils. This suggests that the induction of IL-4 expression in psoriatic skin after UVB irradiation occurs in a similar way as was earlier found in normal skin. It is known that the UVB irradiated skin is invaded by different inflammatory cells, e.g. neutrophils and macrophages. The neutrophil-derived IL-4 together with macrophage-derived IL-10 may alter the microenvironment in the irradiated skin in such a way that the development of type 2 T cell responses will be favoured.

Our results showed that the T cells in psoriatic skin did not show IL-4 expression in situ upon UVB exposure. This result is seemingly in contradiction with the findings of our earlier studies which revealed increased IL-4 and decreased IFN-γ expression upon in vitro stimulation of dermal T cells derived from irradiated psoriatic skin. This could be explained in two ways: Firstly, we have shown in our earlier study that the T cells which were...
recruited into the normal skin upon UVB exposure were not activated. The absence of IL-4 expression on T cells in psoriatic skin after UVB exposure may be due to lack of activation of these T cells. Secondly, it should be kept in mind that T cells do not have the ability to store cytokines once they produce them, thus, the absence of IL-4 in these cells does not necessarily mean that they do not express this cytokine.

Psoriasis vulgaris is widely accepted as a disease with a preferential expression of type 1 cytokines (e.g. IFN-γ) in lesions and peripheral blood. Therapeutic agents which cause the suppression or reversal of type 1 cytokine predominance were reported to be effective in treatment of psoriasis. Related to this, it is an interesting finding that a single high dose of UVB exposure results in IL-4 expression in psoriatic skin. In addition to T cell apoptosis in psoriatic skin, type 2 cytokine switch in the surviving T cells could be another mechanism in the therapeutic effectiveness of UVB in psoriasis. However, one should keep in mind that our experimental set-up does not allow a direct comparison commonly applied UVB therapy. In the treatment of psoriasis, UVB is used repetitively in low doses while in our study we administered locally a single high dose of UVB to the lesions. We currently perform similar experiments in biopsies from psoriasis lesions before and after therapeutic use of UVB to investigate whether type 1/ type 2 cytokine switch occur after UVB therapy of psoriasis.
References


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