Effects of therapies on cytokine patterns in psoriasis

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Chapter 10

Summary and Discussion
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The aim of this thesis was to investigate the possible role of cytokines in the improvement of psoriatic lesional skin mediated by immunosuppressive therapies. The majority of our studies were dedicated to the effects of UVB radiation. In one study, skin biopsies from patients treated with cyclosporine A and methotrexate were included to see the effects of these systemic therapies on the psoriatic skin. As concerns the UVB irradiation, two different radiation methods were used in these studies. Firstly, the skin of psoriatic patients and normal subjects were locally irradiated with a single high (erythema-inducing) dose of UVB to investigate effects of an acute UVB exposure on the skin. Biopsies from the irradiated and unirradiated skin areas were used to detect cell numbers and distribution, and cytokine expression by in situ immunohistochemical stainings and PCR. These biopsies were also used to generate dermal T cell cultures in order to determine intracellular cytokine expression in these cells upon stimulation. Secondly, skin of psoriasis patients was repeatedly treated with low (suberythemal) dose NB-UVB (conventional therapy) in order to monitor effects on the cytokine expression in the psoriatic skin by above mentioned techniques and/or ELISA. In situ effects of therapy with cyclosporine A and methotrexate on T cell numbers and IFN-γ and IL-4 expression were investigated by using immunostaining methods as well. Because the cytokine milieu in the skin can modulate immune response mounted by the local immunocompetent cells, we speculated that changes of cytokine expression in the skin could be one of the therapeutical mechanisms of these therapies.

Effects of a local exposure to a single high dose of BB-UVB radiation on the cytokine expression in normal and psoriatic skin: neutrophils in the game Few years ago, it was demonstrated by di Nuzzo et al that a single high dose of BB-UVB exposure of normal skin results in enhanced development of type 2 T cells in primary dermal cell cultures as determined by intracellular cytokine staining 1. In the studies which are described in chapter 2 of this thesis, we wanted to investigate whether a similar skewing would occur in psoriatic lesional skin after a single high dose of BB-UVB irradiation despite the presence of an ongoing inflammatory process. Indeed, as observed by different techniques, the high expression of type 1 cytokine IFN-γ decreased and the expression of type 2 cytokine IL-4 increased in psoriatic lesional skin after the irradiation. Two days after the exposure, we observed an increased number of T cells in the dermis of the irradiated lesional skin. This change could be explained by the influx of T cells into the dermal skin area after the BB-UVB exposure, as was earlier described to occur in normal skin 2. These T cells could represent a
new population with distinct properties (e.g. cytokine expression) as compared to lesional T cells in psoriatic skin.

Interestingly, the irradiated spot, but not the surrounding area of the same plaque markedly healed after this single exposure. A single high dose of BB-UVB causes an inflammatory response itself and thereby a rapid change in the micromilieu (cells and cytokines), which enabled apparently a relatively fast clinical response as compared to the conventional therapy with repetitive (three times per week for 6 to 10 weeks) low doses of NB-UVB radiation. However, the use of high doses of UVB irradiation is ethically unacceptable as therapy for psoriasis patients because of the acute (i.e. erythema and formation of bullae) and chronic side effects (i.e. possible skin cancer induction). Especially the lower wavelengths in the BB-UVB spectrum are responsible for these adverse effects. Because NB-UVB radiation lacks these lower wavelengths, though is still effective, it would be worth to investigate the use of a local single high dose of NB-UVB radiation to treat psoriasis. This idea is supported by the recent application of excimer laser-derived 308 nm UVB therapy of psoriasis vulgaris lesions which is applied in higher doses with fewer exposures. The reported advantages of this therapy modality are less cumulative doses as compared to the conventional phototherapy with UVB radiation and the local application which spares the nonlesional skin from unnecessary UVB exposure. It was reported that excimer laser-derived 308 nm UVB therapy causes significant depletion of T cells from epidermis and dermis showing that the effect of high doses of this small spectrum radiation in the dermis can be more pronounced than the conventional UVB therapy. The quick clinical response of psoriatic lesions we observed after a single high dose of BB-UVB irradiation could be a result of similar mechanisms that are operational in the excimer laser treatment. To our knowledge, there are no reports in the literature concerning the effects of local high dose UVB therapy on the clinical and immunological features of psoriatic lesions. It would be interesting to investigate whether excimer laser-derived 308 nm UVB therapy induces comparable changes in the cytokine expression in psoriatic lesional skin as we have seen after the high dose BB-UVB exposure.

Dermal T cells from UVB-exposed skin, but not from unirradiated skin, show a type 2 cytokine skewing when they are cultured in the presence of the dermal microenvironment. However, immunohistochemical double staining revealed that only 2% of T cells in situ were positive for IL-4 two days after the UVB exposure. It appeared that the majority of the UVB-induced IL-4+ cells did not display the common T cell marker CD3. This finding prompted us to search for the identity of these IL-4+ cells and possible local factors which could be
responsible for this skewing in the UVB-exposed skin. In the studies described in chapter 4, we found out that the IL-4 expressing cells coexpressed CD15 and CD11b, but not CD3, tryptase, CD56 and CD36, indicating that these cells were neutrophils. When the CD15+ cells were depleted from the dermal cell suspension just before the start of the primary dermal cell culture, dermal T cells did not show type 2 cytokine skewing. For one decade it is known that the CD11b+HLA-DR+ so-called “UV-macrophages”, which invade the skin after UVB exposure, can produce huge amounts of IL-10 and are regarded as the major cells changing the cytokine milieu in the irradiated skin. Our results showed that neutrophils may also have a high impact on the local changes contributing to the immunosuppression in the UVB-radiated skin. To investigate whether these IL-4 expressing neutrophils could also be found in the UVB-exposed psoriatic skin, we performed immunohistochemical stainings on the psoriatic lesional skin and showed that also in this case CD15+CD11b+ cells were responsible for IL-4 expression (chapter 5). As neutrophils and macrophages both infiltrate high dose BB-UVB-exposed skin and both express CD11b, we wondered whether neutrophils could contribute to the UVB-induced IL-10 expression, originally ascribed solely to the UV-macrophages. In chapter 6, we clearly demonstrated that neutrophils in the UVB-irradiated skin also express IL-10. Remarkably, they could express HLA-DR as well indicating that they form part of the HLA-DR+CD11b+ cell population in the UVB-irradiated skin which is generally believed to represent the UV-macrophages. Thus, the publications concerning the “HLA-DR+CD11b+ UV-macrophages” should be cautiously interpreted under the light of our present results. From our studies in chapter 4, 5 and 6 it can be speculated that the quick clinical response that we observed in the local lesional areas treated with a single high dose of UVB could be caused, amongst others, by the IL-4 and IL-10 expression in infiltrating neutrophils. In line with the results from our studies described in chapters 4, 5 and 6 it was very recently reported by other investigators that neutrophils could express IL-4 and IL-10 after exposure to a comparable high dose of UVB radiation. It was also demonstrated that the skin of patients with polymorphous light eruption displays a decreased expression of these cytokines after the UVB exposure. As skin lesions in these patients are formed after the exposure to the UVB radiation, it was speculated by these investigators that the formation of these lesions can be a result of disturbed type 2 cytokine skewing. These findings support our belief that the type 2 cytokine skewing is an important mechanism of the immune modulation after acute high dose UVB exposure (Figure 1).
Effects of NB-UVB therapy on cytokine expression in psoriatic skin The UVB therapy of psoriasis consists of a whole body application of repetitive low doses of NB-UVB radiation. It is tempting to assume that this sort of irradiation of the skin has different immunologic consequences than a single high dose UVB exposure. To investigate whether the type 1/type 2 T cell dichotomy in the psoriatic lesional skin is affected by NB-UVB therapy, we included patients treated with NB-UVB radiation in our study described in chapter 3. In this study, we demonstrated by intracellular cytokine staining that dermal T cells remaining in the lesional skin after the completion of the NB-UVB therapy have a diminished capacity to express IFN-γ, while the expression of IL-4 increases in some individuals. Similarly, in the supernatants of these T cells, we found a decreased expression of IFN-γ with an increased expression of IL-4, IL-10 and TGF-β. The increase in the expression of IL-4 was not as striking as in the acute high-dose UVB-irradiated lesional skin. It is known that epidermal T cells are depleted in lesional psoriatic skin after the therapy with NB-UVB which could explain the clinical response of psoriatic lesions to this therapy. However, many T cells in the dermis survive the NB-UVB treatment. Because of the importance of high IFN-γ expression by lesional T cells in the pathogenesis of psoriasis, it is tempting to assume that reduction of IFN-γ expression in the dermal T cells could be an important factor in the clinical improvement after NB-UVB therapy. Other systemic therapies such as cyclosporine A and methotrexate also caused a reduction in the number of cutaneous T cells, but a consistent change in the IFN-γ and IL-4 expression was however not observed (chapter 9). Although there are no prospective studies comparing the clearance and the duration of the remission induced by UVB therapy and other systemic therapies, it is
generally believed that the UVB therapy results in a higher rate of disease clearance and longer remission period of the disease than other classical systemic therapies. It might be hypothesized that the decreased IFN-γ expression by the dermal T cells remaining in UVB-treated lesions could explain the differences in the clinical response to the UVB therapy and above mentioned systemic therapies.

Because we found that clinical improvement of psoriatic lesions after NB-UVB therapy was associated with downregulation of IFN-γ we investigated factors which can operate in this downregulation. There are several cytokines that promote the development and maintenance of a type 1 phenotype of T cells as explained in the Introduction of this thesis. It was already known that keratinocytes can express the p35 subunit of IL-12 and the common p40 subunit of IL-12 and IL-23. In chapter 7, we proved at mRNA and protein level that keratinocytes are able to express the p19 subunit of IL-23 as well. The IL-23 heterodimer was found in the cell lysates and supernatants of keratinocytes by immune blot analysis and ELISA. We also could detect IL-23 in Langerhans cells and dermal dendritic cells. Recently, the expression of IL-23 was described in dermal dendritic cells in psoriatic lesional skin at mRNA level ¹⁰, confirming our observation. After having defined the expression of IL-23 in human skin, we determined in chapter 8 the presence of this cytokine and other IFN-γ-inducing cytokines in psoriatic lesional skin by immunohistochemical staining and followed their fate after NB-UVB. IL-12, IL-18 and IL-23 which were highly expressed in the lesional skin, showed a significant decrease after therapy with NB-UVB, nicely along with a reduction of IFN-γ expression. The expression of IL-15 showed a less dramatic change with a greater interindividual variability. Unfortunately, we were not able to study the expression of cytokines IL-21 and IL-27 (both contributing to the formation of type 1 phenotype of T cells ¹¹,¹²), because of the unavailability of antibodies against these cytokines. Although our study showed a clear association between reduced expression of IFN-γ-inducing cytokines and a decrease of IFN-γ in the psoriatic lesional skin after NB-UVB therapy, it is still not possible to pinpoint how this reduction of IFN-γ-inducing cytokines takes place. There may be a couple of possibilities: i, cells are directly affected by UVB radiation and express less cytokines; ii, a diminished number of inflammatory cells after the UVB radiation automatically results in a decreased expression of these cytokines, iii, UVB-induced changes of the microenvironment in the irradiated skin eventually affect the cells producing IFN-γ-inducing cytokines (indirect UVB effects). Of course, all these possibilities could be operational at the same time. Because the expression of IL-12 / IL-23 p40, IL-23 p19 and IL-18 in keratinocytes in situ shows a diffuse pattern, the decrease of
the cytokine expression by keratinocytes may be related to the reduction of the epidermal thickness after successful treatment of psoriasis. Although this relationship exists, we also observed a clear reduction of the staining intensity, indicating that UVB exposure caused a reduction of the expression of these cytokines in the epidermis that is independent of the number of cells (Figure 2). Our results give further support to the view that keratinocytes can contribute to the local type 1 / type 2 cytokine balance by means of their cytokine expression.

![Diagram showing UVB exposure affecting cytokine levels in keratinocytes and Langerhans cells](image)

**Figure 2.** NB-UVB therapy reduces type 1 cytokine expression in psoriatic skin. It has been demonstrated that the majority of the epidermal T cells dies shortly after the beginning of the UVB therapy of psoriatic lesions. Our studies ([chapter 3](#)) showed that dermal T cells remaining in the lesional skin after the NB-UVB therapy have a diminished capacity to express IFN-γ as compared to T cells from the untreated skin lesions. This could be explained by a decreased expression of inducers of IFN-γ such as IL-12, IL-18 and IL-23 as we demonstrated in the studies involved in [chapter 8](#).

**Effects of cyclosporine A and methotrexate therapy on T cell number and cytokine-profile in psoriatic skin** The study described in [chapter 9](#), shows that the two most commonly used systemic therapies of psoriasis, cyclosporine A and methotrexate, decrease the number of T cells in the lesional skin of psoriasis patients. The reduction of the number of T cells after cyclosporine A therapy is likely attributed to its very well known T cell suppressor action. It is difficult to judge whether the decreased number of T cells after methotrexate therapy is due to a direct or indirect effect of this drug on cutaneous T cells. In vitro studies revealed that methotrexate can cause apoptosis of proliferating T cells when it is used in comparable doses as used in the clinics[^13^]. On the other hand, it was also demonstrated that methotrexate can reduce the expression and activity of cytokines such as IL-1 and TNF[^14^][^15^]. The decreased expression of these cytokines can have various effects such as a decreased activation and migration of dendritic cells and decreased stimulation.
of keratinocytes. This can result in the dampening of the local inflammatory response leading to a reduction of activation and recruitment of T cells to this cutaneous site.

We did not see a clear effect of either cyclosporine A or methotrexate therapy on the expression of IFN-γ and IL-4 in the psoriatic skin. As it is described in the Introduction of this thesis, studies on the type 1 / type 2 cytokine balance in other diseases did also not provide a clear picture of the possible changes in this balance after cyclosporine A and methotrexate therapy. We determined a decreased expression of IFN-γ in 3 out of 5 patients in each of the cyclosporine A and methotrexate groups. The expression of IL-4 was decreased in 2 and 1 patients of the cyclosporine A and methotrexate patient groups, respectively. Changes in the cytokine expression after therapy did not show a clear association with T cell numbers or the clinical response. These results give the impression that these therapies did not cause a prominent change in the expression of these cytokines in situ. However, it must be noted that we only had a limited number of patients in this study and therefore it is not possible to draw any firm conclusion. Individual variations in the immunological response could be an important reason for the differences in the expression of these cytokines after the therapy with cyclosporine A and methotrexate.

**Concluding remarks** Our studies presented in this thesis point out the importance of the fine balance of the cytokine expression in the skin immune system. Overexpression of type 1 cytokines is associated with the formation and maintenance of psoriatic lesions and reversal of this expression is related to successful therapy. We showed in both normal and psoriatic skin that either a switch from type 1 to type 2 immune response (after exposure to high dose BB-UVB) or just dampening of the type 1 immune response (after NB-UVB therapy) can participate in the therapeutical mechanisms of treatment with UVB radiation. This change in cytokine profile may probably occur in other antipsoriatic therapies as well. We believe that the reduction of type 1 cytokines is not simply due to the reduction of relevant cell numbers, but also, to a great extent, to a diminished ability of the resident and infiltrating cells to produce these cytokines. Our studies indicate that neutrophils can affect the type 1/type 2 cytokine balance in dermal T cells probably by the expression of IL-4 and IL-10. This latter statement is speculative and should be proven in future. Every step, which leads to the determination of factors involved in the pathogenesis of psoriasis and its healing process upon therapy, helps us to have a clearer idea about this disease. This thesis adds another piece to the giant psoriasis puzzle, but at the same time created new questions to be answered. To mention some: i, Which effect on inflammatory cells in the
psoriatic lesional skin after treatment with NB-UVB radiation has most impact on the success of this therapy? Is it just a matter of apoptosis or are the UVB-induced phenotypic changes of cutaneous cells more relevant? ii, It seems likely that keratinocytes are involved in the cutaneous immune response, but how much is the contribution of these cells to chronic character of the inflammatory skin disease psoriasis? iii, What is the impact of UVB-induced changes in cytokine expression on the differentiation and proliferation of keratinocytes? Do these cytokines determine the proliferation inhibition of keratinocytes or is the direct antiproliferative effect of the UVB radiation more important? iv, Do the different antipsoriatic therapies cause different effects on the (type 1 / type 2) cytokine expression pattern in the diseased skin, and can these differences explain the temporary or sustained therapeutical effect? Answers to all these questions may lead to the detection of (a) particular cytokine(s) that play(s) a crucial role in the development and perpetuation of psoriatic lesions. This kind of research may ultimately provide a clue for the development of less harmful therapies to treat psoriasis.
**References**


