chapter 1

General introduction and aims of the thesis
**Introduction to psoriasis and psoriatic arthritis**

Psoriasis is a chronic inflammatory skin disease with an estimated prevalence of 2% worldwide. The term ‘psoriasis’ was first used by the Greek physician Galen, and is derived from the word ‘psora’ meaning itch. Patients suffering from psoriasis may experience physical discomfort as well as a great psychological burden. The clinical manifestations of psoriasis are abundant and diverse, and the intensity of disease activity varies in time. The most common clinical presentation is plaque-type psoriasis or psoriasis vulgaris, which is characterized by sharply demarcated erythematous plaques with silvery scaling presenting symmetrically on the extensor side of the elbows and knees, and the lumbosacral region. This type of psoriasis is often accompanied by involvement of nails and scalp. Other types of psoriasis are divided into those primarily describing the form (guttate, pustular, annular) or the distribution (flexural, palmoplantar, disseminated). Guttate psoriasis is characterized by numerous small coin-sized papules distributed over the whole body, and flexural psoriasis is characterized by erythematous plaques in body folds such as the groins, axillae, and the submammary region. Erythrodermic psoriasis and generalized pustular psoriasis are potentially life-threatening manifestations of psoriasis often requiring hospitalization. An arthritis associated with psoriasis was first recognized in the mid-nineteenth century and in 1860 Paul Bazin coined the term ‘psoriasis arthropathique’\(^1\). It was not until 1964 that the American Rheumatism Association recognised it as a separate entity. Approximately 25-34 percent of patients with skin psoriasis develop psoriatic arthritis (PsA)\(^3\), a seronegative destructive joint disease with 5 different subtypes: asymmetrical oligo-articular arthritis (70%), symmetrical poly-arthritis (15%), distal interphalangeal arthritis (5%), spinal/axial arthritis (5%), and arthritis mutilans (5%)\(^5\). Without proper treatment PsA can lead to joint degeneration and loss of function. Usually the skin psoriasis precedes the arthritis, but in 19% of the cases arthritis is present before skin lesions develop\(^3\).
Immunopathology of psoriasis and psoriatic arthritis

Introduction Although the exact origin of psoriasis and PsA has not been established yet, the immunopathogenetic pathways leading to the development of a psoriatic plaque have been subject to extensive studies in the last decades. In addition, the introduction of new biological therapies for psoriasis has proven to be a powerful tool in the investigation of the psoriatic immune responses. In general, the initiation and exacerbation of both psoriasis and PsA probably result from an interaction of genetic, environmental, and immunological factors. The morphology of lesional psoriatic skin is characterised by 3 major histological changes: 1) epidermal thickening, parakeratosis, and hyperkeratosis; 2) a pronounced dermal vascular plexus; and 3) the presence of inflammatory cells (e.g. T cells and neutrophilic granulocytes) in the superficial dermis and in the epidermis.

Few studies have analyzed the immunohistochemical changes in synovial tissues in PsA. Histological features of synovial tissue include infiltration by macrophages, T cells, and other inflammatory cells, hyperplasia of the synovial lining, and vascular changes, described as tortuosity and higher intensity of villous vascularization. In addition, high IL-8 levels were found in PsA synovial fluid, outlining the importance of this chemokine in the recruitment of inflammatory cells in patients with PsA. In addition to cell-mediated immune responses, humoral immune responses are believed to play a role in the pathogenesis of PsA as well. For example, serum levels of IgA and IgG are higher in PsA patients, and synovial membranes from patients with PsA contain higher numbers of plasma cells positive for IgG or IgA than patients with meniscal tears.

T cells in psoriasis Increasing evidence suggests that T cells play a key role in the pathogenesis of psoriasis. In 1983, Bos et al. showed that the majority of the dermal inflammatory infiltrate consists of partially activated CD4+ and CD8+ T cells. These cells express CD45RO on the surface, indicating their effector / memory status. Most of the infiltrating T cells also express markers such as the interleukin-2 receptor (CD25) and HLA-DR, indicating early and mid-to-late activation, respectively. The infiltration of T cells into the skin precedes epidermal proliferation and endothelial cell activation. The key role for T
lymphocytes in the pathogenesis of psoriasis was supported by reported beneficial effects of specific T-cell targeted therapies, such as cyclosporine A and DAB389 IL-2 toxin, and more recently alefacept\textsuperscript{20-21}. Because of this, psoriasis is now suggested to be a T-cell mediated inflammatory disease.

How do T cells play a role in the development of a psoriatic plaque? To create a better understanding of this process, it can be broken down in three separate steps:

1. the activation of T cells
2. the migration of T cells into the lesional skin
3. the release of cytokines by activated T cells in the skin.

Ad 1) Initial T-cell activation requires stimulation of the T-cell receptor (TCR) by the major histocompatibility complex (MHC I or II) on the antigen presenting cell (APC). Although this is believed to be an antigen-specific process, the antigenic peptide responsible in psoriasis has as yet not been identified. The adhesion of the T cell with the APC is facilitated by the interaction of surface molecules, such as CD2 on the T cell with leukocyte function associated antigen (LFA)-3 on the APC, or LFA-1 (CD11a) on the T cells with intercellular adhesion molecule (ICAM)-1 on the APC\textsuperscript{24}. After the initial activation via the TCR, a second, non-antigen specific signal is needed to complete the activation. This 'costimulatory' signal results from interactions between molecules on the T cell and their ligands on APCs, for example CD2/LFA-3, very-late antigen 4 (VLA-4)/vascular cell adhesion molecule 1 (VCAM-1), LFA-1/ICAM-1, CD40/CD40L, and others\textsuperscript{25-30}. Simultaneous delivery of both signals is essential for T cell activation, and if the costimulatory signal is inhibited, T cells can become unresponsive or 'anergic'.

A number of new biological therapies for psoriasis have been developed to inhibit T cell activation in one way or another, for example efalizumab, a humanized anti-CD11a monoclonal antibody, and alefacept, a LFA-3/IgG1 fusion protein. These and other biologicals will be discussed further on in this introduction.

Ad 2) Once activated, T cells obtain certain cell surface proteins, which are necessary for migration from lymph nodes and blood vessels into extranodal
tissue, such as the skin. T-cell trafficking to the skin is a complicated process that requires intensive interaction between the activated T cell and the endothelium. First, T cells must be slowed down in the bloodstream in order to be immobilized and to bind to the endothelium. This process, called 'tethering', is mediated by the glycoprotein cutaneous lymphocyte antigen (CLA), which is expressed on the surface of activated T cells in psoriasis. CLA is an adhesion molecule that interacts with E-selectin and P-selectin, which are strongly upregulated on endothelium during cutaneous inflammation. Both lesional and non-lesional skin of psoriasis patients shows upregulation of these adhesion molecules. Temporary binding of the selectins with the receptors on the T cell surface creates a rolling motion, which slows the T cell down. This allows the T cell to be exposed to chemokines that activate T-cell surface proteins, such as LFA-1 and very-late antigen (VLA)-4. These integrin receptors form high-affinity bonds with respectively ICAM-1 and VCAM-1 on endothelial cells, resulting in an arrest of the rolling process and subsequent flattening of the activated T cell, which facilitates the diapedesis of the T cell through the blood vessel wall. After extravasation through the blood vessel wall, skin-homing T cells migrate to the dermis and epidermis in response to chemotactic gradients. The chemokines that enhance T-cell trafficking are produced by endothelial cells, keratinocytes, monocytes, and Langerhans cells, and their release is stimulated by interferon (IFN)-γ and tumour necrosis factor (TNF)-α.

**Ad 3)** Activated T cells produce a certain cytokine profile and based on this T cells are generally divided in two types. Type 1 T cells produce the pro-inflammatory cytokines interleukin (IL)-2, IFN-γ, and TNF-α, whereas type 2 T cells produce cytokines such as IL-5, IL-4, and IL-10. Activated T cells isolated from psoriasis lesions showed a predominant type 1 cytokine profile. Both CD4+ and CD8+ T cells can produce type 1 cytokines. There have been several reports describing a predominance of cytotoxic CD8+ T cells in psoriatic lesional epidermis, whereas CD4+ cells are the predominant type in lesional dermis. The secretion of cytokines by activated T cells influences neighbouring cells, such as keratinocytes and dendritic cells, which in turn release additional cytokines, creating a chronic inflammatory cascade. IFN-γ has been shown to
induce epidermal hyperplasia when injected into non-lesional skin of psoriasis patients. Also, IFN-γ stimulates the expression of ICAM-1 by epidermal keratinocytes, facilitating the binding of T cells to keratinocytes. TNF-α is another important proinflammatory cytokine that plays a role in the pathogenesis and maintenance of psoriasis. The role of TNF-α and TNF-α inhibitors will be discussed further on in the introduction.

**T cells in psoriatic arthritis** In synovial tissue, activated memory CD4+ T cells predominate, which are focally distributed near small blood vessels and the intimal lining layer, whereas in synovial PsA fluid activated CD8+ T cells predominate. The demonstration of oligoclonal expansions of T cells derived from synovial fluid of patients with PsA supports the hypothesis that T cells are involved in the pathogenesis of PsA. Findings of common T cell receptor βV expansions in psoriatic skin and synovium suggest an important role for cognate T-cell responses and suggest that the inciting antigens are identical or homologous between afflicted skin and synovium. Numerous chemokines and cytokines, such as TNF-α, IL-1β, IL-2, IL-8, IL-10, IL-15, IL-18, and IFN-γ are believed to play a role in triggering cell proliferation and sustaining joint inflammation in PsA. The expression of the cellular adhesion molecules ICAM-1, VCAM-1, and E-selectin facilitate migration of activated T cells through the vascular endothelium and formation of an infiltrate in synovial PsA tissue. Further evidence that T cells play a role in the pathogenesis is provided by the observation that anti-T-cell targeted therapy has proven to be beneficial in PsA.

**TNF-α in psoriasis** TNF-α is a pro-inflammatory cytokine produced by activated T cells, keratinocytes, monocytes, and dendritic cells in human skin. It exists as a membrane-bound molecule on cells that produce it, as a soluble protein in the circulation, and bound to cell surface receptors on target cells, such as keratinocytes, dendritic cells, T cells, NK cells, and endothelial cells. TNF-α has numerous effects on the immune response, correlating with the clinical and histological pathology seen in psoriatic skin. TNF-α can induce the expression of adhesion molecules, such as ICAM-1, VCAM-1, and E-selectin, and vascular growth factors, such as vascular endothelial growth
factor (VEGF) in the skin, promoting T cell trafficking. In addition, TNF-α has been demonstrated to increase the expression of other proinflammatory cytokines, such as IL-1, IL-5, IL-6, and transforming growth factor, and chemokines, such as IL-8, a member of the CXC chemokine family, thereby enhancing the infiltration of T cells into the epidermis. Nuclear factor κB (NFκB), a nuclear transcription factor that is crucial in inflammation, is also activated by TNF-α. Finally, TNF-α stimulates mature Langerhans cells to migrate from the skin to the lymph nodes, where antigen presentation and T-cell activation take place. Substantial evidence suggests that TNF plays a fundamental role in the pathogenesis of psoriasis. Increased levels of TNF-α compared to controls have been reported in psoriatic lesional skin. Levels of TNF-α in psoriatic lesional skin have been found to correlate with severity of psoriasis. In addition, levels of TNF in skin and serum of psoriasis patients have been demonstrated to decrease after successful therapy. The most convincing evidence, linking TNF-α to psoriasis, is the ability of TNF-α inhibitors such as etanercept, infliximab, and adalimumab to ameliorate clinical symptoms of psoriasis. Clinical trials with infliximab and etanercept for psoriasis and PsA are discussed elsewhere in the introduction.

**TNF-α in psoriatic arthritis** In PsA, TNF-α activates NF-κB, leading to synovial cell proliferation, leukocyte trafficking, further proinflammatory cytokine production, and up-regulation of RANKL-mediated osteoclastogenesis. A significantly higher concentration of TNF-α and its receptors have been reported in PsA synovial fluid compared with osteoarthritis. Examination of serial synovial biopsies in four PsA patients, who participated in an open study on the effects of the chimeric anti-TNF antibody infliximab, has shown that clinical improvement of peripheral arthritis activity is associated with decreased intimal lining layer hyperplasia, reduced vascularity, and reduced polymorphonuclear cell and macrophage infiltration.

**Angiogenesis in psoriasis** Neovascularization appears to play an important role in the evolution of a psoriatic plaque. Epidermal proliferation is closely associated with vascular expansion in the superficial dermis early in the development of a psoriatic plaque. Studies demonstrated that abnormal blood
vessel growth could predict the area of skin to be involved in the inflammatory process. Microvascular changes include exaggerated tortuosity, pronounced dilatation, increased permeability, and endothelial cell proliferation within the capillaries in the dermal papillae. Vascular proliferation is driven by the local expression of angiogenic molecules (mostly derived from keratinocytes), such as transforming growth factor-α, TNF-α, plasminogen activator inhibitor (PAI)-1, platelet-derived endothelial cell growth factor, endothelial cell stimulating angiogenesis factor (ESAF), and vascular endothelial growth factor (VEGF). VEGF induces microvascular hyperpermeability and acts as an endothelial cellspecific mitogen, and is recognized as a central regulator of angiogenesis. Both ESAF and VEGF were found to be elevated in plaques of psoriasis as compared with uninvolved skin and normal skin. Tissue and serum levels of ESAF, PAI-1, and VEGF correlated with the clinical severity of psoriasis, suggesting a pathogenetic role in psoriasis. Indeed, a randomized phase I/II trial with Neovastat, an inhibitor of angiogenesis, revealed a dose-dependent effect of this drug in the improvement in psoriasis.

Angiogenesis in psoriatic arthritis In previous studies of PsA the most significant histological findings were vascular changes, described as tortuosity and higher intensity of villous vascularization, supporting the theory that microvascular changes play an important role in the pathogenesis of PsA. In addition, synovial fluid metalloproteinases such as matrix metalloproteinase (MMP)-9 correlate with the pattern of neo-vascularization and synovial fluid VEGF levels. Elevated VEGF concentrations, produced by macrophages and fibroblast-like synoviocytes, have been reported in serum and synovial fluid of patients with PsA. Other growth factors that control angiogenesis are the angiopoietins (Ang). Ang1 induces stable maturation of blood vessels, whereas Ang2 plays a role in vessel remodelling and maturation. Ang2 and VEGF mRNA expression and protein levels were significantly higher in early PsA compared with rheumatoid arthritis synovium. Angiogenesis also plays a role in bone formation, since invasion of the cartilage by new blood vessels precedes osteoblastic transformation and ossification. However, data on vascular changes in PsA synovial tissue are sparse and sometimes conflicting, possible due to differences in patient’s selection.
Genetics in psoriasis and psoriatic arthritis

Population and twin studies support the concept that psoriasis and PsA have a genetic basis. Genetic linkage studies have confirmed a genetic predisposition. It has been estimated that the HLA-associated allele PSORS1 on chromosome 6p accounts for 30 to 50% of the genetic contribution to psoriasis. This locus contains genes coding for HLA-C, corneodesmosin (Cdsn), and alpha-helix coiled-coil rod homolog (HCR) which were found to be expressed at higher levels in psoriatic lesional skin than in normal skin.

Predisposing loci other than HLA include 17q24-q25 in a variety of Caucasian populations, 4qter (PSORS3) in Irish families, 1q21 (PSORS4) in Italian and U.S. families, 3q21 (PSORS5) in Swedish families and some other loci. Strong evidence of familial aggregation in PsA has been found as well, the risk of PsA was found to be 50 times greater in first-degree relatives of PsA patients than in a control population. However, association of the HLA locus with PsA has been less clear-cut than with psoriasis. HLA B27 has traditionally been associated with spinal inflammation, and associations of PsA with other loci have been described as well. Finally, promoter polymorphisms of the genes encoding TNF-α and IL-1β have been found to be associated with different subtypes of psoriasis characterized by early and late disease onset.

It is clear that the genetic predisposition for psoriasis and PsA is complex and cannot be ascribed to a single gene. For now, the cause of psoriasis and PsA can be considered to be multifactorial, resulting from an interaction of genetic, environmental, and immunological factors.

Biological response modifiers in psoriasis and psoriatic arthritis

Why do we need new therapies for psoriasis and PsA? Current therapies such as photo(chemo)therapy, cyclosporine A, and methotrexate are effective for psoriasis, but are limited in their use because of their potential side-effects. Cyclosporine may cause hypertension and renal failure, and methotrexate commonly causes malaise and hepatotoxicity. Photo(chemo)therapy, in particular PUVA, increases the risk of skin cancer, which limits its use in a chronic disease such as psoriasis. In addition, a National Psoriasis Foundation Survey showed that only 26% of patients with psoriasis are satisfied with their
current treatment. Therapeutic options for patients suffering from PsA have been limited as well during the last decades. In contrast to rheumatoid arthritis, no disease-modifying anti-rheumatic therapy has been available for PsA except for methotrexate. Instead, symptom-modifying drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) are used to ameliorate pain. For these reasons there is a clear need for effective and long-lasting anti-psoriatic and anti-PsA treatments with limited side-effect profiles.

Improved understanding of the immunopathogenetic mechanisms in psoriasis and PsA has led to the rapid development of the so-called biological response modifiers ("biologics"), a versatile group of engineered bioactive proteins. Biologicals can be divided into three main groups according their molecular structure: monoclonal antibodies, fusion proteins, and cytokines. The generic names of the biologicals comply to a strict nomenclature: chimeric monoclonals end with -ximab, humanized monoclonals end with -zumab, human monclonals end with -umab, and receptor-antibody fusion proteins end with -cept. Table 1 shows the biological response modifiers currently approved or under development for psoriasis and/or PsA.

**Monoclonal antibodies**

**anti-adhesion** Efalizumab Efalizumab is a recombinant, humanized monoclonal IgG1 antibody directed against the α subunit (CD11a) of leukocyte function-associated antigen (LFA)-1. In psoriasis, binding of LFA-1 on memory T-cells to ICAM-1 on keratinocytes and vascular endothelial cells leads to T-cell activation as well as trafficking of T cells from the circulation into the skin. This provides the rationale for blocking the LFA-1/ICAM-1 interaction with efalizumab in the treatment of psoriasis. Data from in vitro studies demonstrated that efalizumab inhibits multiple key pathogenic steps in psoriasis: T-cell activation, T-cell trafficking to the skin, and T-cell adhesion to keratinocytes. The biological effects of efalizumab and clinical activity were demonstrated in clinical trials. Gottlieb et al (2000) demonstrated in an open label study that intravenous administered efalizumab resulted in improvement in psoriasis area and severity index (PASI), decreased numbers of epidermal and dermal T cells, decreased expression of ICAM-1 on keratinocytes and blood vessels, and epidermal thinning. There was an inverse
relationship between sustained CD11a down-modulation and saturation and improvement of histological parameters\textsuperscript{118}. Another open-label study by the same author confirmed these observations, and demonstrated a dose-response relationship both clinically and histological. The mean PASI decrease in the highest dosage group was 47 percent\textsuperscript{117}. In the peripheral blood, administration of efalizumab to psoriasis patients resulted in an increase in circulating leukocytes, which was largely caused by an increase in T-cell numbers as opposed to other leukocytes. The largest increase was observed in memory CD8\textsuperscript{+} T cells, suggesting that efalizumab blocks cutaneous entry of memory CD8\textsuperscript{+} T cells\textsuperscript{119}. Immunohistochemical changes induced by efalizumab were mirrored by reports on clinical improvement in psoriasis patients treated with efalizumab. In a randomized, double-blind, placebo-controlled multicentre phase II trial 144 psoriasis patients received either placebo or i.v. efalizumab 0.3 mg/kg for 8 weeks. The percentage of efalizumab-treated patients achieving more than 50\% improvement in physician’s global assessment at day 56 was 48\%, compared to 15\% of the placebo-treated patients. Epidermal thickness was reduced by 37\% in efalizumab-treated patients and 19\% in placebo-treated patients. Treatment was well tolerated; mild to moderate flu-like complaints were the most common adverse events. Depletion of circulating lymphocytes did not occur\textsuperscript{120}. To provide a more convenient mode of administration, a subcutaneous formulation of efalizumab was developed and used in phase III clinical trials. Five hundred ninety-seven patients with chronic plaque psoriasis were treated with either efalizumab (1 or 2 mg/kg s.c.) or placebo once weekly for 12 consecutive weeks in a phase III multicentre, randomized, placebo-controlled, double-blinded study. Depending on the response after 12 weeks, subjects received an additional 12 weeks of treatment with efalizumab or placebo. At week 12, PASI 75 (75\% or more reduction in PASI) was achieved by 22\% of the patients who had received 1 mg/kg of efalizumab and 28\% of those who had received 2 mg/kg of efalizumab, as compared with 5\% of the subjects in the placebo group. Efalizumab-treated subjects had greater improvement than those in the placebo group as early as week 4. After the discontinuation of efalizumab at week 24, an improvement of 50 percent or more in the PASI was maintained in approximately 30\% percent of patients during the 12 weeks of follow-up.
Efalizumab was well tolerated, and adverse events were generally mild to moderate\textsuperscript{121}. Finally, in a recently performed phase III, multicentre, randomized, double-blinded, placebo-controlled study 556 patients with moderate to severe psoriasis were treated with efalizumab 1 mg/kg s.c. or placebo weekly for 12 weeks. At week 12, 27% of efalizumab-treated patients achieved PASI 75, and 59% of efalizumab-treated patients achieved PASI 50 (50% or more reduction in PASI). After the first 12-week treatment period, all patients were treated with 1 mg/kg efalizumab weekly for another 12 weeks. After 24 weeks of continuous efalizumab therapy, 44% of patients achieved PASI 75 and 67% achieved PASI 50, suggesting that extending efalizumab treatment from 12 to 24 weeks leads to improved efficacy. There was a decline in adverse events during the study without evidence of cumulative toxic effects\textsuperscript{122}. In phase III studies, efalizumab has been anecdotally reported to improve PsA. This finding is in agreement with the notion that the interaction between LFA-1 and ICAM-1 represents a major adhesion pathway in lymphocytic homing in PsA\textsuperscript{123}. Recently, efalizumab was tested in a phase II randomized trial in 107 patients with PsA. Preliminary results showed that after 12 weeks of treatment, 28 percent of the efalizumab-treated patients achieved a 20% or more reduction in American College of Rheumatology (ACR\textsuperscript{124}) response criteria, compared to 19 percent of the placebo-treated patients\textsuperscript{125}. Efalizumab is approved for the treatment of moderate to severe psoriasis in the U.S.A. and most countries in Europe.

**anti-cytokine**

**Infliximab** Infliximab is a monoclonal chimeric antibody composed of a murine anti-TNF Fab fragment joined to the constant region of human IgG1. Each molecule of infliximab has two antigen-binding sites for TNF-\(\alpha\), allowing for increased binding avidity to both membrane-bound and soluble TNF-\(\alpha\)\textsuperscript{56}. Infliximab can neutralize both transmembrane-bound TNF-\(\alpha\) on the cells that synthesize it (e.g. T cells, keratinocytes, and dendritic cells), and soluble circulating TNF-\(\alpha\), thereby inhibiting the pro-inflammatory effects of TNF-\(\alpha\). In addition, in vitro studies have suggested that binding of infliximab to membrane-bound TNF-\(\alpha\) could lead to lysis of TNF-\(\alpha\) producing cells via activation of complement-dependent or antibody-dependent cell-mediated
The clinical efficacy of infliximab in psoriasis was first demonstrated when a patient treated with infliximab for Crohn's disease showed a dramatic improvement of her psoriasis lesions as well. Next, the clinical efficacy of infliximab was confirmed in a small investigator-initiated, randomized, double-blinded, placebo-controlled trial of 33 patients with plaque type psoriasis, who were treated with either infliximab i.v. 5 mg/kg, infliximab i.v. 10 mg/kg or placebo at weeks 0, 2, and 6. At week 10, PASI 75 was achieved by 82 percent of the patients in the 5 mg/kg infliximab and 73 percent in the 10 mg/kg infliximab group, compared with 18 percent of patients in the placebo group. The infliximab-treated group showed a safety profile similar to that of the placebo group, with headache being the only adverse event recorded more frequently in the 10 mg/kg group. At the end of this study, 'non-responding' patients in the placebo group were randomized to receive either 5 mg/kg or 10 mg/kg infliximab at weeks 10, 12, and 16, and responding patients in the infliximab groups were evaluated for relapse (loss of at least half of the improvement in PASI at week 10) and retreated with open-label infliximab (5 or 10 mg/kg) as needed. In all, 29 patients received either 5 or 10 mg/kg of infliximab. At week 26, 55 percent of patients maintained a PASI 50 or better, and 48 percent of patients maintained at least PASI 75. Subsequently, the efficacy and safety of infliximab for the treatment of psoriasis were investigated in a phase II trial (SPIRIT trial) from which the results were recently published. In this study, 249 patients with severe plaque psoriasis were randomized to receive infliximab 5 mg/kg i.v., infliximab 3 mg/kg i.v., or placebo at weeks 0, 2, and 6. At week 10, 88 percent of the patients in the 5 mg/kg infliximab group and 72 percent of the patients in the 3 mg/kg infliximab group achieved PASI 75, compared to 6 percent in the placebo group. Furthermore, patients treated with infliximab showed rapid onset of improvement from baseline in psoriasis. Maximum response to infliximab therapy was observed 10 weeks after the first infusion. The duration of response was variable for individual patients, but in general patients started to lose response after 10 weeks (3 mg/kg group) and 14 weeks (5 mg/kg), respectively. Four patients were considered to have serious adverse events related to infliximab therapy, which included squamous cell carcinoma, cholecystitis, diverticulitis, and pyelonephritis with sepsis. Infusion reactions, such as chills, headache, nausea, and dyspnoea,
were reported in 18 and 22 percent of patients in the infliximab (3 and 5 mg/kg) groups, respectively, compared with 2 percent in the placebo group. There were no serious or life-threatening infusion reactions. Although the incidence of infusion reactions at week 26 was approximately 2 to 3-fold higher for patients with antibodies to infliximab relative to those who were negative for antibodies, the majority of patients with antibodies did not have any infusion reaction. The incidence of newly positive anti-nuclear antibodies (ANAs) observed in this study was 22-25 percent, however, no patients in this study developed symptoms of drug-induced lupus or lupus like syndrome. Other safety concerns such as the development of tuberculosis or malignant lymphoma were not observed during this study. In addition to the beneficial effect of infliximab on skin lesions in psoriasis, infliximab has been demonstrated to reduce clinical signs and symptoms of PsA as well. Ten patients with severe polyarticular PsA were treated with infliximab 5 mg/kg i.v. in combination with their current therapy at weeks 0, 2, and 6 in a small open-label study. At week 10, 8 of 10 patients achieved an ACR 70 response. In another open-label study, 9 patients with active PsA were treated with infliximab 3 mg/kg i.v. at weeks 0, 2, 6, 14, and 22. At week 22, ACR 20, ACR 50, and ACR 70 were achieved by 8, 5, and 2 patients, respectively. The observations in these studies led to a larger-scale, 16-week placebo-controlled trial followed by a 34-week open label extension to test the efficacy and safety of infliximab 5 mg/kg in 102 patients with PsA (IMPACT trial). At week 16, ACR 20, ACR 50, and ACR 70 were achieved by 69, 49, and 29 percent of patients in the infliximab group and 8, 0, and 0 percent of patients in the placebo group. The improvement observed in this study was not clearly related to the continued use of concomitant disease-modifying anti-rheumatic drugs. Recently, results were published from the IMPACT2 trial, a phase III study in which the efficacy and safety of infliximab were investigated in a larger population of patients with PsA. A total of 200 patients with active PsA were randomized to receive either placebo or infliximab 5 mg/kg i.v. at weeks 0, 2, 6, 14, and 22, in combination with stable doses of MTX. At week 14, ACR 20, ACR 50, and ACR 70 was achieved by 58, 36, and 15 percent of infliximab-treated patients, and 11, 3, and 1 percent of placebo-treated patients, respectively. The incidence of adverse events was comparable between the
infliximab group and the placebo group. No opportunistic infections, such as tuberculosis, or serious infusion reactions were observed. Five percent of infliximab-treated patients were positive for anti-infliximab antibodies at week 22. Newly positive ANAs were detected in 10 percent of infliximab-treated patients. None of the patients developed a lupus-like condition

Adalimumab Adalimumab is a fully human-derived recombinant monoclonal antibody that binds TNF-α and blocks its interaction with the p55 and p75 cell surface TNF receptors. Initially, adalimumab was developed to treat rheumatoid arthritis, but recently two patients with chronic severe recalcitrant psoriasis and PsA were reported who experienced significant improvement in both skin and joint disease after treatment with adalimumab 40 mg s.c. every other week. The results of a phase II, double-blinded, placebo-controlled, randomized trial were presented as a poster publication at the American Academy of Dermatology meeting in February 2004. Patients with moderate to severe plaque psoriasis (n=148) were treated with adalimumab 40 mg s.c. weekly, adalimumab 40 mg s.c. every other week (eow), or placebo. At week 12, PASI 50 was achieved by 88 percent of patients treated with adalimumab weekly, by 76 percent of patients treated with adalimumab eow, and by 17 percent of placebo-treated patients. PASI 75 was achieved by 80 percent of patients treated with adalimumab weekly, 53 percent of patients treated with adalimumab eow, and by 4 percent of placebo-treated patients. Adalimumab was well tolerated, and injection site reactions were the primary side effect. The effectiveness of adalimumab in PsA was evaluated in a placebo-controlled, double-blind study, called Adalimumab Effectiveness in Psoriatic Arthritis Trial (ADEPT). Three-hundred and thirteen patients with active PsA received placebo or 40 mg of adalimumab s.c. every other week. Of the 69 patients with greater than three percent of body surface involvement who were treated with adalimumab, 42 percent achieved a PASI 90 response at 24 weeks. Sixty percent of patients achieved an ACR 20 response at week 12, and sustained response through week 24. One-fourth of these patients achieved an ACR 70 response at week 24. The rates of adverse events and serious adverse events in the study were comparable for adalimumab and placebo. Results from the ADEPT trial were reported at the American College of Rheumatology congress in San Antonio, Texas in October 2004.
Cases of tuberculosis have been reported in clinical trials with adalimumab and other TNF blockers in rheumatoid arthritis, and patients should be screened for latent tuberculosis prior to treatment. The incidence of lymphoma was also increased during adalimumab therapy in RA patients compared to the incidence in the general population. However, it is known that patients with rheumatoid arthritis have an increased risk of lymphoma compared to the general population. The assumption that adalimumab is less immunogenic than other anti-TNF-α agents remains to be proven by long-term safety studies.

**Fusion proteins**

**Alefacept**

Alefacept is a fully human fusion protein consisting of the first extracellular domain of LFA-3 fused to the hinge, CH₂, and CH₃ sequences of IgG₁. The LFA-3 domain of alefacept binds CD2 on T cells and blocks the costimulatory LFA-3/CD2 interaction, thereby inhibiting T-cell activation and proliferation. In addition, when alefacept binds CD2 on memory T cells and engages with FcγRIII IgG receptors on natural killer cells, granzyme-mediated apoptosis (programmed cell death) of T cells is induced. Because CD2 expression is higher on memory-effector (CD45RO⁺) CD4⁺ and CD8⁺ T cells compared with naïve (CD45RA⁺) T cells, alefacept is thought to produce a selective reduction in memory T cells. In addition, a small population of circulating dendritic cells is also CD2, suggesting that alefacept could have an additional effect on this cell population.

In a phase II, multicentre, randomized, placebo-controlled, double-blinded study, alefacept was evaluated as a treatment for psoriasis. Two hundred twenty-nine patients with chronic plaque psoriasis received either alefacept i.v. or placebo weekly for 12 weeks. Twenty-four percent of patients who had received alefacept were clear or almost clear after 12 weeks of therapy, and the average duration of remission in those patients who were clear was 8 months. Alefacept reduced peripheral-blood memory effector T-cell (CD45RO⁺) counts, which was correlated with the improvement in psoriasis. Alefacept was well tolerated and no serious adverse events related to alefacept were observed. The reduction in levels of circulating memory T-cell subsets after alefacept therapy was confirmed in other studies.
A phase III, randomized, double-blinded, placebo-controlled study was conducted to evaluate efficacy of two courses of alefacept in patients with chronic plaque psoriasis. Five hundred fifty-three patients received two 12-week courses of once-weekly intravenous alefacept 7.5 mg or placebo. During treatment and follow-up, PASI 75 was achieved by 28% of alefacept-treated patients, and PASI 50 was achieved by 56% of alefacept-treated patients. After a single course of alefacept, patients achieving PASI 75 maintained PASI 50 for a median duration of 7 months. In addition, 40% of patients who received 2 courses of alefacept achieved PASI 75 and 71% of patients achieved PASI 50, indicating that a second course of alefacept increases efficacy.

A multicentre, randomized, double-blinded, placebo-controlled phase III trial investigated the efficacy of intramuscular alefacept. A total of 507 patients with chronic plaque psoriasis were treated with alefacept i.m. (10 mg or 15 mg) or placebo once weekly during 12 consecutive weeks. Mean reductions in PASI in the 15-mg alefacept, 10-mg alefacept, and placebo groups reached a maximum of 46%, 41%, and 25%, respectively, at 6 weeks post dosing. Twenty-one percent of the 15-mg dose group achieved PASI 75 at 2 weeks post dosing. Improvement was long-lasting, and 12 weeks after completion of treatment, mean PASI in both alefacept groups had not returned to baseline values. There were no opportunistic infections and no cases of disease rebound.

Preliminary data suggest that the efficacy of alefacept therapy might be enhanced by combination with narrowband or broadband UVB. In addition to improvement of clinical parameters, alefacept therapy has been shown to be associated with improvement of quality of life of patients with psoriasis. Regarding the safety of alefacept therapy, no opportunistic infections or organ toxicity related to alefacept therapy have been reported as to this date. The only adverse events that had a >5% higher incidence in the alefacept group than the placebo group were chills, pharyngitis, and accidental injuries. However, some concerns have risen due to the fact that an adequate secondary immune response to infectious agents or antigens depends on memory-effector (CD45RO+) T cells, which are affected by alefacept therapy. For this reason a study was performed to assess the effect of alefacept therapy on both primary and secondary responses to a newly encountered antigen and the acquired immune response to a recall antigen (tetanus toxicoid).
Results of this study showed that alefacept did not impair primary or secondary antibody responses to a neoantigen or memory responses to a recall antigen\textsuperscript{151}. Data available from patients treated with up to nine cycles of alefacept indicate that there is no increase in toxicity over time. Other safety issues concern the possible development of lymphoproliferative disorders associated with immunosuppressive therapy. Indeed, three cases of lymphomas were reported during the trials. Future use of alefacept in the postmarketing period will elucidate this important issue. Alefacept is approved for the treatment of moderate to severe psoriasis in the U.S.A., and to date more than 9000 patients with psoriasis have been treated\textsuperscript{152}.

**Etanercept** Etanercept is a recombinant molecule comprising the human TNF-\(\alpha\) p75 receptor fused to the Fc portion of human IgG1 molecule. By blocking the binding of TNF-\(\alpha\) to cell surface receptors, etanercept neutralizes the biologic activity of TNF-\(\alpha\). In a double-blinded, placebo-controlled study 60 patients with psoriasis and PsA were randomized to receive either placebo or etanercept 25 mg s.c. twice weekly for 12 weeks. After 12 weeks, the median PASI improvement was 46 percent in etanercept-treated patients versus 9 percent in placebo-treated patients. PASI 75 was achieved by 26 percent of etanercept-treated patients, compared to none of the placebo-treated patients. ACR 20 was achieved by 73 percent of etanercept-treated patients versus 13 percent of placebo-treated patients. No serious adverse events were reported\textsuperscript{153}. Another randomized, double-blinded, placebo-controlled study to investigate the efficacy and safety of etanercept in 112 psoriasis patients showed similar data. After 12 weeks of treatment, 30 percent of the etanercept-treated patients achieved PASI 75, compared to 2% of placebo-treated patients. The observed improvement was sustained in time, and by 24 weeks of treatment 56 percent of etanercept-treated patients versus 5 percent of placebo-treated patients achieved PASI 75. Adverse events were similar among etanercept and placebo groups, except for injection site reactions which occurred more frequently in patients treated with etanercept\textsuperscript{154}. This phase II proof-of-concept study demonstrated that etanercept in patients with psoriasis was well tolerated and significantly improved the signs and symptoms of disease\textsuperscript{155}. Based on these results, a larger trial followed. In a 24-
week, double-blinded, phase III study 652 psoriasis patients were treated with either etanercept s.c. at a low dose (25 mg once weekly), a medium dose (25 mg twice weekly), a high dose (50 mg twice weekly), or with placebo. After 12 weeks, patients in the placebo-group began treatment with etanercept 25 mg twice weekly. At week 12, PASI 75 was achieved by 14 percent of patients in the low-dose group, 34 percent of patients in the medium dose group, and 49 percent in the high dose group, compared to 4 percent of patients in the placebo-group. The clinical responses continued to improve with longer treatment. At week 24, PASI 75 was achieved by 25 percent of patients in the low-dose group, 44 percent of the patients in the medium-dose group, and 59 percent of patients in the high-dose group. No occurrence of opportunistic infections or tuberculosis was reported during the course of the study. Eight etanercept-treated patients had serum samples that tested positive for non-neutralizing anti-etanercept antibodies, but no differences in efficacy or adverse events were observed in these patients compared to patients without anti-etanercept antibodies. The efficacy of etanercept in PsA was confirmed in a placebo-controlled, double-blinded trial in which 205 patients with active PsA received either placebo or 25 mg etanercept s.c. twice weekly plus a stable dose of methotrexate for 24 weeks. Differences in clinical response between the groups were evident at week 4, and were maintained throughout the treatment period. At 12 weeks, ACR 20, ACR 50, and ACR 70 was achieved by 59, 38, and 11 percent of patients in the etanercept group and 15, 4, and 0 percent of patients in the placebo group, respectively. After 24 weeks of treatment, ACR 20, ACR 50, and ACR 75 was achieved by 50, 37, and 9 percent of etanercept-treated patients, whereas the corresponding placebo responses were 13, 4, and 1 percent, respectively. In general etanercept therapy was well tolerated, however one patient in the etanercept group developed multiple sclerosis at the end of the study. Regarding the safety profile of etanercept, some published reports have reported drug-induced systemic lupus erythematosus (SLE) in association with etanercept therapy. However, the lupus-like symptoms in all reported cases resolved following discontinuation of therapy. Etanercept is approved for treatment of psoriasis and PsA in the U.S.A and Europe.
Cytokines and chemokines

rhIL-4 Psoriasis is characterized by the presence of type-1 cytokine-producing T cells in lesional skin\(^39\). In experimental animal models of type-1 mediated autoimmune diseases immune deviation of type-1 into anti-inflammatory type-2 responses generally improves the disease, without inducing general immunosuppression. Recombinant human IL-4 (rhIL-4), which induces a type-2 phenotype, has been developed for treatment of psoriasis. In a prospective dose-escalating study, rhIL-4 s.c. was administered to 20 patients with severe psoriasis 3 times daily, 5 days a week, for 6 weeks. PASI decreased in all patients treated with rhu-IL-4; psoriasis improved more than 50\% (PASI 50) in 19 patients. On immunohistochemical evaluation of lesional skin, rhu-IL-4 induced a switch from type-1 to type-2 responses, with close correlation between clinical improvement and reversal of the IFN-\(\gamma\)/IL-4 ratio. Adverse events were mild and included fever, headache, and oedema\(^{160}\).

rhIL-11 Recombinant human IL-11 (rhIL-11) has demonstrated anti-inflammatory effects in vitro and in vivo. In animal models, treatment with rhIL-11 reduced pro-inflammatory cytokine levels produced by T cells and macrophages, such as IFN-\(\gamma\) and TNF-\(\alpha\)\(^{161}\), and polarized the T-cell response toward a type-2 response with increased IL-4 production\(^{162}\). In an open-label, dose-escalating, phase I clinical trial rhIL-11 s.c. was administered to 12 patients with psoriasis every day for 8 weeks. Eleven of 12 patients experienced reduction in PASI, ranging from 20 to 80 percent. Amelioration of disease by rhIL-11, as shown by reduced keratinocyte proliferation and cutaneous inflammation, was associated with decreased expression of products of disease-related genes, including k16, iNOS, IFN-\(\gamma\), IL-8, IL-12, TNF-\(\alpha\), IL-1\(\beta\), and CD8, and with increased expression of endogenous IL-11\(^{163}\).

Other

Tadekinig-alpha (rh-IL-18 BP) Tadekinig-alpha is a recombinant unmodified form of the naturally occurring human IL-18 binding protein that is capable of neutralising the biological activity of IL-18. IL-18 was first identified as an IFN-\(\gamma\)-inducing factor\(^{164,165}\), and it has costimulatory functions on other type-1 cytokines such as TNF-\(\alpha\) and IL-1 as well. It is believed that by reducing the levels of these
pro-inflammatory cytokines by rh-IL-18 BP, immunological balance in psoriasis and PsA will be restored. Phase I studies of r-hIL-18 binding protein are now completed, and a phase II clinical study is on-going in psoriasis and PsA.

**Aims of the studies**

In the last decade a whole range of new 'biological' response modifiers have emerged for the treatment of psoriasis and PsA. Although clinical efficacy has been monitored in multicentre, randomized, placebo-controlled, double-blinded trials, less is known about the actual mechanism of action of these drugs in lesional skin and synovium of patients with psoriasis and PsA in situ. Because biological response modifiers act on very specific steps in the immunological cascade, investigation of changes in immunohistochemical markers in lesional skin and synovium might provide us with more insight into the immunopathogenesis in psoriasis and PsA. Traditionally, the evaluation of the cellular infiltrate and protein expression 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 in this situation we compared the assessment of immunohistochemically stained skin sections with the more traditional manual quantification and semi-quantitative analysis (chapter 2). The digital image analysis was used in subsequent studies with biological response modifiers. In chapter 3, we investigated the immunohistochemical changes in lesional psoriatic skin after alefacept therapy, a LFA-3/IgG1 fusion protein that interferes with the activation and proliferation of T cells by binding to the CD2 receptor on their surfaces. We focussed on lesional memory-effector T cells in particular, since it is known that alefacept selectively reduces memory-effector T cells in peripheral blood. In a similar way, we investigated changes in the inflammatory infiltrate, in particular memory-effector T cells, in synovial tissue in patients with PsA after alefacept therapy. This was the first time alefacept was administered to patients with PsA, and the clinically efficacy of this drug in PsA was reported as well (chapter 4). Next to biological response modifiers interfering with T cell activation, such as alefacept, the use of TNF-α-inhibitory drugs has proven to induce rapid and profound
improvement in clinical signs and symptoms of psoriasis and PsA. The mechanism of action of infliximab, a chimeric anti-TNF-α antibody, has not been fully elucidated yet. In vitro studies suggest that besides neutralization of TNF-α, infliximab might be able to induce apoptosis of TNF-α producing cells via activation of complement-dependent or antibody-dependent cell-mediated toxicity. In chapter 5, we first investigated the early effects (after 48 hours) of infliximab on serial skin and synovial tissue biopsy samples of patients with PsA, focussing in particular on apoptosis of T cells. Next, we evaluated the influence of infliximab on T cell infiltration and expression of adhesion molecules after 4 weeks of infliximab therapy in the same group of PsA patients (chapter 6). Another TNF-α-inhibitory drug that is known to improve clinical signs and symptoms of psoriasis and PsA is etanercept, 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. To compare the mechanism of action of etanercept with infliximab, we studied the effects of etanercept therapy on the T-cell infiltrate, expression of adhesion molecules, and expression of angiogenesis markers in lesional skin of patients with chronic plaque psoriasis in a double-blinded placebo-controlled study (chapter 7).
Table 1. List of biological response modifiers that are approved or under development for psoriasis and/or psoriatic arthritis.

<table>
<thead>
<tr>
<th>Binding characteristics</th>
<th>Generic name</th>
<th>Brand name</th>
<th>Molecular structure</th>
<th>Mode of action</th>
<th>Indication / Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monoclonal antibodies</strong></td>
<td>Anti-CD11a</td>
<td>Efalizumab</td>
<td>Humanized Mab</td>
<td>Binds to α subunit of LFA-1; blocks LFA-1 / ICAM-1 interaction</td>
<td>Psoriasis, FDA and EMEA approved</td>
</tr>
<tr>
<td></td>
<td>Anti-TNF-α</td>
<td>Infliximab</td>
<td>Chimeric Mab</td>
<td>Neutralizes TNF-α; mediated lysis of TNF-α* cells</td>
<td>Psoriasis and PsA, phase III</td>
</tr>
<tr>
<td></td>
<td>Adalimumab</td>
<td>Humira</td>
<td>Humanized Mab</td>
<td>Neutralizes TNF-α</td>
<td>Psoriasis and PsA, phase II and III</td>
</tr>
<tr>
<td><strong>Fusion proteins</strong></td>
<td>Anti-CD2</td>
<td>Alefacept</td>
<td>Fusion protein of extracellular domain of human LFA-3 and Fc part of human IgG1</td>
<td>Inhibits LFA-3 / CD2 interaction, induces NK-cell mediated T-cell apoptosis</td>
<td>Psoriasis, FDA approved</td>
</tr>
<tr>
<td></td>
<td>Anti-TNF-α</td>
<td>Etanercept</td>
<td>Fusion protein of the extracellular domain of the human TNF-αR and Fc part of human IgG1</td>
<td>Neutralizes TNF-α</td>
<td>Psoriasis and PsA, FDA and EMEA approved</td>
</tr>
<tr>
<td><strong>Recombinant cytokines and chemokines</strong></td>
<td>IL-4</td>
<td>Rhu-IL-4</td>
<td>?</td>
<td>Recombinant human IL-4</td>
<td>Immune deviation from Th1 to Th2</td>
</tr>
<tr>
<td></td>
<td>IL-11</td>
<td>Rh-IL-11</td>
<td>?</td>
<td>Recombinant IL-11</td>
<td>Immune deviation from Th1 to Th2; suppression of inflammation</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>IL-18BP</td>
<td>Tadecinig -alpha</td>
<td>?</td>
<td>Recombinant human IL-18 binding protein</td>
<td>Neutralizes IL-18</td>
</tr>
</tbody>
</table>
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