Effects of biological response modifiers in psoriasis and psoriatic arthritis
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chapter 8

Summary and conclusions
Summary

The aim of this thesis was to investigate the changes in lesional psoriatic skin and synovial tissue of patients with plaque psoriasis and psoriatic arthritis (PsA) after treatment with biological response modifiers. On the one hand this provides a tool to assess the efficacy of new drugs besides clinical assessment tools such as the psoriasis area and severity index (PASI) and American College of Rheumatology (ACR) response criteria. On the other hand this approach may help to understand the immunopathogenetic mechanisms of both psoriasis and PsA. Since psoriasis and PsA are considered to be T-cell mediated diseases, we were in particular interested in changes in T-cell subsets after therapy. In addition, because psoriasis and PsA are characterized by activated endothelium and increased vascularity in lesional skin and synovial tissue, we investigated changes in expression of adhesion molecules (involved in migration of T cells), angiogenesis markers, and growth factors (both involved in neovascularization) after therapy.

Traditionally, the evaluation of the cellular infiltrate and expression of proteins in skin tissue sections is done by manual quantification. However, for reliable evaluation of histology in the development of new anti-psoriatic treatments there is a need for a more time-efficient and reproducible method. To test the use of digital image analysis (DIA) in this situation we compared the assessment of immunohistochemically stained skin sections with manual quantification (MQ) and semi-quantitative analysis (SQA) in chapter 2. The number of CD3+ T cells and the expression of E-selectin were evaluated in stained paired skin biopsies from 11 patients with chronic plaque psoriasis before and after initiation of anti-psoriasis therapy. We observed significant correlations between MQ and DIA for the number of T cells. Both DIA and MQ were equally effective in detecting reductions of T-cell numbers in active-treated patients. MQ took considerable more time to complete than DIA. We also observed significant correlations between SQA and DIA for the expression of E-selectin although DIA was more sensitive than SQA to detect (early) changes. SQA took considerable more time to complete than DIA. From this study we concluded that the quantification of the inflammatory infiltrate in psoriatic lesional skin by DIA generated similar results as MQ and SQA in a reliable, reproducible and more time efficient fashion. We used DIA
to evaluate changes in the inflammatory infiltrate in lesional skin and synovium after alefacept therapy. Alefacept, a LFA-3/IgG1 fusion protein, interferes with the activation and proliferation of T cells by binding to the CD2 receptor on their surfaces. The clinical efficacy of this drug has been demonstrated in chronic plaque psoriasis. We performed a single-centre, open-label study to investigate the clinical efficacy and immunohistochemical effects on the T-cell population in psoriatic lesional skin and synovium (chapters 3 and 4). Eleven patients with plaque psoriasis and PsA all received twelve weekly doses of 7.5 mg alefacept intravenously. Skin biopsies were obtained at baseline and at day 8, 43 and 92, and synovial biopsies were obtained at baseline and at day 29 and 92. Skin and synovial tissue was evaluated by DIA after immunohistochemical staining. After completion of treatment, the majority of patients experienced a PASI reduction ≥ 50% compared to baseline. Immunohistochemical analysis of lesional skin displayed a gradual decrease in the number of cutaneous T cells during therapy, in particular epidermal CD8⁺ cells and dermal CD4⁺ cells. Patients with a PASI reduction ≥ 50% after therapy had a clearance of effector / memory (CD45RO⁺) T cells from the epidermis, in contrast to patients with < 50% PASI reduction. In addition to improvement of skin lesions, the majority of the patients fulfilled the Disease Activity Score (DAS) response criteria for arthritis. There was a significant reduction in CD4⁺T cells in the synovial tissue after therapy. In addition, patients who fulfilled the DAS response criteria after treatment displayed a significant reduction in effector / memory (CD45RO⁺) T cells compared with non-responding patients. These findings support the hypothesis that effector / memory T cells play a prominent role in the pathogenesis of psoriasis and PsA, and that alefacept is capable of reducing these cells in lesional psoriatic skin and synovium. This is also the first time alefacept therapy was demonstrated to improve symptoms of PsA.

In the following studies we focused on anti-TNF-α therapy, and the clinical and immunohistochemical effects of these drugs on lesional psoriatic skin and synovium. TNF-α plays a key role in the inflammatory cascade in psoriasis and PsA as illustrated by the increased TNF-α expression in psoriatic skin lesions and inflamed synovial tissue. Consistent with this notion, infliximab, an anti-TNF-α monoclonal antibody, has been reported to be clinically effective for both psoriasis and PsA, but the mechanism of action is not
precisely known. In vitro studies suggest that the binding of infliximab to membrane-bound TNF-α could lead to lysis of TNF-α producing cells via activation of complement-dependent or antibody-dependent cell-mediated toxicity. Indeed, an increase in the number of apoptotic cells in the lamina propria of the gut has been detected after infliximab therapy in Crohn’s disease. To investigate whether apoptosis of T cells played a role in the mechanism of action of infliximab in psoriasis and PsA as well, we performed a single-centre, randomized, placebo-controlled study to investigate the early changes at the site of inflammation (chapter 5). Twelve patients with both active psoriasis and PsA were randomized to receive a single infusion of either infliximab (3mg/kg) or placebo intravenously. Synovial tissue biopsies and lesional skin biopsies were obtained at baseline and 48 hours after initiation of treatment, and evaluated by digital image analysis after immunohistochemical staining. A significant reduction in T cell numbers was found in both lesional epidermis and synovial tissue after infliximab treatment, but not after placebo treatment. However, the changes in cell numbers could not be explained by induction of apoptosis at the site of inflammation (investigated by both caspase-3-staining and TUNEL assay). Neutralization of the effects of TNF-α appears sufficient to induce clinical improvement in psoriasis and PsA even without induction of apoptosis at the site of inflammation, for example by reduced T cell trafficking to lesional skin and synovium. In line with this idea, studies in rheumatoid arthritis patients have shown that infliximab therapy results in decreased expression of adhesion molecules and chemokines, molecules that are essentially involved in cell migration. Blood vessels in both psoriatic skin and synovial tissue express a variety of adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), and E-selectin. In addition, overexpression of vascular endothelial growth factor (VEGF), which is involved in neoangiogenesis, and its endothelial cell receptors have been reported in psoriatic skin and synovium. To investigate the influence of infliximab on T-cell trafficking and vascularity, we treated 11 patients with psoriasis and PsA with infliximab (3 mg/kg) at baseline, and at weeks 2, 6, 14 and 22 in an open-label study (chapter 6). Clinical assessments, including PASI, and DAS, were performed at baseline and every 2 weeks afterwards. In
addition, skin biopsies from a target psoriatic plaque and synovial tissue biopsies from a target joint were taken before treatment and at week 4. The number of T cells, the number of blood vessels, the expression of adhesion molecules, and the presence of vascular growth factors were investigated by digital image analysis. At week 16, the mean PASI was reduced considerably from 12.3 at baseline to 1.8. The mean DAS was strongly reduced as well. Because the dosage of infliximab was lower (3mg/kg) than usual (5mg/kg), we found some fluctuations in DAS response compared to the change in PASI, the latter showing a steady decrease over time. After 4 weeks, the cell infiltrate was reduced in both skin and synovium, with a significant reduction in the number of blood vessels in dermis and synovium, and a significant reduction in the expression of alpha-v-beta-3 integrin (αvβ3), a marker of neovascularisation, in both skin and synovium. In addition, a significant reduction in the expression of adhesion molecules was observed in both skin and synovium at week 4. We also observed a trend towards reduced expression of vascular endothelial growth factor (VEGF) in both skin and synovium. The effects of infliximab on vascularity might be particularly important in psoriasis and PsA because of the prominent role of hypervascularization and the typical tortuous morphology of the capillaries in these diseases. In conclusion, low-dose infliximab treatment led to decreased neoangiogenesis and deactivation of the endothelium resulting in decreased cell infiltration and clinical improvement in psoriasis and PsA. To investigate whether these effects are restricted to infliximab therapy or apply to anti-TNF-alpha therapy in general, we performed a similar study with etanercept therapy in chapter 7. Etanercept is a fusion protein consisting of two identical chains of a recombinant human TNF receptor (p75) monomer fused to the Fc portion of human IgG1. By competitive inhibition of the interaction of circulating TNF-α with cell surface-bound TNF-receptors, etanercept is thought to prevent TNF-mediated cellular responses by rendering TNF biologically inactive. Compared to infliximab, the inhibition of biological activity of TNF-α by etanercept appears less effective. This might explain differences in efficacy between infliximab and etanercept. We studied the effects of etanercept in lesional psoriatic skin in situ in a double-blind placebo-controlled study. Six patients were treated with either 50 mg s.c. twice weekly, 25 mg s.c. twice
weekly, or placebo. Skin biopsies were performed at baseline, after 4, and after 12 weeks of treatment. The results suggest that etanercept is an effective treatment for moderate to severe plaque psoriasis. All patients treated with etanercept in our study achieved more than 50% reduction in PASI after 12 weeks of treatment, and one patient even more than 75% reduction in PASI after 12 weeks of treatment. We observed a strong reduction in infiltrating T cells in lesional epidermis and dermis after 4 weeks in etanercept-treated patients, in contrast to placebo-treated patients. There was a reduction in the expression of the adhesion molecules E-selectin, VCAM-1, and ICAM-1 in the etanercept-treated patients. However, in contrast to infliximab therapy, no changes in the expression of vWF, \( \alpha_4\beta_3 \), and VEGF were observed, suggesting that etanercept did not seem to have a profound effect on vascularity and angiogenesis in lesional psoriatic skin. In conclusion, etanercept induces a decreased influx of activated T cells in lesional skin of psoriasis patients in association with a downregulation of the expression of adhesion molecules on endothelial cells.

**Concluding remarks**

The aetiology of psoriasis and psoriatic arthritis (PsA) is still not known. Initially, psoriasis was thought to be a disorder of the keratinocytes, since hyperproliferation and abnormal differentiation of keratinocytes result in the silvery scales that are typical for psoriasis. The reason for disturbed keratinisation, however, could not be identified, nor could this hypothesis explain the joint disease associated with psoriasis. Approximately two decades ago the emphasis of psoriasis research shifted from keratinocytes to T cells because of two important observations. First, a remarkable improvement in clinical signs and symptoms of psoriasis was observed during cyclosporine treatment, a known T-cell inhibitor. The success of T-cell targeted therapy was recently confirmed when alefacept, an LFA-3/IgG1 fusion protein that inhibits T-cell activation, was found to ameliorate symptoms of both psoriasis and PsA (this thesis). Second, immunohistochemical staining of the inflammatory infiltrate of psoriasis revealed that activated effector/memory T cells were the first cells to arrive in early skin lesions and were present in lesional synovium as well. It is therefore suggested that psoriasis and PsA are
T-cell mediated diseases, in which activated T cells responding to an unknown antigen drive the initiation of the inflammatory cascade, including abnormal keratinocyte proliferation and differentiation. Many established and experimental anti-psoriatic treatments are based on this principle, such as cyclosporine A, UVB, methotrexate, and alefacept. Surprisingly, when anti-TNF-α therapy emerged in other inflammatory diseases, such as rheumatoid arthritis and Crohn’s disease, it was discovered that both psoriasis and PsA responded remarkably well to these therapies, suggesting that TNF-α might play a more important role in the pathogenesis of psoriasis and PsA than previously anticipated. This suggestion is supported by the observations that the expression of TNF-α is increased in serum, skin, and synovium of patients with psoriasis and PsA. TNF-α is not only a regulator of the inflammatory response, but also plays an important role in bone metabolism by enhancing osteoclastogenesis. With TNF-α inhibition, there is a domino effect of decreased expression of other proinflammatory cytokines and decreased recruitment of activated cells, with consequent down-regulation of inflammation in both skin and joints. It should be noted, however, that severe infections in some of the patients treated with anti-TNF-α therapy underline the need for careful control. In addition, many of these treatments generally do not achieve complete remission in all patients, and it would be interesting to identify which patients are suitable for each specific anti-TNF-α therapy, perhaps by using pharmacogenomic techniques that will be tested in the near future.

Recently, another cell type with pathogenic potential has been identified in psoriasis and PsA, the natural killer-like T (NK-T) cell. NK-T cells express both a conserved αβ T cell receptor and a natural killer cell receptor, and are part of the innate immune system. NK-T cells can act without prior sensitization. It has been suggested that interaction of NK-T cell receptors with keratinocytes is the mechanism of keratinocytic hyperproliferation via the production of IFN-γ.

In conclusion, recent developments in psoriasis research, for instance the remarkable improvements of psoriasis and PsA after anti-TNF-α therapy, have shifted the focus from T-cells towards innate immunity. A possible hypothesis would be that in psoriasis there is a genetic deficiency in down-regulation of the innate immunity, in particular TNF-α, leading to increased angiogenesis and expression of adhesion molecules on endothelial cells which cause
increased trafficking of activated effector / memory T cells and NK-T cells towards lesional skin and synovium. These cells stimulate keratinocytes to hyperproliferation, and in synovial tissue increased levels of TNF-α induce osteoclastogenesis. The age of onset of psoriasis and PsA might be, in part, dependent on specific environmental or physical triggers, which are as yet unknown. NK and NK-T cells act as a first-line of defence against virally infected cells, which are lysed upon recognition by activated NK cells. It is possible that a viral antigen may be important in the pathogenesis and actual onset of psoriasis and PsA. Future research in psoriasis and PsA should focus more on the role of the innate immune system, in particular NK-T cells.