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1 GENERAL INTRODUCTION AND AIMS OF THE THESIS

INTRODUCTION

Psoriasis is one of the most common skin diseases and has been recognized since ancient times. Although sometimes mixed up with for example leprosy in early times, psoriasis is viewed as a separate entity since 1841[1]. Psoriasis is estimated to have an incidence of 1-3% worldwide and prevalence rates varies from 0.5% in China to 4.8% in Norway [2].

This thesis focuses on psoriasis vulgaris, also known as chronic plaque psoriasis, and this is the most common form of psoriasis occurring in more than 80% of cases. It is characterized by symmetrical red scaly well-demarcated plaques that are typically found on elbows, knees, the scalp, buttocks and genitalia. There are clinical variants of psoriasis, defined as subsets, with identical histopathological changes in the skin. These subsets are described based on their form (guttate, pustular, annular) or their distribution (inversa, palmoplantar, erythrodermic). Occasionally combinations of the different types develop simultaneously or sequentially over time in the same patient. About 50% of patients with psoriasis have distinctive nail changes, e.g. pitting, onycholysis, oil-spots and dystrophy, related to the disease [3].

In addition to the skin and nail involvement, a seronegative inflammatory arthritis can develop in 7-39% of psoriasis patients [4-7]. This wide range is probably due to variable methods of assessment. More recent studies suggest that the prevalence of psoriatic arthritis tends towards the higher end of this range. The course of psoriatic arthritis varies, with some having mild changes and others severe, rapid destruction of joints. Usually the skin lesions precedes the involvement of the joints or tendons, but in 19% the arthritis is present before skin lesions appear [8].

Psoriasis is classified as mild, moderate, or severe. This classification takes account of the severity of cutaneous manifestations, which are usually rated with the Psoriasis Area and Severity Index (PASI) [9-11]. This index is based on the degree of erythema, infiltration, and scaling and the extent of involvement of the four body areas (head, trunk, arms and legs). Psoriasis is classified as mild if the PASI is below 10, and moderate to severe if it is 10 or above; the highest possible PASI value is 72. Total body surface area (BSA) is another method to classify the severity of psoriasis, and then BSA >10 is the criterion for moderate to severe psoriasis [12].

Once psoriasis appears, it is usually a life-long disease characterized by a variable and unpredictable course and spontaneous remissions is uncommon [13]. Psoriasis patients need long-term therapy, which often give rise to a variety of side-effects, including organ-toxicity. Although psoriasis itself is not life-threatening, it causes significant psychosocial morbidity and a decrease in health-related quality of life [14-18]. Quality of life in psoriasis patients equals or exceeds that due to other severe disorders such as diabetes, rheumatoid arthritis, or cancer [15;18;19]. The combined costs of long-term therapy and social costs of the disease due to reduced levels of employment have a major impact on health care systems and on society in general.

There is increasing awareness that psoriasis as a disease is more than skin deep [3]. Co-morbidities thought to have an increased prevalence in psoriasis include cardiovascular disease, lymphoma, non-melanoma skin cancer, Crohn's disease and metabolic disorder [20-32]. The relative influence of known confounders like concomitant therapy with immunosuppressants and phototherapy, smoking and alcohol is currently unknown [33-36].

Although there is currently no cure for psoriasis, there are several treatment options comprising topical therapy, phototherapy and systemic agents. In Table 1 an overview of currently applied (and widely approved) treatments of psoriasis is given.

Table 1

Topical	Photo(chemo)therapy	Systemic
Corticosteroids[186]	Broadband UVB (290-320nm)[187]	Methotrexate[188]
Dithranol[189]	Narrowband UVB (311 nm)[190]	Cyclosporine A[53]
Tar[191]	PUVA (320-400 nm)[192]	Acitretin[193]
Tazarotene[194]	Excimer laser (308)[195]	Fumaric acid[196]
Vitamine D analogues[197]		Biologicals[198]

IMMUNOPATHOGENESIS

The cause of psoriasis can be considered multifactorial, resulting from an interaction of genetic [37;38], environmental [39;40], and immunological factors.

Twin and family studies have shown that psoriasis has a strong genetic component although the inheritance pattern is still unclear. In patients with childhood psoriasis 71% have a positive family history [41]. Siblings and first-degree relatives of psoriasis patients show a four-fold or more increased risk in developing psoriasis [42]. Studies of disease concordance among twins show a risk of psoriasis that is two to three times as high among monozygotic twins as among dizygotic twins [43-45]. At least ten chromosomal loci have been identified that are evidently linked to psoriasis (PSOR1-10), the most important of which is PSOR1 on chromosome 6p21 and is considered to be responsible for up to 50% genetic susceptibility [46].

Environmental factors that trigger psoriasis in genetically predisposed individuals are suggested with the finding that psoriasis often manifests itself initially, or is worsened at some point in its further course, after exposure with factors of various types. The main ones that have been identified are streptococcal upper respiratory infections, certain medications (beta-blockers, ACE-inhibitors, lithium salts, interferon-alpha, anti-malarials) and stress [47;48].

With regard to immunological factors, the hypothesis of the pathogenesis of psoriasis is continuously evolving with new developments achieved in immunology.

Until the early 1980s, psoriasis was believed to be a disease primarily of epidermal keratinocyte proliferation. Along with the development of immunohistochemical staining techniques, it was discovered that the leukocyte infiltrate consists mainly of CD4 positive and CD8 positive T cells [49-51]. Furthermore, reported beneficial effects of specific T-cell therapies, such as cyclosporin A and DAB389IL-2 suggesting a prominent role for T cells in the pathogenesis of psoriasis [52-56]. Some years later, when the T cells were divided into Th1 and Th2 on the basis of the cytokine production profile, the T-cell hypothesis was refined and psoriasis was considered a Th1-cell-associated disease with a prominent role for interferon (IFN)-γ [57-59].

In recent years, clinical and basic science observations have shown that innate immunity as well as adaptive immunity is crucial in the initiation and maintenance of psoriatic plaques [60;61]. Innate immunity comprises the immediate response against pathogens and usually precedes the adaptive immunity, which requires several days to develop. The division in innate and adaptive immunity is not so clear-cut, however, as some overlap exists. In addition, these two branches do not operate independently, but rather are able to influence each other [62]. Practically all cellular and humoral elements of the innate part of the skin immune system are upregulated or increased in lesional skin of psoriasis patients [63]. Keratinocytes, neutrophils, natural killer (NK) cells, NK T cells and dendritic cells are all part of the cutaneous inflammation in psoriasis [60]. Of the dendritic cells specifically plasmacytoid dendritic cells are of importance. This specific type of dendritic cell accumulates in the skin of psoriasis patients and produces IFN-α early in the development of psoriasis. This activates and expands the autoimmune T cell cascade leading to psoriasis, and may provide an unique link between the innate and adaptive immune system in driving inflammation in psoriasis [64].

In 2005, a new type of T cell, Th17, was described [65]. Th17 cells are distinguished from both Th1 and Th2 cells in that they secrete a distinct set of proinflammatory cytokines, including IL-17A (IL-17), IL-17F, IL-6, and, to a lesser extent, TNF-α and IL-22 [66-70]. Th17 cells and Th17-related cytokines play a pivotal role in the pathogenesis of psoriasis [65;71;72]. IL-17 potently stimulates keratinocytes to produce proinflammatory cytokines and IL-22 induces proliferation of keratinocytes and production of antimicrobial peptides as well as chemokines [71;73] . Furthermore, successful anti-TNF treatment reduces Th17 cells in psoriasis lesions [74]. Th17 cells activation is induced by IL-23, which is overproduced by activated dendritic cells in psoriasis lesions [75;76] and keratinocytes [77]. Biologically active IL-23 is a heterodimer molecule consisting of a unique p19 subunit and a p40 subunit shared with heterodimer IL-12, which combines the p40 subunit with a specific p35 subunit. IL-12 is a cytokine that promotes Th1 cell differentiation and production of IFN-γ [78]. Both IL-23 p19 and IL12/IL-23 p40 mRNA are increased in skin lesions of psoriasis patients, but in contrast IL-12 p35 mRNA expression is decreased compared with uninvolved skin [62].

More recently, in 2009, inflammatory CD4+ T cells were described, that produced IL-22, but do not express IL-17A or IFN-γ, the so-called Th22 cells [79-82], which are

also increased in psoriasis [81;83]. Concentration of plasma IL-22 is higher in psoriatic patients and levels are highly reflective of skin disease activity [84]. In the skin, IL-22 induces antimicrobial peptides, promotes keratinocyte proliferation, and inhibits keratinocyte differentiation, which suggests a role in remodeling wound healing and in innate defense mechanisms [85].

At present it is unknown whether Th1, Th17 and Th22 cells might cooperate in order to amplify immune responses or whether these cells are involved at different stages during development of the inflammation in psoriasis. In model systems cross-regulation between Th1 and Th17 was demonstrated, suggesting important functional interactions [86;87]. Lowes et al. demonstrated Th17 to be a discrete population localized predominantly to the dermis of psoriasis skin lesions, separate from Th1 cells, suggesting a mixed Th1 and Th17 inflammatory environment [88]. Th17 cells might participate in the initial acute inflammation, while Th1 cells are involved in prolonging and perpetuating tissue inflammation [89]. The clinical relevance of Th22 remains to be determined [84].

During the last decade a tremendous progress in understanding of the pathogenesis of psoriasis was made [78]. Discovery of new cell types (e.g. plasmacytoid dendritic cells, NK T cells), new cytokines, and other developments in fundamental and clinical immunology have all been incorporated in the etiology of this skin disease. The current model comprises a complex network with many different cell types that can reciprocally stimulate each other by a still growing list of bound and soluble factors (see Figure 1). What causes this cascade of immunological events in skin of psoriasis patients is still a mystery. Due to its complexity it is as yet (and perhaps will always be) impossible to pinpoint a single cell, factor, or gene as the culprit of psoriasis.

CHEMOKINES AND CHEMOKINE RECEPTORS IN PSORIASIS

For psoriatic lesions to develop, inflammatory cells must be able to migrate into lesional skin. Chemokines are small (8-14kD) chemotactic peptides that have an important role in host defence by regulating the migration of passing immune cells. More than 45 chemokines have been identified [90] and their secretion can be controlled by various agents including cytokines and lipopolysaccharide [91]. They have been grouped into four classes -CXC, CC, CX_3C and C- on the basis of their disposition and number of invariant cysteines [92]. A common feature of most chemokines is a heparin-binding domain comprised mainly, but not exclusively, of residues in an α-helical region near the C-terminus [93]. This allows chemokines in the blood to bind to glycosamineglycans that are exposed on the cell surface of endothelial cells, thus rapidly forming a solid-phase chemotactic gradient. By this means they are able to attract and activate passing immune cells [94]. Chemokines exert their biological effects by binding to and activating cell-surface receptors that belong to the G-protein-coupled receptor (GPCR) superfamily. Currently, 19 chemokine receptors have been identified [95]. They are

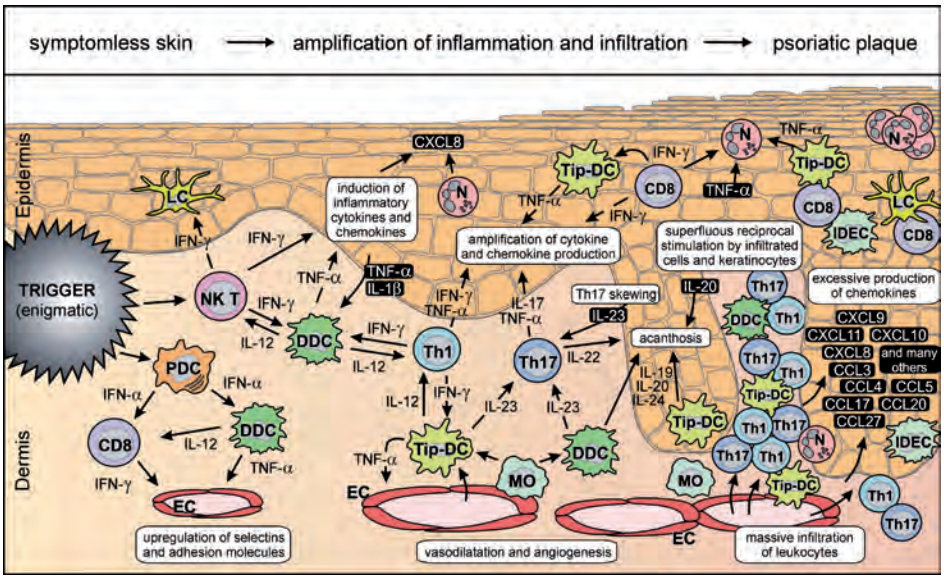

Figure 1 The immunopathogenesis of psoriasis in a nutshell. A still enigmatic trigger (injury, infection, otherwise?) of the innate immune system initiates a cascade of events in normal- appearing skin of psoriatic individuals. IFN-α released from activated plasmacytoid dendritic cells (PDC) stimulates myeloid dermal dendritic cells (DDC) to produce cytokines that support TH1 development. In addition, CD8+ cutaneous psoriatic T cells (Th1-prone and autoreactive) have an increased sensitivity to IFN-α and are activated to produce IFN-γ. Triggered NK-T cells also release IFN-γ and in concert with the TNF-α from dermal dendritic cells these cytokines stimulate keratinocytes to synthesize inflammatory cytokines and chemokines. As a result an array of leukocytes are recruited into the skin, like neutrophils (N), all kinds of T cells, Langerhans cells (LC), inflammatory dendritic epidermal cells (IDEC), TNF/iNOS-producing- dendritic cells (Tip-DC), and monocytes (MO). The latter may differentiate into dendritic cells under inflammatory conditions of the tissue. Activated resident DDC and infiltrating Tip-DC produce IL-12 and IL-23, which stimulate Th1 and Th17 cells to release IFN-γ and IL-17, respectively. TNF-α, IFN-γ, and IL-17 activate synergistically keratinocytes to produce huge amounts of inflammatory cytokines and chemokines, which leads to an amplification of the infiltration and activation of leukocytes. IL-20 subfamily cytokines released by DDC, Tip-DC, and T cells cause hyperproliferation of the keratinocytes. Likely due to a lack of sufficient downregulatory capacity of the skin immune system the superfluous reciprocal activation signals between numerously infiltrated leukocytes and keratinocytes ends up in a vicious circle of inflammation and a psoriatic plaque develops. Cytokines released by leukocytes are depicted in black letters, while the factors produced by keratinocytes are in white letters on a black background. *Reprinted with permission from Edizioni Minerva Medica: Giornale Italiano di Dermatologia e Venereologia. Teunissen MBM, Piskin G, Res PCJM, De Groot M, Picavet DI, De Rie MA, Bos JD. State of the art in the immunopathogenesis of psoriasis. Giornale Italiano di Dermatologia e Venereologia 2007;142(3):229-242.*

expressed on a variety of cells including immune cells, endothelial cells and neurons [96], and are either constitutively activated or induced by agents such as cytokines [97] and lipopolysaccharide [98]. Each receptor has a repertoire of chemokine ligands that activates it. These range from CCR1, which has at least nine ligands that bind with

high affinity, to specific receptors such as CCR8, which only has one ligand [92]. So, it seems that there is a great degree of redundancy in the chemokine receptor system. This is accentuated even further by the fact that some chemokines can bind with high affinity to more than one receptor. For example, CCL5 (also known as RANTES) can bind to CCR1, CCR3 and CCR5. By contrast, others such as CCL1 (also known as I-309) only bind a single receptor (CCR8). In addition, chemokines that are agonists for one receptor can be natural antagonists for others [99].

Various studies have documented a strong chemokine expression in keratinocytes in psoriatic skin, and the production of chemokines by keratinocytes may contribute relevantly to the formation of the inflammatory infiltrate [100]. The intra-epidermal accumulation of neutrophils, a characteristic feature of psoriasis, is caused by CXCL8 (also known as IL-8) and CXCL1(also known as GRO-α), which were found to be in a high content within psoriatic scales [101-103]. In addition, the infiltrating neutrophils in psoriatic skin express the corresponding receptors CXCR1 and CXCR2, of which CXCR2 is overexpressed in psoriatic skin [104-106].The monocytes and Th1 cells found in psoriatic lesions are attracted predominantly by CCL2 (also known as MCP-1), CCL5, CXCL9 (also known as Mig) and CXCL10 (also known as IP-10) [107-110].

The predominant chemokine receptors expressed on Th1-cells are CCR5 and CXCR3 [63;111-116]. Besides its preferential expression on Th1 cells, CCR5 is also expressed on monocytes, macrophages, natural killer and dendritic cells: all thought to be significant elements in the pathogenesis of psoriasis [117-127]. The ligands of CCR5 (CCL3, CCL4 and CCL5 (also known as MIP1α, MIP1β and RANTES, respectively)) are highly expressed by keratinocytes in psoriatic tissue [128-131]. Furthermore, it has been demonstrated that the proinflammatory cytokines IFN-γ and TNF-α can induce the expression of these chemokines [129;130] and that treatment of psoriasis resulted in a significant decrease of CCL5, as well as a reduction of CCR5+ T cells in the skin [130;132;133].

The other chemokine receptor predominantly expressed on Th1 lymphocytes, CXCR3, has been suggested to be one of the major chemokine receptors responsible for their recruitment to inflamed sites *in vivo* [134]. In addition, it can also be expressed by natural killer (NK) cell, B cells, plasmacytoid dendritic cells and myeloid dendritic cells [135-141]. The presence of infiltrating $CXCR3^+$ T cells in psoriasis as well as other inflammatory skin disorders has been reported [142-147]. The cognate ligands of CXCR3, CXCL9, CXCL10 and CXCL11 (also known as ITAC) are induced by IFN-γ and have been shown to be expressed by inflammatory cells and/or keratinocytes in psoriatic skin and lesional skin of other inflammatory skin disease [107;143;148].

Another important chemokine receptor in the pathogenesis of psoriasis is CCR6[149]. This chemokine receptor is expressed on the Th17 subsets of CD4+ T cells[150] and, together with its ligand, CCL20 (also known as MIP-3α), it is expressed at statistically higher levels in lesional psoriatic skin than in non-lesional or normal donor skin[151]. Furthermore, studies have shown that CCR6 is necessary for the pathology induced in a mouse model of psoriasis-like inflammation[152]. A nice overview on chemokines and chemokine receptors that have been associated with psoriasis is published by Homey[153].

NEW DIRECTIONS IN PSORIASIS THERAPY

Although there are several systemic treatments for patients with moderate to severe psoriasis, they do not fully meet the needs of patients [154]. The toxicity, inefficacy and often inconvenience of current conventional treatments, in addition to the impaired quality of life in psoriasis patients, call for new therapeutics options.

With the growing knowledge and understanding of the pathogenesis in psoriasis, more specific immunomodulating therapies are being developed, the so-called 'biologic response modifiers' or 'biologics'. These custom-made, protein-like molecules can target specific parts of the activated immune system in psoriasis, e.g. activation, co-stimulation, or proliferation of T cells, their trafficking into the skin, or effector cytokines [155;156]. Since 1989 several biological therapies have been tested for psoriasis and in January 2003 Alefacept (Amevive®), anti-CD2, was the first biological that was approved by the US Food and Drugs Administration (FDA). In Table 2 an overview is given of all biological therapies registrated by the European Medicines Agency (EMEA) and the FDA for the treatment of moderate to severe plaque type psoriasis.

Alefacept is an intravenously or intramuscularly administered fusion protein which contains of human immunoglobin (IgG) and the binding site of lymphocyte function-associated antigen-3 (LFA-3). It binds to the CD-2 receptor located on T cells, resulting in inhibition and memory T cell activation and proliferation [157]. Beside T cells, CD2 is also expressed on NK cells and a small population of CD14+ DC's [158]. Two randomized, double-blind, placebo-controlled trials showed an improvement of PASI with 75% (PASI-75) after 12 weeks of treatment in 28 to 40% of the patients [159;160]. Alefacept is currently approved in the USA and in Europe only in Switzerland.

Another anti-CD2 therapy, which is currently still under investigation, is siplizumab (MEDI-507). In vitro studies of this humanized monoclonal anti-CD2 antibody have shown that high doses of siplizumab cause depletion of lymphocytes, whereas lower doses induce T-cell hyporesponsiveness. Currently, clinical studies in psoriasis patients are going on [161;162].

Table 2

Generic name	Brand name	Mode of action	registrated indications beside psoriasis
alefacept	Amevive®	anti-CD2	-
efalizumab	Raptiva®	anti-CD11a	-
etanercept	Enbrel®	anti-TNF-α	Pediatric plaque psoriasis, RA, PsA, JIA, AS
infliximab	Remicade®	anti-TNF-α	RA, PsA, AS, Crohn's disease (childeren and adults), ulcerative colitis
adalimumab	Humira®	anti-TNF-α	RA, PsA, JIA, AS, Crohn's disease (adults)
ustekinumab	Stelara®	anti-p40 IL12/IL23	-

RA, rheumatoid arthritis; PsA, psoriatic arthritis; JIA, polyarticular juvenile idiopathic arthritis; AS, ankylosing spondylitis

Efalizumab was also designed to interfere with T-cell adhesion and co-stimulation. This humanized murine monoclonal antibody targets CD11a, which is a subunit of leukocyte function-antigen-1 (LFA-1) that is expressed on all leukocytes. In order for leukocytes to bind to other cell types, CD11a needs to bind to intercellular adhesion molecule-1 (ICAM-1). Efalizumab blocks this binding, which interrupts many processes, including the activation of T cells, adhesion of T cells to endothelial cells and migration of T cells to sites of inflammation, including psoriatic skin [163]. In clinical trials PASI-75 was achieved in 22-39% of patients after 12 weeks of treatment with 1 mg kg^{-1}/wk efalizumab subcutaneously [164]. In February 2009 the European Medicines Agency (EMEA) has recommended the suspension of the marketing authorization for efalizumab after concluding that the benefits of efalizumab no longer outweighed its risks, because of safety concerns, including the occurrence of progressive multifocal leukoencephalopathy (PLM) in three patients treated with efalizumab [165].

Currently there are three registered TNF-α inhibitors for the treatment of psoriasis. Etanercept was the first to be approved and is a human TNF-α receptor and immunoglobulin fusion protein that binds TNF-α and lymphotoxin-α, rendering TNF-α biologically inactive. As a result etanercept modulates multiple biological responses induced or regulated by TNF-α, including serum levels of cytokines, expression of adhesion molecules responsible for leukocyte migration and, to a lesser extent, ICAM-1 [166]. About 30% of patients treated in doses of 25 mg twice weekly and 50% of patients treated in doses of 50 mg twice weekly achieve PASI 75 in 12 weeks. Continuing therapy up to 6 months improves response rates further to 43% and 57% for 25 mg biweekly and 50 mg biweekly, respectively [167-170].

While etanercept is a fusion protein, infliximab and adalimumab are both monoclonal antibodies directed at TNF-α. Infliximab is a chimeric monoclonal antibody given intravenously every 2 months after a loading period. Onset of action is rapid, with evidence of significant improvement within the first 2 weeks of treatment and maximum benefit by week 10, when 79% of patients achieve PASI-75 [171-173].

Adalimumab is a human monoclonal antibody given subcutaneously once every other week. As with infliximab, onset of action is rapid, with significant improvements in disease severity within 2 weeks of treatment initiation. At week 12, 69% of patients treated with 40 mg every other week achieve PASI-75 [174;175].

Currently, two new monoclonal antibodies against TNF-α are on the horizon, golimumab and certolizumab. Golimumab (Simponi®) is the first transgenic human monoclonal antibody against TNF-α approved for the treatment of rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis. It is synthesized using conventional hybridoma technique after immunizing transgenic mice containing human immunoglobin genes. The constant region of golimumab is identical to that of infliximab, but the variable regions of golimumab have fully human sequences [176]. Certolizumab (Cimzia®) is a PEGylated Fab'fragment of an anti-TNF-α monoclonal antibody. The compound binds to TNF-α and prevents binding to cell surface receptors. As certolizumab does not contain an Fc region, unlike infliximab and adalimumab, it does not fix complement or cause antibody-dependent cell-mediated

cytotoxicity in vitro [177]. It has been approved for the treatment of Crohn's disease and rheumatoid arthritis [178]. Although both medications are currently not registered for the treatment of psoriasis, given their mechanisms of action, it is likely to have similar benefits as other TNF-α inhibitors and therefore in the future they may be added to the treatment options for patients with moderate to severe psoriasis.

More recently, monoclonal antibodies directed against p40, a polypeptide shared by IL-12 and IL-23, are being tested in clinical trials. Ustekinumab is the first registered IL-12/IL-23 inhibitor in Europe and the US for the treatment of moderate to severe plaque psoriasis. In total 2,666 patients were investigated in three large randomized controlled clinical trials and both doses of ustekinumab (i.e. 45 mg and 90 mg) were highly effective in psoriasis. Onset of action is evident within 2 weeks, with 67% and 72% of patients achieving PASI 75 by week 12 for the 45 mg and 90 mg doses, respectively, and maximal efficacy evident between week 20 and week 24 [179-181]. Remarkable is the low dosing frequency: at week 12 only two administrations of the drug have been given to the patients. Ustekinumab has a median half-time of approximately 3 weeks, yet the dosing frequency is once every three months, after a loading period. Although this appears to be favorable for the patient, in case of an infection or non-elective surgery treatment cannot be antagonized. Recently, ustekinumab was compared with etanercept in chronic plaque psoriasis in a large phase II randomized controlled trial. The percentage of patients achieving PASI 75 by week 12 with ustekinumab 90 mg and 45 mg at week 0 and 4 was 74% and 68%, respectively, compared with 56% for patients randomized to etanercept 50 mg biweekly for 12 weeks [182]. Besides psoriasis, ustekinumab is currently also being evaluated for the treatment of psoriatic arthritis [183].

ABT-874 (Briakinumab®) is another monoclonal antibody directed against p40 IL-12/IL-23 and is in the pre-registration phase for treatment of chronic plaque psoriasis [184]. A phase II randomized placebo-controlled trial showed that over 90% of patients receiving more than one dose (100 or 200 mg) of ABT-874 achieved a PASI 75 by 12 weeks [185].

With the increasing knowledge and understanding of psoriasis, new treatment options are now available without the cumulative organ toxicity of systemic treatments like methotrexate and cyclosporine. The usefulness of this new generation of sophisticated therapeutical agents is a good example that fundamental research in immunology is indispensable for human health. With these more specific immunomodulating therapies, the treatment of patients with moderate to severe psoriasis has shifted to long-term disease management, and hence, requires long-term evaluation of efficacy and safety. For this purpose patient registers are indispensable.

And although tremendous progress has been made in the last decade regarding the understanding of the pathogenesis of psoriasis, and subsequently the treatment of psoriasis patients, psoriasis still remains a chronic disease, which we cannot cure. Therefore, the ultimate challenge for the next decade will be the determination of the factor or factors that actually trigger psoriasis, enabling the possibility to prevent this skin disorder in genetically predisposed individuals.

AIMS OF THE STUDIES

The first part of this thesis considers the clinical and/or the effects on different leukocyte subsets in situ of several registered biological treatments for psoriasis. The last two chapters consider possible treatment targets for psoriasis patients.

Although biologics are a big leap forward for the treatment of moderate to severe psoriasis, these effective treatments still show side-effects. In **Chapter 2a** a case-report on a clinical side-effect is described of a psoriasis patient treated with efalizumab. **Chapter 2b** is a written response to a comment made in the discussion of another case-report, which referred to the case-report mentioned in 2a.

Randomized clinical trials regarding biologics show large clinical improvement in patients with moderate to severe psoriasis. Yet, patients included in these studies did not have to fit strict criteria regarding unresponsiveness to several systemic treatments, contrary to patients in daily practice. **Chapter 3** describes the results of a retrospective clinical study evaluating the non-trial based clinical response of normal and high dosed etanercept treatment in psoriasis patients.

The remarkable improvements of psoriasis seen in clinical trials with tumor necrosis factor (TNF)-α antagonist, together with the discovery of activation of several cellular and humoral components of the innate immune system, suggests that aggravation of innate immunity plays an important role in the pathogenesis of psoriasis. We hypothesizes that, if malfunctioning of the innate immune system is somehow a pivotal initiator of psoriasis, successful treatment of psoriasis would diminish the expression of markers of innate immunity. The in situ effects of etanercept treatment in psoriasis patients, specifically on different inflammatory markers, are described in **Chapter 4**.

In **Chapter 5a**, the same inflammatory markers as in Chapter 4 are examined in psoriasis skin lesions, but now after treatment with adalimumab in a prospective, randomized, placebo-controlled study in patients with psoriatic arthritis. **Chapter 5b** describes the results of study in synovial tissue. Both studies were done in order to identify biomarkers associated with effective treatment.

Although the specific effector cells responsible for the inflammatory process in psoriasis are not known, T cells play a role in the pathogenesis. The trafficking of T cells from blood to tissues is essential in chronic inflammatory diseases such as psoriasis. Key factors in this migration are chemo-attractant cytokine molecules known as chemokines and their receptor. The predominant chemokine receptors expressed on Th1-cells are CCR5 en CXCR3. In **Chapter 6** the expression of the chemokine receptor CCR5 and its ligands in lesional and non-lesional psoriatic skin are described. In addition, the clinical and immunohistochemical results of a randomized placebo controlled trial with a CCR5 inhibitor are presented. In **Chapter 7**, the expression of another chemokine receptor, namely CXCR3, and its ligands are investigated in lesional and non-lesional psoriatic skin.

REFERENCE LIST

1. van de Kerkhof PCM. Clinical features. In: *Textbook of psoriasis* (van de Kerkhof,PCM, ed), 2nd edn. Oxford: Blackwell Publishing, 2003: 3-29.
2. Raychaudhuri SP, Farber EM. The prevalence of psoriasis in the world. *J Eur Acad Dermatol Venereol* 2001; **15**: 16-7.
3. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. *Lancet* 2007; **370**: 263-71.
4. Brockbank JE, Schentag C, Rosen C *et al*. Psoriatic arthritis (PsA) is commom among patients with psoriasis and family medicine clinic attendees. *Arthritis Rheum* 2001; **44**: S94.
5. Gladman DD, Brockbank J. Psoriatic arthritis. *Expert Opin Investig Drugs* 2000; **9**: 1511-22.
6. Leonard DG, O'Duffy JD, Rogers RS. Prospective analysis of psoriatic arthritis in patients hospitalized for psoriasis. *Mayo Clin Proc* 1978; **53**: 511-8.
7. Shbeeb M, Uramoto KM, Gibson LE *et al*. The epidemiology of psoriatic arthritis in Olmsted County, Minnesota, USA, 1982-1991. *J Rheumatol* 2000; **27**: 1247-50.
8. Scarpa R, Oriente P, Pucino A *et al*. Psoriatic arthritis in psoriatic patients. *Br J Rheumatol* 1984; **23**: 246-50.
9. Garduno J, Bhosle MJ, Balkrishnan R *et al*. Measures used in specifying psoriasis lesion(s), global disease and quality of life: a systematic review. *J Dermatolog Treat* 2007; **18**: 223-42.
10. Puzenat E, Bronsard V, Prey S *et al*. What are the best outcome measures for assessing plaque psoriasis severity? A systematic review of the literature. *J Eur Acad Dermatol Venereol* 2010; **24 Suppl 2**: 10-6.
11. Schmitt J, Wozel G. The psoriasis area and severity index is the adequate criterion to define severity in chronic plaque-type psoriasis. *Dermatology* 2005; **210**: 194-9.
12. Reich K, Mrowietz U. Treatment goals in psoriasis. *J Dtsch Dermatol Ges* 2007; **5**: 566-74.
13. de Jong EM. The course of psoriasis. *Clin Dermatol* 1997; **15**: 687-92.
14. Choi J, Koo JY. Quality of life issues in psoriasis. *J Am Acad Dermatol* 2003; **49**: S57-S61.
15. de Korte J, Sprangers MAG, Mombers FMC *et al*. Quality of Life in Patients with Psoriasis: A Systematic Literature Review. *J Invest Dermatol Symposium Proceedings* 2004; **9**: 140-7.
16. Dubertret L, Mrowietz U, Ranki A *et al*. European patient perspectives on the impact of psoriasis: the EUROPSO patient membership survey. *Br J Dermatol* 2006; **155**: 729-36.
17. Heydendael VMR, de Borgie CAJM, Spuls PI *et al*. The Burden of Psoriasis Is Not Determined by Disease Severity Only. *J Invest Dermatol Symposium Proceedings* 2004; **9**: 131-5.
18. Rapp SR, Feldman SR, Exum ML *et al*. Psoriasis causes as much disability as other major medical diseases. *J Am Acad Dermatol* 1999; **41**: 401-7.
19. Fortune DG, Richards HL, Griffiths CE. Psychologic factors in psoriasis: consequences, mechanisms, and interventions. *Dermatol Clin* 2005; **23**: 681-94.
20. Boffetta P, Gridley G, Lindelof B. Cancer risk in a population-based cohort of patients hospitalized for psoriasis in Sweden. *J Invest Dermatol* 2001; **117**: 1531-7.
21. Gelfand JM, Berlin J, Van VA *et al*. Lymphoma rates are low but increased in patients with psoriasis: results from a population-based cohort study in the United Kingdom. *Arch Dermatol* 2003; **139**: 1425-9.
22. Gelfand JM, Neimann AL, Shin DB *et al*. Risk of myocardial infarction in patients with psoriasis. *JAMA* 2006; **296**: 1735-41.
23. Gelfand JM, Shin DB, Neimann AL *et al*. The Risk of Lymphoma in Patients with Psoriasis. *J Invest Dermatol* 2006.
24. Hannuksela-Svahn A, Pukkala E, Laara E *et al*. Psoriasis, its treatment, and cancer in a cohort of Finnish patients. *J Invest Dermatol* 2000; **114**: 587-90.

25. Mallbris L, Akre O, Granath F *et al*. Increased risk for cardiovascular mortality in psoriasis inpatients but not in outpatients. *Eur J Epidemiol* 2004; **19**: 225-30.
26. Margolis D, Bilker W, Hennessy S *et al*. The risk of malignancy associated with psoriasis. *Arch Dermatol* 2001; **137**: 778-83.
27. Najarian DJ, Gottlieb AB. Connections between psoriasis and Crohn's disease. *J Am Acad Dermatol* 2003; **48**: 805-21.
28. Neimann AL, Shin DB, Wang X *et al*. Prevalence of cardiovascular risk factors in patients with psoriasis. *J Am Acad Dermatol* 2006; **55**: 829-35.
29. Nijsten TE, Stern RS. The increased risk of skin cancer is persistent after discontinuation of psoralen+ultraviolet A: a cohort study. *J Invest Dermatol* 2003; **121**: 252-8.
30. Wakkee M, Thio HB, Prens EP *et al*. Unfavorable cardiovascular risk profiles in untreated and treated psoriasis patients. *Atherosclerosis* 2007; **190**: 1-9.
31. Wakkee M, Herings RM, Nijsten T. Psoriasis may not be an independent risk factor for acute ischemic heart disease hospitalizations: results of a large population-based Dutch cohort. *J Invest Dermatol* 2010; **130**: 962-7.
32. Kimball AB, Wu Y. Cardiovascular disease and classic cardiovascular risk factors in patients with psoriasis. *Int J Dermatol* 2009; **48**: 1147-56.
33. Lebwhol M, Callen JP. Obesity, smoking, and psoriasis. *JAMA* 2006; **295**: 208-10.
34. Lindelof B, Sigurgeirsson B, Tegner E *et al*. PUVA and cancer risk: the Swedish follow-up study. *Br J Dermatol* 1999; **141**: 108-12.
35. Paul CF, Ho VC, McGeown C *et al*. Risk of malignancies in psoriasis patients treated with cyclosporine: a 5 y cohort study. *J Invest Dermatol* 2003; **120**: 211-6.
36. Poikolainen K, Karvonen J, Pukkala E. Excess mortality related to alcohol and smoking among hospital-treated patients with psoriasis. *Arch Dermatol* 1999; **135**: 1490-3.
37. Bowcock AM, Barker JN. Genetics of psoriasis: the potential impact on new therapies. *J Am Acad Dermatol* 2003; **49**: 51-6.
38. Valdimarsson H. The genetic basis of psoriasis. *Clin Dermatol* 2007; **25**: 563-7.
39. Eddy DD, Aschheim E, Farber E. Experimental analysis of isomorphic (Koebner) response in psoriasis. *Arch Dermatol* 1964; **89**: 579.
40. Gudjonsson JE, Elder JT. Psoriasis: epidemiology. *Clin Dermatol* 2007; **25**: 535-46.
41. Morris A, Rogers M, Fischer G *et al*. Childhood psoriasis: a clinical review of 1262 cases. *Pediatr Dermatol* 2001; **18**: 188-98.
42. Farber EM, Nall ML. The natural history of psoriasis in 5,600 patients. *Dermatologica* 1974; **148**: 1-18.
43. Brandrup F, Hauge M, Henningsen K *et al*. Psoriasis in an unselected series of twins. *Arch Dermatol* 1978; **114**: 874-8.
44. Farber EM, Nall ML, Watson W. Natural history of psoriasis in 61 twin pairs. *Arch Dermatol* 1974; **109**: 207-211.
45. Pisani M, Ruocco V. 'Twin' psoriasis in monozygotic twins. *Arch Dermatol* 1984; **120**: 1418-9.
46. Nair RP, Stuart PE, Nistor I *et al*. Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. *Am J Hum Genet* 2006; **78**: 827-51.
47. Diluvio L, Vollmer S, Besgen P *et al*. Identical TCR beta-chain rearrangements in streptococcal angina and skin lesions of patients with psoriasis vulgaris. *J Immunol* 2006; **176**: 7104-11.
48. Fry L, Baker BS. Triggering psoriasis: the role of infections and medications. *Clin Dermatol* 2007; **25**: 606-15.
49. Bos JD, Hulsebosch HJ, Krieg SR *et al*. Immunocompetent cells in psoriasis. In situ immunophenotyping by monoclonal antibodies. *Arch Dermatol Res* 1983; **275**: 181-9.

50. Bos JD, Hagenaars C, Das PK *et al*. Predominance of "memory" T cells (CD4+, CDw29+) over "naive" T cells (CD4+, CD45R+) in both normal and diseased human skin. *Arch Dermatol Res* 1989; **281**: 24-30.

51. Morganroth GS, Chan LS, Weinstein GD *et al*. Proliferating cells in psoriatic dermis are comprised primarily of T cells, endothelial cells, and factor XIIIa+ perivascular dendritic cells. *J Invest Dermatol* 1991; **96**: 333-40.

52. Griffiths CE, Powles AV, Leonard JN *et al*. Clearance of psoriasis with low dose cyclosporin. *Br Med J (Clin Res Ed)* 1986; **293**: 731-2.

53. Bos JD, Meinardi MM, van JT *et al*. Use of cyclosporin in psoriasis. *Lancet* 1989; **2**: 1500-2.

54. Ellis CN, Fradin MS, Messana JM *et al*. Cyclosporine for plaque-type psoriasis. Results of a multidose, double-blind trial. *N Engl J Med* 1991; **324**: 277-84.

55. Mueller W, Hermann B. Cyclosporin A for psoriasis. *N Eng J Med* 1976; **301**: 355.

56. Gottlieb SL, Gilleaudeau P, Johnson R *et al*. Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nat Med* 1995; **1**: 442-7.

57. Austin LM, Ozawa M, Kikuchi T *et al*. The majority of epidermal T cells in Psoriasis vulgaris lesions can produce type 1 cytokines, interferon-gamma, interleukin-2, and tumor necrosis factor-alpha, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. *J Invest Dermatol* 1999; **113**: 752-9.

58. Schlaak JF, Buslau M, Jochum W *et al*. T cells involved in psoriasis vulgaris belong to the Th1 subset. *J Invest Dermatol* 1994; **102**: 145-9.

59. Uyemura K, Yamamura M, Fivenson DF *et al*. The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. *J Invest Dermatol* 1993; **101**: 701-5.

60. Gaspari AA. Innate and adaptive immunity and the pathophysiology of psoriasis. *J Am Acad Dermatol* 2006; **54**: S67-S80.

61. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med* 2009; **361**: 496-509.

62. Chan JR, Blumenschein W, Murphy E *et al*. IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. *J Exp Med* 2006; **203**: 2577-87.

63. Bos JD, de Rie MA, Teunissen MB *et al*. Psoriasis: dysregulation of innate immunity. *Br J Dermatol* 2005; **152**: 1098-107.

64. Nestle FO, Conrad C, Tun-Kyi A *et al*. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* 2005; **202**: 135-43.

65. Langrish CL, Chen Y, Blumenschein WM *et al*. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005; **201**: 233-40.

66. Fitch E, Harper E, Skorcheva I *et al*. Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines. *Curr Rheumatol Rep* 2007; **9**: 461-7.

67. Aggarwal S, Ghilardi N, Xie MH *et al*. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J Biol Chem* 2003; **278**: 1910-4.

68. Kastelein RA, Hunter CA, Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol* 2007; **25**: 221-42.

69. Langrish CL, McKenzie BS, Wilson NJ *et al*. IL-12 and IL-23: master regulators of innate and adaptive immunity. *Immunol Rev* 2004; **202**: 96-105.

70. Liang SC, Tan XY, Luxenberg DP *et al*. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006; **203**: 2271-9.

71. Teunissen MB, Koomen CW, de Waal MR *et al*. Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J Invest Dermatol* 1998; **111**: 645-9.
72. Di CA, Di MP, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol* 2009; **129**: 1339-50.
73. Zheng Y, Danilenko DM, Valdez P *et al*. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 2007; **445**: 648-51.
74. Zaba LC, Cardinale I, Gilleaudeau P *et al*. Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J Exp Med* 2007; **204**: 3183-94.
75. Lee E, Trepicchio WL, Oestreicher JL *et al*. Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J Exp Med* 2004; **199**: 125-30.
76. Wilson NJ, Boniface K, Chan JR *et al*. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* 2007; **8**: 950-7.
77. Piskin G, Sylva-Steenland RM, Bos JD *et al*. In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. *J Immunol* 2006; **176**: 1908-15.
78. Teunissen MBM, Piskin G, Res PCJM *et al*. State of the art in the immunopathogenesis of psoriasis. G Ital Dermatol Venereol 2007; **142**(**3**): 229-242.
79. Duhen T, Geiger R, Jarrossay D *et al*. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat Immunol* 2009; **10**: 857-63.
80. Liu Y, Yang B, Zhou M *et al*. Memory IL-22-producing CD4+ T cells specific for Candida albicans are present in humans. *Eur J Immunol* 2009; **39**: 1472-9.
81. Nograles KE, Zaba LC, Shemer A *et al*. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. *J Allergy Clin Immunol* 2009; **123**: 1244-52.
82. Trifari S, Kaplan CD, Tran EH *et al*. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat Immunol* 2009; **10**: 864-71.
83. Harper EG, Guo C, Rizzo H *et al*. Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: implications for psoriasis pathogenesis. *J Invest Dermatol* 2009; **129**: 2175-83.
84. Kagami S, Rizzo HL, Lee JJ *et al*. Circulating Th17, Th22, and Th1 cells are increased in psoriasis. *J Invest Dermatol* 2010; **130**: 1373-83.
85. Boniface K, Bernard FX, Garcia M *et al*. IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *J Immunol* 2005; **174**: 3695-702.
86. Nakae S, Iwakura Y, Suto H *et al*. Phenotypic differences between Th1 and Th17 cells and negative regulation of Th1 cell differentiation by IL-17. *J Leukoc Biol* 2007; **81**: 1258-68.
87. Park H, Li Z, Yang XO *et al*. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; **6**: 1133-41.
88. Lowes MA, Kikuchi T, Fuentes-Duculan J *et al*. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J Invest Dermatol* 2008; **128**: 1207-11.
89. Bettelli E, Oukka M, Kuchroo VK. TH-17 cells in the circle of immunity and autoimmunity. *Nat Immunol* 2007; **8**: 345-50.
90. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity* 2000; **12**: 121-7.
91. Widney DP, Xia YR, Lusis AJ *et al*. The murine chemokine CXCL11

(IFN-inducible T cell alpha chemoattractant) is an IFN-gamma- and lipopolysaccharide-inducible glucocorticoid-attenuated response gene expressed in lung and other tissues during endotoxemia. *J Immunol* 2000; **164**: 6322-31.

92. Murphy PM, Baggiolini M, Charo IF *et al*. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev* 2000; **52**: 145-76.
93. Lau EK, Paavola CD, Johnson Z *et al*. Identification of the glycosaminoglycan binding site of the CC chemokine, MCP-1: implications for structure and function in vivo. *J Biol Chem* 2004; **279**: 22294-305.
94. Baggiolini M. Chemokines and leukocyte traffic. *Nature* 1998; **392**: 565-8.
95. Proudfoot AE, Power CA, Schwarz MK. Anti-chemokine small molecule drugs: a promising future? *Expert Opin Investig Drugs* 2010; **19**: 345-55.
96. Horuk R. Chemokine receptors. *Cytokine Growth Factor Rev* 2001; **12**: 313-35.
97. Loetscher P, Seitz M, Baggiolini M *et al*. Interleukin-2 regulates CC chemokine receptor expression and chemotactic responsiveness in T lymphocytes. *J Exp Med* 1996; **184**: 569-77.
98. Sica A, Saccani A, Borsatti A *et al*. Bacterial lipopolysaccharide rapidly inhibits expression of C-C chemokine receptors in human monocytes. *J Exp Med* 1997; **185**: 969-74.
99. Ogilvie P, Bardi G, Clark-Lewis I *et al*. Eotaxin is a natural antagonist for CCR2 and an agonist for CCR5. *Blood* 2001; **97**: 1920-4.
100. Zlotnik A, Morales J, Hedrick JA. Recent advances in chemokines and chemokine receptors. Crit Rev Immunol 1999; 19: 1-47.
101. Gillitzer R, Berger R, Mielke V et al. Upper keratinocytes of psoriatic skin lesions express high levels of NAP-1/IL-8 mRNA in situ. J Invest Dermatol 1991; 97: 73-9.
102. Kojima T, Cromie MA, Fisher GJ et al. GRO-alpha mRNA is selectively overexpressed in psoriatic epidermis and is reduced by cyclosporin A in vivo, but not in cultured keratinocytes. J Invest Dermatol 1993; 101: 767-72.
103. Gillitzer R, Ritter U, Spandau U et al. Differential expression of GRO-alpha and IL-8 mRNA in psoriasis: a model for neutrophil migration and accumulation in vivo. J Invest Dermatol 1996; 107: 778-82.
104. Schulz BS, Michel G, Wagner S et al. Increased expression of epidermal IL-8 receptor in psoriasis. Down-regulation by FK-506 in vitro. J Immunol 1993; 151: 4399-406.
105. Beljaards RC, Van BP, Nieboer C et al. The expression of interleukin-8 receptor in untreated and treated psoriasis. Arch Dermatol Res 1997; 289: 440-3.
106. Kulke R, Bornscheuer E, Schluter C et al. The CXC receptor 2 is overexpressed in psoriatic epidermis. J Invest Dermatol 1998; 110: 90-4.
107. Gottlieb AB, Luster AD, Posnett DN et al. Detection of a gamma interferon-induced protein IP-10 in psoriatic plaques. J Exp Med 1988; 168: 941-8.
108. Deleuran M, Buhl L, Ellingsen T et al. Localization of monocyte chemotactic and activating factor (MCAF/MCP-1) in psoriasis. J Dermatol Sci 1996; 13: 228-36.
109. Gillitzer R, Wolff K, Tong D et al. MCP-1 mRNA expression in basal keratinocytes of psoriatic lesions. J Invest Dermatol 1993; 101: 127-31.
110. Goebeler M, Toksoy A, Spandau U et al. The C-X-C chemokine Mig is highly expressed in the papillae of psoriatic lesions. J Pathol 1998; 184: 89-95.
111. Bonecchi R, Bianchi G, Bordignon PP et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 1998; 187: 129-34.
112. Loetscher P, Uguccioni M, Bordoli L et al. CCR5 is characteristic of Th1 lymphocytes. Nature 1998; 391: 344-5.
113. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory

immune responses. Annu Rev Immunol 2000; 18: 593-620.

114. Kunkel EJ, Boisvert J, Murphy K et al. Expression of the chemokine receptors CCR4, CCR5, and CXCR3 by human tissue-infiltrating lymphocytes. Am J Pathol 2002; 160: 347-55.
115. Qin S, Rottman JB, Myers P et al. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest 1998; 101: 746-54.
116. Loetscher P, Moser B, Baggiolini M. Chemokines and their receptors in lymphocyte traffic and HIV infection. Adv Immunol 2000; 74: 127-80.
117. Bonish B, Jullien D, Dutronc Y et al. Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-gamma production by NK-T cells. J Immunol 2000; 165: 4076-85.
118. Cameron AL, Kirby B, Fei W et al. Natural killer and natural killer-T cells in psoriasis. Arch Dermatol Res 2002; 294: 363-9.
119. Cameron AL, Kirby B, Griffiths CE. Circulating natural killer cells in psoriasis. Br J Dermatol 2003; 149: 160-4.
120. Nickoloff BJ, Wrone-Smith T, Bonish B et al. Response of murine and normal human skin to injection of allogeneic blood-derived psoriatic immunocytes: detection of T cells expressing receptors typically present on natural killer cells, including CD94, CD158, and CD161. Arch Dermatol 1999; 135: 546-52.
121. Nickoloff BJ, Bonish B, Huang BB et al. Characterization of a T cell line bearing natural killer receptors and capable of creating psoriasis in a SCID mouse model system. J Dermatol Sci 2000; 24: 212-25.
122. Sozzani S, Allavena P, Vecchi A et al. Chemokines and dendritic cell traffic. J Clin Immunol 2000; 20: 151-60.
123. Ottaviani C, Nasorri F, Bedini C et al. CD56brightCD16(-) NK cells accumulate in psoriatic skin in response to CXCL10 and CCL5 and exacerbate skin inflammation. Eur J Immunol 2006; 36: 118-28.
124. Homey B. Chemokines and chemokine receptors as targets in the therapy of psoriasis. Curr Drug Targets Inflamm Allergy 2004; 3: 169-74.
125. Fraziano M, Cappelli G, Santucci M et al. Expression of CCR5 is increased in human monocyte-derived macrophages and alveolar macrophages in the course of in vivo and in vitro Mycobacterium tuberculosis infection. AIDS Res Hum Retroviruses 1999; 15: 869-74.
126. Mack M, Cihak J, Simonis C et al. Expression and Characterization of the Chemokine Receptors CCR2 and CCR5 in Mice. J Immunol 2001; 166: 4697-704.
127. Homey B, Dieu-Nosjean MC, Wiesenborn A et al. Up-regulation of macrophage inflammatory protein-3 alpha/CCL20 and CC chemokine receptor 6 in psoriasis. J Immunol 2000; 164: 6621-32.
128. Raychaudhuri SP, Jiang WY, Farber EM et al. Upregulation of RANTES in psoriatic keratinocytes: a possible pathogenic mechanism for psoriasis. Acta Derm Venereol 1999; 79: 9-11.
129. Giustizieri ML, Mascia F, Frezzolini A et al. Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. J Allergy Clin Immunol 2001; 107: 871-7.
130. Fukuoka M, Ogino Y, Sato H et al. RANTES expression in psoriatic skin, and regulation of RANTES and IL-8 production in cultured epidermal keratinocytes by active vitamin D3 (tacalcitol). Br J Dermatol 1998; 138: 63-70.
131. Nomura I, Gao B, Boguniewicz M et al. Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. J Allergy Clin Immunol 2003; 112: 1195-202.
132. Ghoreschi K, Thomas P, Breit S et al. Interleukin-4 therapy of psoriasis induces Th2 responses and improves

human autoimmune disease. Nat Med 2003; 9: 40-6.

133. Uchida T, Suto H, Ra C et al. Preferential expression of Th2-type chemokine and its receptor in atopic dermatitis. Int Immunol 2002; 14: 1431-8.

134. Kunkel EJ, Boisvert J, Murphy K et al. Expression of the chemokine receptors CCR4, CCR5, and CXCR3 by human tissue-infiltrating lymphocytes. Am J Pathol 2002; 160: 347-55.

135. Garcia-Lopez MA, Sanchez-Madrid F, Rodriguez-Frade JM et al. CXCR3 chemokine receptor distribution in normal and inflamed tissues: expression on activated lymphocytes, endothelial cells, and dendritic cells. Lab Invest 2001; 81: 409-18.

136. Jones D, Benjamin RJ, Shahsafaei A et al. The chemokine receptor CXCR3 is expressed in a subset of B-cell lymphomas and is a marker of B-cell chronic lymphocytic leukemia. Blood 2000; 95: 627-32.

137. Kohrgruber N, Groger M, Meraner P et al. Plasmacytoid dendritic cell recruitment by immobilized CXCR3 ligands. J Immunol 2004; 173: 6592-602.

138. Muehlinghaus G, Cigliano L, Huehn S et al. Regulation of CXCR3 and CXCR4 expression during terminal differentiation of memory B cells into plasma cells. Blood 2005; 105: 3965-71.

139. Ocana E, Delgado-Perez L, Campos-Caro A et al. The prognostic role of CXCR3 expression by chronic lymphocytic leukemia B cells. Haematologica 2007; 92: 349-56.

140. Qin S, Rottman JB, Myers P et al. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest 1998; 101: 746-54.

141. Vanbervliet B, Bendriss-Vermare N, Massacrier C et al. The inducible CXCR3 ligands control plasmacytoid dendritic cell responsiveness to the constitutive chemokine stromal cell-derived factor 1 (SDF-1)/CXCL12. J Exp Med 2003; 198: 823-30.

142. Iijima W, Ohtani H, Nakayama T et al. Infiltrating CD8+ T cells in oral lichen planus predominantly express CCR5 and CXCR3 and carry respective chemokine ligands RANTES/CCL5 and IP-10/CXCL10 in their cytolytic granules: a potential self-recruiting mechanism. Am J Pathol 2003; 163: 261-8.

143. Rottman JB, Smith TL, Ganley KG et al. Potential role of the chemokine receptors CXCR3, CCR4, and the integrin alphaEbeta7 in the pathogenesis of psoriasis vulgaris. Lab Invest 2001; 81: 335-47.

144. Teraki Y, Miyake A, Takebayashi R et al. Homing receptor and chemokine receptor on intraepidermal T cells in psoriasis vulgaris. Clin Exp Dermatol 2004; 29: 658-63.

145. Wenzel J, Uerlich M, Haller O et al. Enhanced type I interferon signaling and recruitment of chemokine receptor CXCR3-expressing lymphocytes into the skin following treatment with the TLR7-agonist imiquimod. J Cutan Pathol 2005; 32: 257-62.

146. Wenzel J, Peters B, Zahn S et al. Gene expression profiling of lichen planus reflects CXCL9+-mediated inflammation and distinguishes this disease from atopic dermatitis and psoriasis. J Invest Dermatol 2008; 128: 67-78.

147. Wenzel J, Wiechert A, Merkel C et al. IP10/CXCL10-CXCR3 interaction: a potential self-recruiting mechanism for cytotoxic lymphocytes in lichen sclerosus atrophicans. Acta Derm Venereol 2007; 87: 112-7.

148. Flier J, Boorsma DM, van Beek PJ et al. Differential expression of CXCR3 targeting chemokines CXCL10, CXCL9, and CXCL11 in different types of skin inflammation. J Pathol 2001; 194: 398-405.

149. Hedrick MN, Lonsdorf AS, Hwang ST et al. CCR6 as a possible therapeutic target in psoriasis. Expert Opin Ther Targets 2010.

150. Annunziato F, Cosmi L, Liotta F et al. The phenotype of human Th17 cells and their precursors, the cytokines that mediate their differentiation and

the role of Th17 cells in inflammation. Int Immunol 2008; 20: 1361-8.

151. Homey B, eu-Nosjean MC, Wiesenborn A et al. Up-regulation of macrophage inflammatory protein-3 alpha/CCL20 and CC chemokine receptor 6 in psoriasis. J Immunol 2000; 164: 6621-32.
152. Hedrick MN, Lonsdorf AS, Shirakawa AK et al. CCR6 is required for IL-23-induced psoriasis-like inflammation in mice. J Clin Invest 2009; 119: 2317-29.
153. Homey B. Chemokines and chemokine receptors as targets in the therapy of psoriasis. Curr Drug Targets Inflamm Allergy 2004; 3: 169-74.
154. Nijsten T, Margolis DJ, Feldman SR et al. Traditional systemic treatments have not fully met the needs of psoriasis patients: results from a national survey. J Am Acad Dermatol 2005; 52: 434-44.
155. Gniadecki R, Calverley MJ. Emerging drugs in psoriasis. Expert Opin Emerg Drugs 2002; 7: 69-90.
156. Vena GA, Cassano N. Emerging drugs for psoriasis. Expert Opin Emerg Drugs 2006; 11: 567-96.
157. Koo J, Khera P. Update on the mechanisms and efficacy of biological therapies for psoriasis. J Dermatol Sci 2005; 38: 75-87.
158. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. Nature 2007; 445: 866-73.
159. Krueger GG, Papp KA, Stough DB et al. A randomized, double-blind, placebo-controlled phase III study evaluating efficacy and tolerability of 2 courses of alefacept in patients with chronic plaque psoriasis. J Am Acad Dermatol 2002; 47: 821-33.
160. Lebwohl M, Christophers E, Langley R et al. An international, randomized, double-blind, placebo-controlled phase 3 trial of intramuscular alefacept in patients with chronic plaque psoriasis. Arch Dermatol 2003; 139: 719-27.
161. Bissonnette R, Langley RG, Papp K et al. Humanized anti-CD2 monoclonal antibody treatment of plaque psoriasis: efficacy and pharmacodynamic results of two randomized, double-blind, placebo-controlled studies of intravenous and subcutaneous siplizumab. Arch Dermatol Res 2009; 301: 429-42.
162. Langley RG, Papp K, Bissonnette R et al. Safety profile of intravenous and subcutaneous siplizumab, an anti-CD2 monoclonal antibody, for the treatment of plaque psoriasis: results of two randomized, double-blind, placebo-controlled studies. Int J Dermatol 2010; 49: 818-28.
163. FDA. Raptiva [efalizumab] drug label 2005.
164. Schmitt J, Zhang Z, Wozel G et al. Efficacy and tolerability of biologic and nonbiologic systemic treatments for moderate-to-severe psoriasis: meta-analysis of randomized controlled trials. Br J Dermatol 2008; 159: 513-26.
165. EMEA. Efalizumab suspension PRESS RELEASE. 19-02-2009.
166. FDA. Enbrel [etanercept] drug label 2005.
167. Leonardi CL, Powers JL, Matheson RT et al. Etanercept as monotherapy in patients with psoriasis. N Engl J Med 2003; 349: 2014-22.
168. Papp KA, Tyring S, Lahfa M et al. A global phase III randomized controlled trial of etanercept in psoriasis: safety, efficacy, and effect of dose reduction. Br J Dermatol 2005; 152: 1304-12.
169. Tyring S, Gottlieb A, Papp K et al. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. Lancet 2006; 367: 29-35.
170. Yamauchi PS, Gindi V, Lowe NJ. The treatment of psoriasis and psoriatic arthritis with etanercept: practical considerations on monotherapy, combination therapy, and safety. Dermatol Clin 2004; 22: 449-59, ix.
171. Reich K, Nestle FO, Papp K et al. Infliximab induction and maintenance therapy for moderate-to-severe psoriasis:

a phase III, multicentre, double-blind trial. Lancet 2005; 366: 1367-74.

172. Gottlieb AB, Evans R, Li S et al. Infliximab induction therapy for patients with severe plaque-type psoriasis: a randomized, double-blind, placebo-controlled trial. J Am Acad Dermatol 2004; 51: 534-42.
173. Chaudhari U, Romano P, Mulcahy LD et al. Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. Lancet 2001; 357: 1842-7.
174. Gordon KB, Langley RG, Leonardi C et al. Clinical response to adalimumab treatment in patients with moderate to severe psoriasis: Double-blind, randomized controlled trial and open-label extension study. J Am Acad Dermatol 2006; 55: 598-606.
175. Menter A, Tyring SK, Gordon K et al. Adalimumab therapy for moderate to severe psoriasis: A randomized, controlled phase III trial. J Am Acad Dermatol 2008; 58: 106-15.
176. Hutas G. Golimumab as the first monthly subcutaneous fully human anti-TNF-alpha antibody in the treatment of inflammatory arthropathies. Immunotherapy 2010; 2: 453-60.
177. Melmed GY, Targan SR, Yasothan U et al. Certolizumab pegol. Nat Rev Drug Discov 2008; 7: 641-2.
178. FDA. Certolizumab drug label 2010.
179. Krueger GG, Langley RG, Leonardi C et al. A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. N Engl J Med 2007; 356: 580-92.
180. Papp KA, Langley RG, Lebwohl M et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). Lancet 2008; 371: 1675-84.
181. Leonardi CL, Kimball AB, Papp KA et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). Lancet 2008; 371: 1665-74.
182. Griffiths CE, Strober BE, van de Kerkhof P et al. Comparison of ustekinumab and etanercept for moderate-to-severe psoriasis. N Engl J Med 2010; 362: 118-28.
183. Gottlieb A, Menter A, Mendelsohn A et al. Ustekinumab, a human interleukin 12/23 monoclonal antibody, for psoriatic arthritis: randomised, double-blind, placebo-controlled, crossover trial. Lancet 2009; 373: 633-40.
184. Lima XT, Abuabara K, Kimball AB et al. Briakinumab. Expert Opin Biol Ther 2009; 9: 1107-13.
185. Kimball AB, Gordon KB, Langley RG et al. Safety and efficacy of ABT-874, a fully human interleukin 12/23 monoclonal antibody, in the treatment of moderate to severe chronic plaque psoriasis: results of a randomized, placebo-controlled, phase 2 trial. Arch Dermatol 2008; 144: 200-7.
186. Katz HI, Hien NT, Prawer SE et al. Superpotent topical steroid treatment of psoriasis vulgaris--clinical efficacy and adrenal function. J Am Acad Dermatol 1987; 16: 804-11.
187. Eells LD, Wolff JM, Garloff J et al. Comparison of suberythemogenic and maximally aggressive ultraviolet B therapy for psoriasis. J Am Acad Dermatol 1984; 11: 105-10.
188. Weinstein GD, Frost P. Methotrexate for psoriasis. A new therapeutic schedule. Arch Dermatol 1971; 103: 33-8.
189. Buckley DB. A double-blind comparison of 0.1% dithranol in a 17% urea base ("Psoradrate") and base alone in the treatment of active chronic psoriasis. Curr Med Res Opin 1978; 5: 489-94.
190. van Weelden H, De La Faille HB, Young E et al. A new development in UVB phototherapy of psoriasis. Br J Dermatol 1988; 119: 11-9.
191. Young E. The external treatment of psoriasis. A controlled investigation of

the effects of coal tar. Br J Dermatol 1970; 82: 510-5.

192. Weismann K, Howitz J, Bro-Jorgensen A. Treatment of resistant psoriasis with oral 8-methoxypsoralen and longwave ultraviolet light (PUVA). A treatment schedule and a follow-up study. Acta Derm Venereol 1977; 57: 73-6.
193. Gollnick H, Bauer R, Brindley C et al. Acitretin versus etretinate in psoriasis. Clinical and pharmacokinetic results of a German multicenter study. J Am Acad Dermatol 1988; 19: 458-68.
194. Weinstein GD, Krueger GG, Lowe NJ et al. Tazarotene gel, a new retinoid, for topical therapy of psoriasis: vehicle-controlled study of safety, efficacy, and duration of therapeutic effect. J Am Acad Dermatol 1997; 37: 85-92.
195. Asawanonda P, Anderson RR, Chang Y et al. 308-nm excimer laser for the treatment of psoriasis: a dose-response study. Arch Dermatol 2000; 136: 619-24.
196. Altmeyer PJ, Matthes U, Pawlak F et al. Antipsoriatic effect of fumaric acid derivatives. Results of a multicenter double-blind study in 100 patients. J Am Acad Dermatol 1994; 30: 977-81.
197. Kragballe K, Beck HI, Sogaard H. Improvement of psoriasis by a topical vitamin D3 analogue (MC 903) in a double-blind study. Br J Dermatol 1988; 119: 223-30.
198. Rozenblit M, Lebwohl M. New biologics for psoriasis and psoriatic arthritis. Dermatol Ther 2009; 22: 56-60.

2a

DERMATITIS DURING EFALIZUMAB TREATMENT IN A PATIENT WITH PSORIASIS VULGARIS

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Efalizumab is a fully humanized monoclonal antibody against CD11a, the α-chain of the lymphocyte function associated (LFA)-1 adhesion molecule. By binding to CD11a, LFA-1 is prevented from binding with its ligand, intercellular adhesion molecule (ICAM-1). This inhibits various T-cell processes believed to be important in the pathogenesis of psoriasis, including T-cell activation, T-cell adhesion to endothelial cells and T-cell migration. Clinical trials demonstrate that efalizumab, given subcutaneously once weekly, provides clinical benefit in patients with moderate to severe plaque psoriasis [1-6]. We report a case in which a patient with psoriasis vulgaris developed dermatitis during efalizumab therapy.

A 48-year-old male, diagnosed with psoriasis in 1991, received weekly subcutaneously injections efalizumab (0.7 mg/kg) for 12 weeks. Apart from budesonide and formoterol inhalation medication, the patient used no other medication.

During week 9 of the treatment the patient developed multiple moderately defined erythemato-squamous papules varying in size from 1 to 2 centimeters on the extremities and trunk, next to the classical psoriasis lesions. The majority of those lesions showed excoriations, as seen in Figure 1.

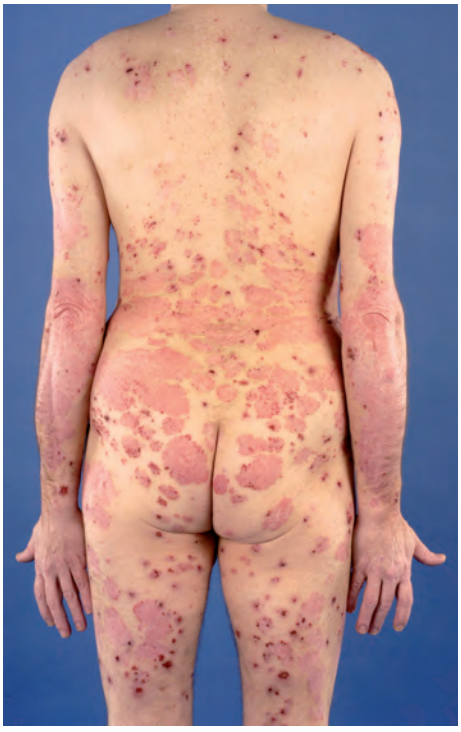

Figure 1 Multiple moderately defined erythematosqamous papules, covered with crusts next to excoriations, and classical psoriasis lesions on the trunk and extremities

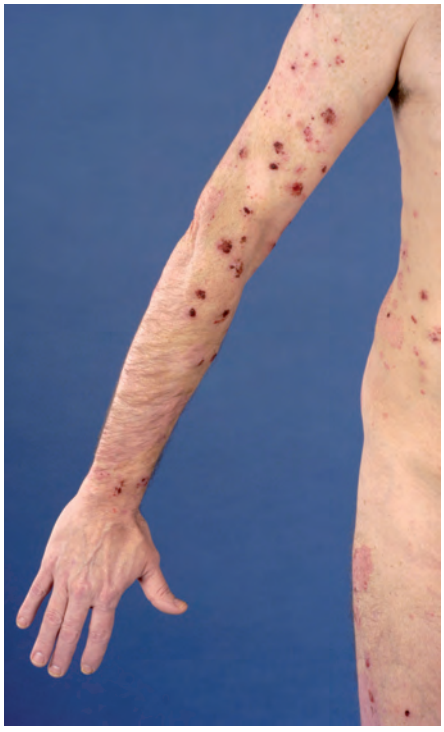

Figure 2 Detail of the right arm with multiple moderately defined erythematosqamous papules, covered with crusts next to excoriations

A skin biopsy taken from a papule of the wrist on week 10 showed reactive epidermal changes which were compatible with the diagnosis lichen simplex chronicus.

One month after the patient had his last injection of efalizumab, a second biopsy was taken from another lesion. This showed a widened acanthotic epidermis with parakeratosis and a chronic inflammation infiltrate in the dermis with local neutrophilic and eosinophilic granulocytes infiltrating the upper epithelium.

Simultaneously with the dermatitis the patient developed unbearable pruritus for which he was admitted to the dermatology ward. After treatment with UVB (311 nm) in combination with topical betamethason twice daily and daily baths with bath oil, the atypical lesions disappeared and the psoriasis improved.

Efalizumab is one of the new biological therapies targeting T-lymphocyte activity for the treatment of chronic plaque psoriasis. Common adverse events include headaches, nonspecific infection, nausea, chills and fever [1;2;5]. A variety of unusual forms of psoriasis have been observed in patients receiving efalizumab, such as guttate psoriasis, psoriatic erythroderma and pustular psoriasis[7]. However, all these manifestations occured after withdrawal of efalizumab and the lesions did not resemble dermatitis. In contrast, our patient developed lesions of dermatitis during treatment with efalizumab.

Although drug eruptions due to efalizumab have not been reported yet, it can not be ruled out that this was the case in our patient. Our patient developed dermatitis 9 weeks after entering the open label phase and this could not be attributed to change of medication. No rechallenge was performed.

Our patient was known to be atopic. His medical history showed asthma for several years as well as a positive family history for atopic disorders. Laboratory results showed a very high IgE of 18200 U ml^{-1} (normal range < 100U ml^{-1}), particularly positive for grass, trees and house dust mite, as well as eosinophily (1569 $10E^6$ l^{-1}, normal range 11-330 $10E^6$ l^{-1}), confirming atopy. Although the concomitant manifestion of atopic dermatitis and psoriasis is very rare, this could be the case in this patient.

Alternatively, since efalizumab influences the natural course of psoriasis, the lesions could be an atypical presentation of new developing psoriasis lesions during the treatment.

REFERENCE LIST

1. Cather JC, Cather JC, Menter A. Modulating T cell responses for the treatment of psoriasis: a focus on efalizumab. *Expert Opin Biol Ther* 2003; **3**: 361-70.
2. Gordon KB, Papp KA, Hamilton TK *et al*. Efalizumab for patients with moderate to severe plaque psoriasis: a randomized controlled trial. *JAMA* 2003; **290**: 3073-80.
3. Gottlieb AB, Krueger JG, Wittkowski K *et al*. Psoriasis as a model for T-cell-mediated disease: immunobiologic and clinical effects of treatment with multiple doses of efalizumab, an anti-CD11a antibody. *Arch Dermatol* 2002; **138**: 591-600.
4. Gottlieb AB, Miller B, Lowe N *et al*. Subcutaneously administered efalizumab (anti-CD11a) improves signs and symptoms of moderate to severe plaque psoriasis. *J Cutan Med Surg* 2003; **7**: 198-207.
5. Lebwohl M, Tyring SK, Hamilton TK *et al*. A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. *N Engl J Med* 2003; **349**: 2004-13.
6. Leonardi CL. Efalizumab: an overview. *J Am Acad Dermatol* 2003; **49**: S98-104.
7. Gaylor ML, Duvic M. Generalized pustular psoriasis following withdrawal of efalizumab. *J Drugs Dermatol* 2004; **3**: 77-9.

2b

DRESS SYNDROME CAUSED BY EFALIZUMAB

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We read with great interest the article entitled 'DRESS syndrome caused by efalizumab'[1]. In which a 52-year old male with treatment-resistant severe psoriasis is presented. He developed a papulovesicular rash after 4 weeks of treatment with efalizumab, and also had high peripheral eosinophilia, abnormal liver function, malaise and fever. This patient as having drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome. In the discussion, White et al. referred to our previously published case-report on a 48-old male patient with psoriasis who, during efalizumab treatment, developed multiple, moderately defined, erythematosquamous papules on extremities and trunk, in close proximity to his classical psoriasis lesions[2]. White et al. suggested the patient described in our report could have had less severe manifestatons of DRESS.

Currently, there is no consensus over specific diagnostic criteria for this diagnosis. According to Peyrière et al., who conducted a large retrospective study on drug-induced cutaneous side-effects, there are no strict diagnostic criteria for DRESS[3]. However, a Japanese consensus group states that there are seven diagnostic criteria, including fever, liver abnormalities, leucocyte abnormalities and lymphadenopathy, and that the diagnosis is confirmed by the presence of at least five of these criteria [4].

Our patient did not have fever, liver abnormalities, leucocyte abnormalities or lymphadenopathy. Furthermore, his hypereosinophilia existed, to a lesser extent, prior to the cutaneous manifestations and persisted after the cutaneous lesions had disappeared. Together with his medical history of asthma, hayfever and a positive family history of atopy, we believe that the hypereosinophilia was in concordance with his atopic constitution and not due to the DRESS syndrome.

REFERENCE LIST

1. White JM, Smith CH, Robson A *et al*. DRESS syndrome caused by efalizumab. *Clin Exp Dermatol* 2008; **33**: 50-2.
2. de Groot M, de Rie MA, Bos JD. Dermatitis during efalizumab treatment in a patient with psoriasis vulgaris. *Br J Dermatol* 2005; **153**: 843-4.
3. Peyriere H, Dereure O, Breton H *et al*. Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? *Br J Dermatol* 2006; **155**: 422-8.
4. Shiohara T, Iijima M, Ikezawa Z *et al*. The diagnosis of a DRESS syndrome has been sufficiently established on the basis of typical clinical features and viral reactivations. *Br J Dermatol* 2007; **156**: 1083-4.

3

INITIAL EXPERIENCE WITH ROUTINE ADMINISTRATION OF ETANERCEPT IN PSORIASIS

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ABSTRACT

Background: Etanercept and efalizumab recently became available and reimbursed for routine use in severe psoriasis in the Netherlands. The criteria for reimbursement are Psoriasis Area and Severity Index (PASI) ≥10 (or Skindex-29 ≥35 if PASI ≥8 and <10) and ineffectiveness of ultraviolet (UV) B/psoralenen plus UVA, methotrexate and cyclosporin, or a contraindication to or serious side effect(s) during these treatments.

Objectives: We hypothesized (1) that efficacy would be lower than those obtained in published phase II and III studies because (a) resistance to all conventional therapies as a reimbursement condition would select for more resistant cases; and (b) inclusion would be more restricted to severe cases (higher PASI); and (2) that efficacy would be lower in obese patients due to the possible role of adipose tissue in tumour necrosis factor (TNF)-α homeostasis.

Methods: We treated 50 patients (38 men, 12 women, mean PASI 15.8) with etanercept 25 or 50 mg twice weekly and evaluated in a retrospective analysis the efficacy and safety in comparison with data from published trials. Additionally we related the clinical effect to the body mass index (BMI), for adipose tissue is thought to have a possible role in TNF-α homeostasis.

Results: Based on the literature, 30% and 49% of the patients treated with etanercept 25 mg and 50 mg twice weekly, respectively, should have achieved 75% or more improvement in PASI compared to baseline (PASI 75), and 10% and 21%, respectively, should have achieved 90% or more improvement (PASI 90). Our data showed that 21% in the 2 x 25 mg group and 23% in the 2 x 50 mg group achieved PASI 75. PASI 90 was only attained in 7% in patients treated with 25 mg and 6% of those treated with 2 x 50 mg. Contrary to our hypothesis the mean initial PASI was comparable to the mean PASI mentioned in the phase II and III clinical trials. Although fatigue is not identified as a side-effect of etanercept, 10% of our patients reported fatigue as an adverse event during etanercept treatment. High BMI, indicating overweight or obesity, was found both in patients with little efficacy and in patients achieving PASI 75 or better.

Conclusion: Use of etanercept in real practice gives impressive results, but these are generally less favourable than those published in clinical trial reports. This is probably due to the stringent conditions for reimbursement, which select for more treatment-resistant patients. Fatigue as a possible side- effect of etanercept should also be an issue for further investigation. Finally, the BMI does not seem to influence the patients' response to etanercept, although further investigations would be needed to confirm this.

INTRODUCTION

As of early 2005, two biologicals (etanercept, efalizumab) became available and also reimbursed for patients with moderate to severe psoriasis in The Netherlands. Other biologicals like alefacept, adalimumab or infliximab are not available yet. The criteria for reimbursement are Psoriasis Area and Severity Index (PASI) ≥10 and ineffectiveness of ultraviolet (UV) B/ psoralen plus UVA (PUVA), methotrexate and cyclosporin, or a contraindication to or serious side-effect(s) during these treatments (Table 1).

In addition to PASI as a criterion for reimbursement, a quality-of-life questionnaire (Skindex-29) is used as indicator for the severity of psoriasis. The Skindex-29 is a self-administered instrument that measures the effects of skin diseases on patients' quality of life.[1] On a scale of 0-100, patients with a Skindex-29 ≥35 are eligible for biological therapy if the PASI is ≥8. The use of a quality of life questionnaire as selection mechanism for reimbursement of a treatment is new in dermatology in The Netherlands, and presumably elsewhere as well.

We hypothesized: (i) that efficacy results would be less than those obtained in published phase 2 and 3 studies until January 2006 because (a) resistance to all conventional therapies as a reimbursement condition would select for more resistant

Table 1 National reimbursement criteria for treatment of chronic plaque psoriasis with biologicals in the Netherlands

Psoriasis Area and Severity Index	≥ 10*
Skindex-29	≥ 35*
Methotrexate	
response	< 50%
dosing	22,5 mg weekly
duration	16 weeks
Cyclosporin	
response	< 50%
dosing	3-5 mg kg^{-1}daily
duration	16 weeks
PUVA / UVB	
response	< 50%
dosing	2 times weekly (PUVA)
	3 times weekly (UVB)
duration	10 weeks
Contraindication/serious side-effects to UVB/PUVA, methotrexate and/or cyclosporine	

UV, ultraviolet; PUVA, psoralen plus UVA. * Skindex ≥ 35 is used as an indicator of the severity of psoriasis if PASI is ≥ 8 and < 10.

cases and (b) inclusion would be more restricted to severe cases (higher PASI), and (ii) that efficacy would be lower in obese patients due to the possible role of adipose tissue in tumour necrosis factor (TNF)-α homeostasis.

PATIENTS AND METHODS

Since the introduction of etanercept and efalizumab in our Department of Dermatology, Academic Medical Center of the University of Amsterdam, a sequential algorithm is used for the treatment of psoriasis with these biological agents. Patients who are eligible for biological treatment are first given the option of etanercept, to be followed by efalizumab when etanercept is contra-indicated, not effective or has caused serious side effects in the past. This decision is based on our view that TNF-α plays a central role in the innate immunity activation that is critical to psoriasis pathogenesis, as compared to inhibition of CD11a.[2]

We performed a retrospective analysis on 50 consequetive psoriasis patients treated for 12 weeks with etanercept at our department. Results were compared with the data from four randomized controlled trials (Pubmed search with MeSH Major topic 'TNFR-Fc fusion protein' and 'psoriasis' and limits: randomized controlled trial, humans).

In the pretreatment phase, psoriasis patients who were initially selected for treatment with etanercept were screened for the national reimbursement and safety criteria.

Patients were treated with either a weekly dose of 2 x 25 mg or 2 x 50 mg etanercept given subcutaneously. The start dose of 2 x 50 mg per week was given in case of obesity or very severe psoriasis. In case of clinically inadequate improvement or worsening of psoriasis the initial dose of 2 x 25 mg weekly could be increased to 2 x 50 mg weekly. This was done on the clinical impression and no strict criteria were applied. Patients were allowed to use concomitant topical or systemic medication.

To determine the severity of psoriasis, PASI, Body Surface Area (BSA) and Skindex-29 were calculated before the start of etanercept therapy and after 12 weeks of treatment. The Skindex-29 has been selected on the basis of a systematic review of quality-of-life indices for psoriasis.[3] There are 29 question items which are divided over 3 scales of quality of life: symptoms, emotions and functioning. Each question has 5 answer options, varying from 'never' to 'always'. According to the reimbursement criteria, patients with a Skindex ≥ 35 (on a scale of 0 – 100) are eligible for biological therapy if the PASI is ≥ 8.

The measure of efficacy was the proportion of patients in each treatment group attaining 50% or more improvement compared to baseline (PASI 50), 75% or more improvement (PASI 75) and 90% or more improvement (PASI 90).

The safety criteria screening included blood tests (full blood count, liver function, kidney function and biochemistry screen), urine tests (protein, glucose and hemoglobin), and a chest X-ray and a tuberculin skin test to screen for tuberculosis. When suspect or positive, a specialist in pulmonary medicine was consulted. During

the treatment phase these tests were repeated every 4 to 6 weeks. Side-effects, the percentage of patients that had to discontinue and reasons for drop-out were documented. Side-effects were considered mild, when daily activities were not influenced; moderate when daily activities were somewhat affected and severe when daily activities were impeded.

To investigate whether efficacy results would be less in obese patients we used the Body Mass Index (BMI) as a measure for obesity. A BMI of 20-24.9 kg m^{-2} was considered normal weight, a BMI of 25-29.9 kg m^{-2} as overweight and a BMI of 30 kg m^{-2} or more as obesity.[4]

RESULTS

From February until October 2005, 68 patients with psoriasis were initially selected for treatment with etanercept (Figure 1). Fifty patients started treatment with etanercept. Of these 50 patients 36 were referred to us by other dermatologists and 14 were referred to our outpatients clinic from our local recruitment area. By January 2006 50 patients had been treated for the duration of 3 months. Demographic data of these patients are shown in Table 2.

The majority of patients who were eligible for the reimbursement criteria, had a PASI of 10 or more. Only 3 patients started treatment with etanercept on account of a Skindex ≥ 35 with a PASI ≥ 8.

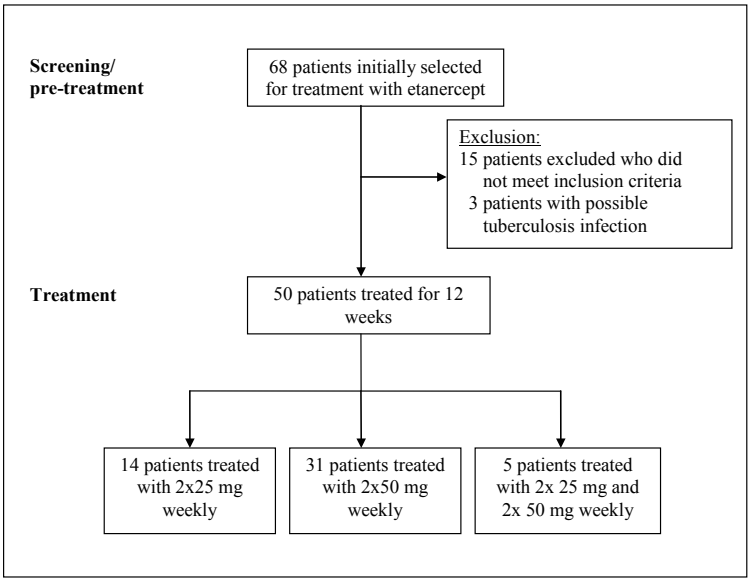

Figure 1 Disposition of patients.

Table 2 Demographic and clinical data of patients at baseline (n=50)

Parameter	2x25 mg weekly (n=14)	2x50 mg weekly (n=31)	2x25 mg → 2x50 mg weekly (n=5)
Age (years)*	45.8 (25-68)	43.7 (25-61)	44.2 (36-54)
Male: female ratio	11:3	23:8	4:1
Body Mass Index (BMI)*	29.4 (21.4-39)	28.5 (18-46.6)	26.3 (21.3-26.4)
Duration of skin disease (years)*	21.4 (5-52)	20.2 (2-51)	20.2 (5-26)
Psoriatic arthritis (n)	3	12	1

* mean (range)

Table 3 Previous systemic dermatologic treatments (n=50)

		Discontinuation due to	
Systemic treatment	Number of patients	Lack of efficacy n (%)	Side effects/ safety concerns n (%)
UVB	43	37 (86)	6 (14)
PUVA	37	30 (81)	7 (19)
methotrexate	50	28 (56)	22 (44)
cyclosporin	45	26 (58)	19 (42)
acitretin	28	23 (82)	1 (4)
fumaric acid ester	16	15 (94)	0 (0)
alefacept	6	6 (100)	0 (0)
onercept	5	5 (100)	0 (0)
etanercept	5	0 (0)	0 (0)
efalizumab	4	3 (75)	1 (25)
sulfasalazine	3	2 (67)	1 (33)
anti-CD3 mAB	1	1 (100)	0 (0)
siplizumab	1	1 (100)	0 (0)

UV, ultraviolet; PUVA, psoralen plus UVA; onercept, recombinant human tumor necrosis factor-α binding protein; mAB, monoclonal antibody; siplizumab, anti-CD2 mAB.

The majority of patients had used other systemic treatments in addition to the required systemic treatments, as shown in Table 3. The main reason for discontinuation of the therapies was lack of efficacy. Thirteen patients had been treated with one or more biologicals previously through participation in clinical trials. With the exception of etanercept, all other biologicals previously used had shown unsatisfactory responses.

Initially a weekly dose of 2 x 25 mg was given to 19 patients and 31 patients used a weekly dose of 2 x 50 mg subcutaneously. Due to clinically inadequate improvement

or worsening of psoriasis, the initial dose of 2 x 25 mg weekly was increased to 2 x 50 mg weekly in 5 patients. In one patient this occurred as soon as 2 weeks after the initiation of etanercept treatment. In the remaining patients the dose was increased 5 to 9 weeks after starting with etanercept.

Throughout the treatment with etanercept, 43 out of 50 patients needed concomitant treatment besides the etanercept therapy. In most patients concomitant topical treatment was sufficient; however, 9 patients required concomitant systemic therapy temporarily or continuously throughout etanercept treatment. Due to psoriatic arthritis, methotrexate was used simultaneously with etanercept by 3 patients. The methotrexate dosages used were 5 mg (with 2 x 50 mg etanercept), 15 mg (with 2 x 25 mg etanercept) and 20 mg (with 2 x 50 mg etanercept) weekly. The dosages for methotrexate were determined previously by the patients reumatologists and were based on the extent of arthritis and the tolerance of methotrexate. The dosages of etanercept were based on the degree of adiposity and the severity of psoriasis. Abnormal liver function tests resulted in discontinuation of methotrexate in the patient using 5 mg weekly. For fear of exacerbation of their already severe psoriasis, methotrexate was used simultaneously by 3 other patients. Their dosages, varying from 7.5 mg to 25 mg weekly, were reduced with 1 tablet per week after starting with 2 x 50 mg etanercept weekly. Cyclosporin, 3 mg kg^{-1} daily with 2 x 25 mg etanercept, and fumaric acid, 1080 mg daily with 2 x 25 mg etanercept, were also used simultaneously for fear of exacerbation in two patients and were stopped, after gradual dose reduction, at 2 and 9 weeks, respectively. Furthermore, acitretin 20 mg daily was used by one patient as prophylaxis for recurrent squamous cell carcinoma caused by years of extensive phototherapy. No additional monitoring was performed in patients using concomitant systemic therapy, as patients already had blood and urine tests every 4 to 6 weeks. So far, no particular side effects occurred.

In Table 4 an overview is given of the efficacy results after 12 weeks of etanercept for the 2 x 25 mg, 2 x 50 mg and the 2 x 25 mg increased to 2 x 50 mg group. An improvement of < 50% occurred twice (14%) in the 2 x 25 mg weekly group, nine times (29%) in the 2 x 50 mg weekly group and twice in the 2 x 25 mg increased to 2 x 50 mg group (40%). Alteration to efalizumab treatment after insufficient response occurred in 5 out of 9 patients in the 2 x 50 mg weekly group and 2 out of 5 patients in the 2 x 25 mg increased to 2 x 50 mg weekly group. The remaining patients who attained an improvement of < 50% continued etanercept treatment in a dose of 2 x 50 mg weekly. Their improvement after 12 weeks was at least 40%.

Of the 7 patients changed to efalizumab therapy, 6 patients completed 12 weeks of treatment: 3 patients displayed a improvement of 54 to 77% and in 3 patients the psoriasis deteriorated even further (-16 to -57%). One patient just recently started efalizumab treatment and no clinical evaluation has yet been made.

Of the patients who deteriorated, 2 patients have recently restarted methotrexate; this time with intramuscular injections to prevent earlier experienced side effects. The clinical effects are not known at this point. The other patient was treated with infliximab successfully.

Table 4 Clinical data of patients after 12 weeks of etanercept treatment

		2x25 mg (n=14)	2x50 mg (n=31)	2x25 mg → 2x50mg (n=5)
PASI	Baseline*	13.7 (8.2-22.2)	17.4 (7.6-39)	11.9 (10.2-14.4)
	Week 12*	5.5 (0-16.2)	7.4 (1.2-20.8)	7.1 (4.8-10.8)
	Improvement (%)	60.0	57.5	40.3
	PASI 50 (n)	12	22	3
	PASI 75 (n)	3	7	0
	PASI 90 (n)	1	2	0
BSA	Baseline*	17.0 (5.5-40.3)	23.1 (6-76.5)	9.8 (7.1-16.4)
	Week 12*	7.8 (0-27)	8.7 (1.0-27.2)	8.0 (3.9-13.1)
	Improvement (%)	54.1	62.3	18.4
Skindex	Baseline*	66.5 (50-97)	60.6 (30-100)	71.2 (54-91)
	Week 12*	29.9 (0-88)	36.1 (1-72)	67.0 (50-85)
	Improvement (%)	55.0	40.4	5.9

* mean (range)

The improvements in Skindex-29 accomplished in the groups attaining < PASI 50, PASI 50, PASI 75 and PASI 90, regardless of the dosing, were 9.2% (-44% to 49.1%), 44.2% (-51.5% to 100%), 75.5% (46.6% to 96.7%) and 61.6% (43.6% to 93.1%).

The Skindex-29 of the three patients eligible for reimbursement on behalf of their Skindex, showed improvements of 49 % (from 51 to 26), 75% (from 72 to 18) and 80% (from 97 to 19).

The adverse events mentioned by the patients during etanercept treatment are shown in Table 5. Most events were considered as of mild or moderate and occurred in similar proportions of patients in each dosage group during the initial 12 weeks of treatment (data not shown). Laboratory tests remained normal during treatment with etanercept.

The BMI of the patients attaining a response < PASI 50 (n=13), was increased in all but one patient. The BMI of this patient was 21.3 kg m^{-2}, whereas the BMI of the other patients ranged from 25.8 to 39.0 kg m^{-2}. In the patient group which showed a PASI improvement of 50 to 75% (n=27), one patient had a BMI < 20 (18 kg m^{-2}) and six patients had a BMI between 20 and 25. A high BMI between 25 and 30 was found in 13 patients of this group and a BMI of more than 30 in seven patients (33.2 to 45.8 kg m^{-2}). In patients attaining PASI 75 or better (n= 10) one had a BMI of 19.5 kg m^{-2}, three had a normal BMI varying from 21.8 to 24.7 kg m^{-2} and six were considered overweight or obese according to their BMI, varying from 25.4 to 31.7 kg m^{-2}. Of the six overweight or obese patients who showed a significant improvement of their psoriasis after 12 weeks of etanercept, two had been dosed with 2 x 25 mg every week.

Table 5 Summary of reported adverse events

Description adverse event	Number of reports (%)
Upper airway infections	13 (26)
Gastrointestinal complaint	7 (14)
Fatigue	5 (10)
Injection side reaction	5 (10)
Worsening of psoriasis	3 (6)
Itch	3 (6)
Headache	3 (6)
Influenza-like symptoms	2 (4)
Joint pain	2 (4)
Fever	2 (4)
Folliculitis	2 (4)
Periodontitis	1 (2)
Urinary tract infection	1 (2)
Kidney stones	1 (2)
Weight gain	1 (2)

Reported adverse events of all patients until January 2006 (n=50).

DISCUSSION

In order to compare our data to the literature, an overview of the studies with etanercept is given in Table 6.[5-8] The percentage of our patients achieving < PASI 50 and PASI 50 is similar or even better, but the groups attaining PASI 75 and PASI 90 are apparently smaller.

The mean PASI of the patients participating in phase 2 and 3 trials is comparable with that in our patients; however, the history of previous psoriasis treatments and the concomitant medication used during etanercept treatment are not. Due to the reimbursement criteria we are forced to treat patients with more therapy-resistant psoriasis compared to the patients included in the above-mentioned clinical trials. This might account for the less favorable results.

Jacob et al. performed a systemic retrospective chart review in order to determine whether etanercept therapy enables long-term psoriasis patients to discontinue their traditional systemic psoriatic therapy.[9] Their results showed that 50.9% of the patients were able to discontinue, and 22.6% were able to decrease their traditional therapy while on etanercept. As additional systemic therapy is still needed in a large proportion of patients, these data of Jacob et al. also give the impression that daily practice gives less favorable results in comparison with previously published clinical trials. However, Jacob et al. mention nothing on the degree of unmanageability by

Table 6. Overview of etanercept treatment in initial practical use and randomized controlled trials

	Present report		Papp et al.[7] 2005		Leonardi et al.[6] 2003		Gottlieb et al.[5] 2003	Tyring et al.[8] 2006
Inclusion criteria	- PASI ≥ 10 (Skindex-29 ≥ 35 if PASI ≥ 8) - Ineffectiveness of / contraindication to / serious side effects during UVB / PUVA, methotrexate and cyclosporine		- BSA ≥ 10 - PASI ≥ 10 - ≥ 1 previous phototherapy or systemic therapy for psoriasis or candidate for these treatments		- BSA ≥ 10 - PASI ≥ 10 - ≥ 1 previous phototherapy or systemic therapy for psoriasis or candidate for these treatments		- BSA ≥ 10 - ≥ 1 previous phototherapy or systemic therapy for psoriasis or candidate for these treatments	- PASI ≥ 10 - ≥ 1 previous phototherapy or systemic therapy for psoriasis or candidate for these treatments
Allowed concomitant medication	Topical corticosteroids, vitamin D analogues, systemic psoriasis treatment		Moderate strength topical corticosteroids for scalp, axilla and groin		Low or moderate strength topical corticosteroids for scalp, axilla and groin		Lower-potency topical corticosteroids and tar-compounds for scalp, axilla and groin	Low or moderate strength topical corticosteroids for scalp, axilla and groin
Dosing (mg/wk)	2x25	2x50	2x25	2x50	2x25	2x50	2x25	2x50
Patients (n)	14	31	196	194	162	164	57	311
Mean PASI baseline	13.7	17.4	16.9	16.1	18.4	18.5	17.8	18.3
< PASI 50 (%)*	14.3	29.0	36	23	42	26	30	-
PASI 50 (%)*	85.7	71.9	64	77	58	74	70	74
PASI 75 (%)*	21.4	22.6	34	49	34	49	30	47
PASI 90 (%)*	7.1	6.3	11	21	19	22	10	21

PASI, Psoriasis Area and Severity Index; PASI 50, 50% or more improvement compared with baseline; PASI 75, 75% or more improvement; PASI 90, 90% or more improvement; BSA, body surface area involvement; UV, ultraviolet; PUVA, psoralen plus UVA. * After 12 weeks of treatment.

the other systemic therapies, the dosing of etanercept and the duration of treatment with etanercept in this group of patients.

Although the PASI was sufficient for reimbursement in the majority of our patients (n=47), a SKINDEX ≥35 was the decisive factor for reimbursement of treatment with biologicals for three patients (6%). The Skindex-29 showed the largest improvement in the patients attaining PASI 75 and the smallest improvement in the patients attaining < 50% improvement.

A perhaps previously under-reported side-effect of etanercept treatment is fatigue. In our population 10% reported fatigue related to etanercept treatment. However, fatigue is not mentioned as a side-effect in the instruction leaflet of etanercept. In a phase 3 clinical trial in psoriatic arthritis patients, fatigue was reported in 4 out of 30 patients (13%) treated with etanercept, although not statistically more as compared to the patients receiving placebo.[10] On the website of the Food and Drug Administration (FDA), it is reported that fatigue was reported as adverse event during post-approval use of etanercept.[11] As these adverse events are reported voluntarily from a population of uncertain size, it is not possible to estimate its frequency reliably. Several studies have indicated that serum levels of TNF-α might be related to the extent of fatigue.[12;13] However, several other investigators found no significant correlation between general fatigue and changes in levels of TNF-α or improvement after anti-TNF-α therapy.[14-18] It could be that changes in fatigue levels noted in clinical trials of anti-TNF-α therapy reflect general improvement in pain, function and psychological status rather than any direct interference with cytokines controlling fatigue. This is confirmed in a recent investigation in which the effect of etanercept was assessed on fatigue and depression. This study showed that improvements in fatigue were correlated with decreasing joint pain and less well correlated with improvement in PASI.[8] Of the patients who reported fatigue in our patient group (n=5), only one showed almost no psoriasis improvement according to the PASI after 12 weeks (16%). The other PASI improvements attained ranged from 46 to 53%. The Skindex-29, however, was deteriorated or showed just little improvement (-52% to 13%) in the majority (n=4) of these patients. Only one patient showed a remarkable improvement in the Skindex-29 of 80%.

Clinical studies have shown that adipocytes are able to produce TNF-α and that the level of TNF-α is up-regulated in obese subjects.[19-21] As the therapeutic effect of etanercept is to eliminate the surplus of TNF-α found in the circulation and in the skin lesions of psoriasis by competitively binding to this proinflammatory cytokine,[22;23] we hypothesized that the response to etanercept would be less in obese patients. Oral communication with Wyeth affirmed our hypothesis. Unpublished analysis done for registration showed that improvement in PASI in patients with a high bodyweight (> 105 kg) was less as compared to patients with normal weight, even on the maximum dose of etanercept. The high BMI found in our group of patients with less than 50% improvement supports our hypothesis. However, the fact that the majority of patients with significant improvement, of which two even had the standard dose (2 x 25 mg),

had high BMI as well, contradicts this idea. Besides the upregulated level of the TNF-α in obese subjects, the soluble receptors in the circulation are also elevated.[19] Perhaps TNF-α and the soluble receptors are equally increased in obese subjects, preventing an extra surplus due to obesity. Also, the small number of patients could be accountable for not revealing a probable relation of obesity and response to etanercept.

In conclusion, these initial practical and nontrial-based clinical results of routine administration of etanercept in patient with psoriasis are less favourable as might be expected from the phase 2 and 3 clinical trials. Although the mean severity of psoriasis (PASI) in our patient group did not differ from that described in these published clinical trials, our patients were more therapy resistant due to the strict reimbursement criteria.

Fatigue may be a previously underreported side-effect of etanercept.

The BMI does not seem to be influence the patient's response to etanercept.

The Skindex-29 is of decisive value as a selection mechanism for reimbursement in 5% of the patient population. Future studies are necessary for affirmation of these conclusions.

REFERENCE LIST

1. Chren MM, Lasek RJ, Quinn LM *et al*. Skindex, a quality-of-life measure for patients with skin disease: reliability, validity, and responsiveness. *J Invest Dermatol* 1996; **107**: 707-13.
2. Bos JD, de Rie MA, Teunissen MB *et al*. Psoriasis: dysregulation of innate immunity. *Br J Dermatol* 2005; **152**: 1098-107.
3. de Korte J, Mombers FMC, Sprangers MAG *et al*. The Suitability of Quality-of-Life Questionnaires for Psoriasis Research: A Systematic Literature Review. *Arch Dermatol* 2002; **138**: 1221-7.
4. WHO. Obesity:preventing and managing the global epidemic. Report on a WHO Consultation on Obesity, Geneva, 3-5 June, 1997. WHO/NUT/NCD/98.1. Technical Report Series Number 894.World Health Organization, Geneva. 2000.
5. Gottlieb AB, Matheson RT, Lowe N *et al*. A randomized trial of etanercept as monotherapy for psoriasis. *Arch Dermatol* 2003; **139**: 1627-32.
6. Leonardi CL, Powers JL, Matheson RT *et al*. Etanercept as monotherapy in patients with psoriasis. *N Engl J Med* 2003; **349**: 2014-22.
7. Papp KA, Tyring S, Lahfa M *et al*. A global phase III randomized controlled trial of etanercept in psoriasis: safety, efficacy, and effect of dose reduction. *Br J Dermatol* 2005; **152**: 1304-12.
8. Tyring S, Gottlieb A, Papp K *et al*. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet* 2006; **367**: 29-35.
9. Jacob SE, Sergay A, Kerdel FA. Etanercept and psoriasis, from clinical studies to real life. *Int J Dermatol* 2005; **44**: 688-91.
10. Mease PJ, Goffe BS, Metz J *et al*. Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet* 2000; **356**: 385-90.
11. Enbrel (etanercept) for subcutaneous injections. www.fda.gov/medwatch/

safety/2005/jul_PL/Enbrel_PL.pdf. 9-8-0005.

12. Borish L, Schmaling K, DiClementi JD *et al*. Chronic fatigue syndrome: identification of distinct subgroups on the basis of allergy and psychologic variables. *J Allergy Clin Immunol* 1998; **102**: 222-30.
13. Patarca R, Klimas NG, Lugtendorf S *et al*. Dysregulated expression of tumor necrosis factor in chronic fatigue syndrome: interrelations with cellular sources and patterns of soluble immune mediator expression. *Clin Infect Dis* 1994; **18 Suppl 1**: S147-S153.
14. Vgontzas AN, Papanicolaou DA, Bixler EO *et al*. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J Clin Endocrinol Metab* 1997; **82**: 1313-6.
15. Vgontzas AN, Chrousos GP. Sleep, the hypothalamic-pituitary-adrenal axis, and cytokines: multiple interactions and disturbances in sleep disorders. *Endocrinol Metab Clin North Am* 2002; **31**: 15-36.
16. Ahlberg K, Ekman T, Gaston-Johansson F. Levels of fatigue compared to levels of cytokines and hemoglobin during pelvic radiotherapy: a pilot study. *Biol Res Nurs* 2004; **5**: 203-10.
17. Geinitz H, Zimmermann FB, Stoll P *et al*. Fatigue, serum cytokine levels, and blood cell counts during radiotherapy of patients with breast cancer. *Int J Radiat Oncol Biol Phys* 2001; **51**: 691-8.
18. Wolfe F, Michaud K. Fatigue, rheumatoid arthritis, and anti-tumor necrosis factor therapy: an investigation in 24,831 patients. *J Rheumatol* 2004; **31**: 2115-20.
19. Hauner H, Bender M, Haastert B *et al*. Plasma concentrations of soluble TNF-alpha receptors in obese subjects. *Int J Obes Rel Metab Disord* 1998; **22**: 1239-43.
20. Hotamisligil GS, Arner P, Caro JF *et al*. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995; **95**: 2409-15.
21. Kern PA, Saghizadeh M, Ong JM *et al*. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 1995; **95**: 2111-9.
22. Ettehadi P, Greaves MW, Wallach D *et al*. Elevated tumour necrosis factor-alpha (TNF-alpha) biological activity in psoriatic skin lesions. *Clin Exp Immunol* 1994; **96**: 146-51.
23. Mussi A, Bonifati C, Carducci M *et al*. Serum TNF-alpha levels correlate with disease severity and are reduced by effective therapy in plaque-type psoriasis. *J Biol Regul Homeost Agents* 1997; **11**: 115-8.

4

REDUCTION OF DIFFERENT INFLAMMATORY CELL TYPES OF THE INNATE IMMUNE SYSTEM IN PSORIATIC SKIN DURING ETANERCEPT TREATMENT

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ABSTRACT

To investigate whether specific markers for innate immunity would diminish with successful treatment in psoriasis, we analyzed lesional and non-lesional skin biopsies taken from patients with moderate to severe psoriasis during 12 weeks of treatment with etanercept in correlation with the clinical response. In the clinical responders (PASI reduction > 50%), all markers (CD3, CD68, CD161, elastase, BDCA-2, TNF-α) showed a decline during treatment, indicating a pivotal role for innate immunity in the pathogenesis of psoriasis.

BACKGROUND AND QUESTION ADDRESSED

Over the years, various hypotheses on psoriasis pathogenesis have been proposed, varying from keratinocyte-centered, to T-cell mediated, to aggravation at the level of innate immunity [1-3]. The latter is based on the remarkable improvements seen in clinical trials with tumor necrosis factor (TNF)- α antagonists [4-6], together with the discovery of activation of several cellular elements and humoral components of the innate immune system in lesional and non-lesional psoriatic skin [7-17].

We hypothesized that, if malfunctioning of the innate immune system is somehow a pivotal initiator of psoriasis, successful treatment of psoriasis would diminish the expression of markers of innate immunity.

EXPERIMENTAL DESIGN

We analyzed lesional and non-lesional skin biopsies, taken on baseline, weeks 3 and 12, from 6 patients with moderate to severe psoriasis treated with etanercept (a humanized TNF-α receptor) 50 mg subcutaneous twice weekly for 3 months. This investigation was a biopsy substudy of the study registered at www.clinicaltrials.gov under NCT00195507. The Psoriasis Area and Severity Index (PASI) and the Body Surface Area (BSA) were assessed at baseline, weeks 3 and 12. Skin biopsies were immunohistochemically stained for CD3 (T cells), CD68 (macrophages), CD161 (NK-T cells), elastase (neutrophils), BDCA-2 (plasmocytoid dendritic cells) and TNF-α. All sections were randomly coded and were analyzed through manual quantification of the twenty high power fields per section. Manual quantification was done by two independent observers blinded for order, patient and clinical data. The epidermal and dermal regions were separately counted. Positive staining of CD3, CD68, CD161, BDCA-2, elastase and TNF-α was expressed as positive cells/mm^2.

RESULTS AND CONCLUSIONS

In the lesional skin biopsies of the clinical responders (Table 1, n=3, PASI reduction > 50%), all investigated markers were clearly reduced after 12 weeks of treatment with etanercept (Figure 1A), whereas no reduction of these markers was seen in the non-responders (Figure 1B). Particulary CD68, CD161, elastase and BDCA2-positive cells declined early during treatment (Figure 2).

Early reduction of CD68-positive cells was also seen by Gottlieb et al. during etanercept treatment, by Marble et al. during adalimumab treatment and by Markham et al. during infliximab treatment [18-20].

A rapid decline of CD161-positive cells during etanercept treatment was also described by Van Lingen et al.[21]. CD161 is a marker for NK-T cells. Activation of NK-T

Tabel 1 Demografics and clinical respons

	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6
Gender	M	M	M	F	M	M
Age (years)	43	52	62	49	56	47
Duration psoriasis (years)	18	45	41	45	32	10
PASI wk 0	19.6	11.3	13.6	11.8	11.4	19.4
PASI wk 3	17.3	9.4	13.6	8.0	na	na
PASI wk 12	10.4	11.7	3.8	7.3	4.3	16.5
Improvement of PASI on wk 3 (%)	12	17	0	32	na	na
Improvement of PASI on wk 12 (%)	47	-4	72	38	62	15
BSA wk 0	17.9	14.5	22.5	10.2	8.2	14.5
BSA wk 3	32.5	14.7	19.1	8.3	na	na
BSA wk 12	8.0	12.5	5.7	8.8	1.0	8.0
Improvement of BSA on wk 3 (%)	-81.6	-1.4	15.1	18.6	na	na
Improvement of BSA on wk 12 (%)	55.3	13.8	74.7	13.7	87.8	44.8

PASI, Psoriasis Area and Severity Index; BSA, Body Surface Area; na, data not available

cells results in prompt release of high levels of cytokines like INF-γ and TNF-α, and NK-T cells have mutual interaction with dendritic cells and keratinocytes, which are thought to be relevant in psoriasis. Remarkably, Bovenschen et al. described a case in which immuhistochemical analyses in a non-responsive patient on infliximab showed a correlation between the number of epidermal NK-T cells and the lack of clinical efficacy, supposing a pathogenic role for these cells in psoriasis [22].

When looking at elastase-positive cells in clinical responders during etanercept, our results are corresponding with Gottlieb et al.[18]. Vincek et al. reported a rapid decline of neutrophils in lesional skin already one day after an infusion with infliximab [23].

No previous investigations are present on the respons of plasmacytoid dendritic cells in psoriatic skin during etanercept treatment. During adalimumab treatment a similar fast response was noted by Marble et al.[19] BDCA-2-positive cells are mostly plasmacytoid dendritic cells, which are present in normal-appearing skin of psoriasis patients, but in contrast, absent in normal skin of healthy individuals [24].

Our results show a clear decline in CD3-positive cells at week 12, which is in line with Mahiques et al. [25]. According to Gottlieb et al. and Zaba et al. this decrease of T cells can be appreciated at a much earlier time point during etanercept treatment [18,26]. Previous studies done with infliximab all showed a rapid decline of CD3-positive cells in lesional skin [20,23,27,28].

To our surprise we did not find a clear effect of TNF-α inhibitor etanercept on the TNF-α expression in the skin *in situ* in responders. Studies with another TNF-α inhibitor, infliximab, did show a decline in TNF alpha-positive cells [20,23,29]. These contradictionary results could be due to the marker used to detect TNF-α. TNF-α is a

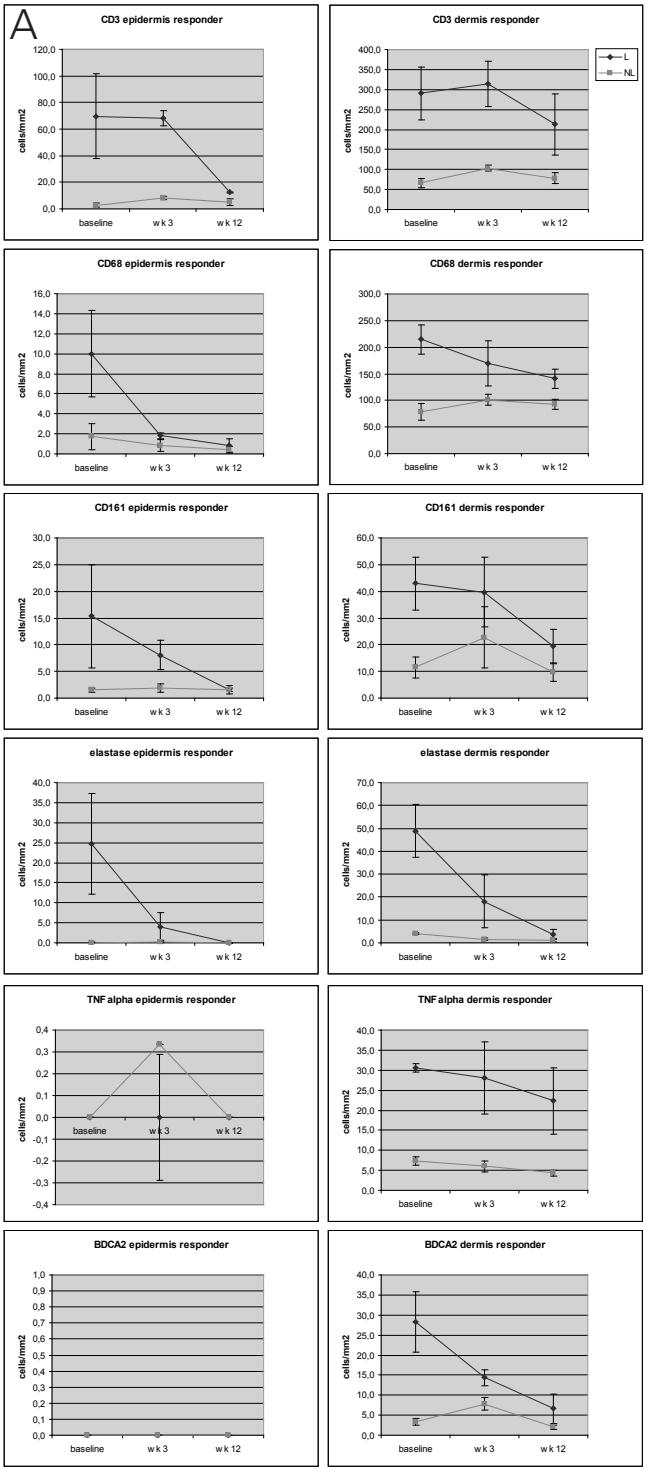

Figure 1 Effects on cell numbers in lesional and non-lesional epidermis and dermis over time in responders (**A**) and non-responders (**B, next page**) during treatment with etanercept. Data are shown as means with SD. L, lesional; NL, non-lesional.

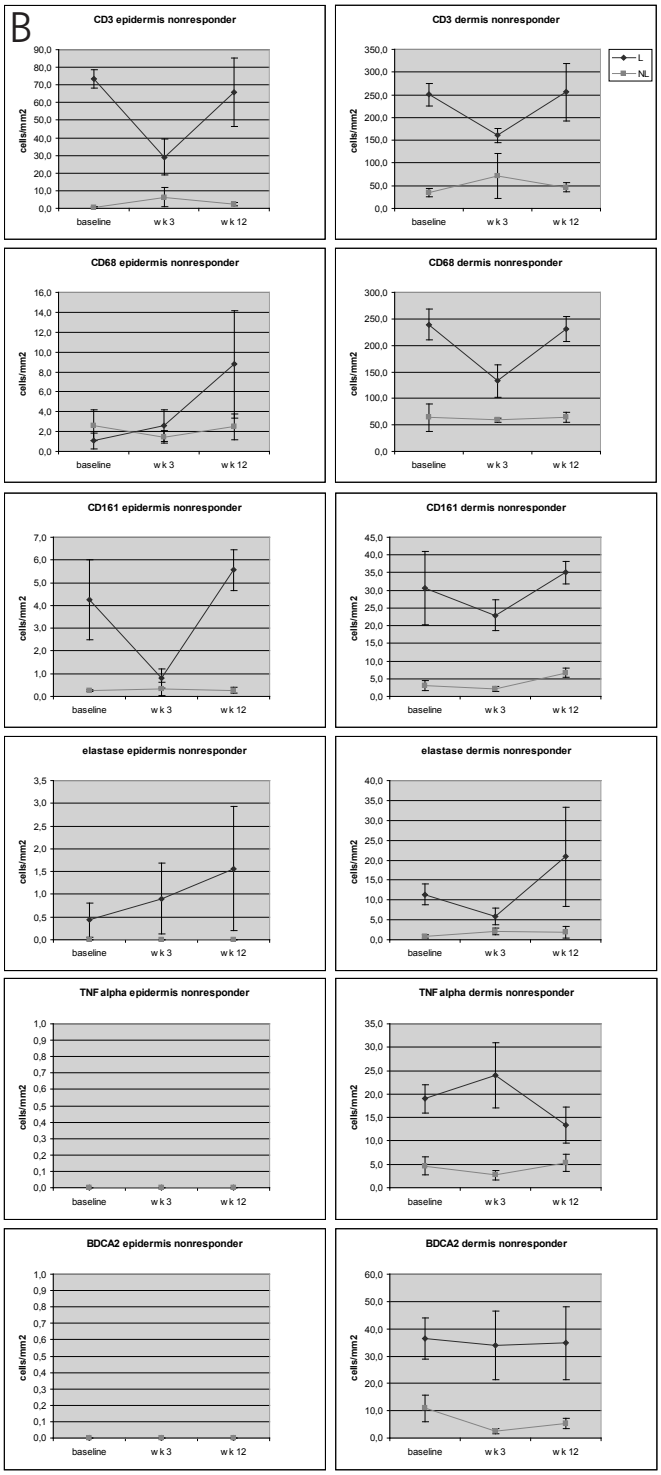

Figure 1 Effects on cell numbers in lesional and non-lesional epidermis and dermis over time in responders (**A, previous page**) and non-responders (**B**) during treatment with etanercept. Data are shown as means with SD. L, lesional; NL, non-lesional.

cytokine produced by many different cell types and different immunohistochemical markers will demonstrate TNF-α in various different ways, as shown by Van der Laan et al. [30]. Furthermore, it has been described that levels of TNF-α in the circulation are stable or even increase after etanercept treatment, probably due to an increased stability of etanercept-bound TNF-α [31]. Moreover, immunohistochemistry gives no information about the biological activity of the detected TNF-α.

Information on the effect of etanercept treatment on the different leukocyte subsets in lesional and non-lesional psoriatic skin and possible relationship to the clinical response is very limited. Gottlieb et al. showed after one month of treatment a rapid and complete reduction of IL-1 and IL-8 (immediate/early genes), followed by progressive reductions in many other inflammation-related genes, and finally somewhat slower reductions in infiltrating myeloid cells ($CD11c^+$ cells) and T lymphocytes [18]. Zaba et al. observed reduction of inflammatory dendritic cell products that drive Th17 cell proliferation (IL-23), as well as Th17 cell products and downstream effector molecules (IL-17, IL-22, CC chemokine ligand 20, β-defensin 4) [26].

Mahiques et al. showed a significant decrease of $CD4^+$ and $CD8^+$ T cells after 12 weeks of treatment in the epidermis and dermis of psoriatic skin [25], whereas Van Lingen et al. only showed a significant decrease of $CD8^+$ T cells in the dermis after 12 weeks in responders [21], as well as a significant decrease of CD161 in the dermis of responders.

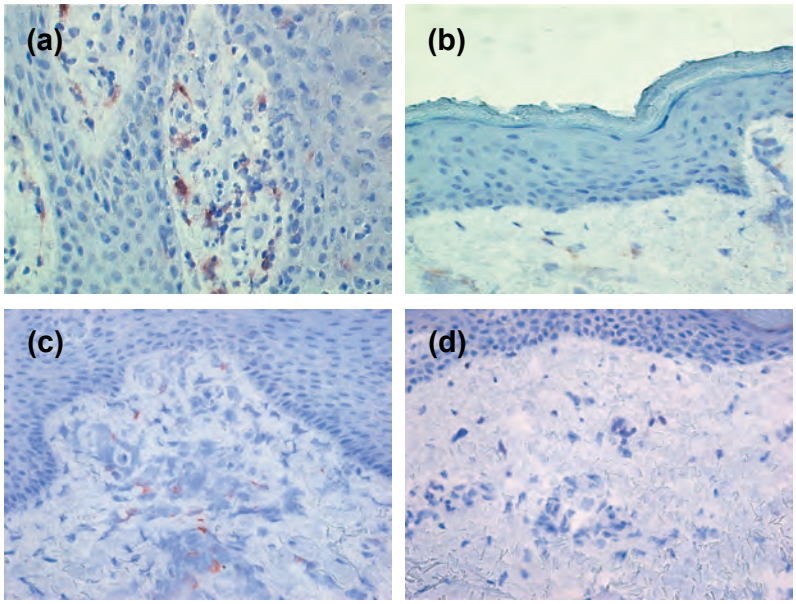

Figure 2 Immunohistochemical staining of psoriatic skin in a responding patient before and after treatment with etanercept. CD68-positive cells at baseline (a) and week 12 (b). BDCA-2-positive cells at baseline (c) and week 12 (d).

In addition to the earlier studies describing the immunohistochemical effects of treatment with etanercept in psoriasis, this is the first study in which $CD68^+$ cells and BDCA-2^+ cells were investigated. Our data are in concordance with previous similar studies and show that several innate immunity markers diminish during effective treatment with etanercept, indicating that innate immunity might play a role in the pathogenesis of psoriasis. Further investigation is needed to understand the involvement of other cell types and cytokines of innate immunity.

REFERENCE LIST

1. Bos J D, de Rie M A, Teunissen M B, Piskin G. Psoriasis: dysregulation of innate immunity. *Br J Dermatol* 2005: **152**: 1098-1107.
2. Bos J D. Psoriasis, innate immunity, and gene pools. *J Am Acad Dermatol* 2007: **56**: 468-471.
3. Schön M P. Animal models of psoriasis: a critical appraisal. *Exp Dermatol* 2008: **17**: 703-712.
4. Gordon K B, Langley R G, Leonardi C *et al*. Clinical response to adalimumab treatment in patients with moderate to severe psoriasis: Double-blind, randomized controlled trial and open-label extension study. *J Am Acad Dermatol* 2006: **55**: 598-606.
5. Leonardi C L, Powers J L, Matheson R T *et al*. Etanercept as monotherapy in patients with psoriasis. *N Engl J Med* 2003: **349**: 2014-2022.
6. Reich K, Nestle F O, Papp K *et al*. Infliximab induction and maintenance therapy for moderate-to-severe psoriasis: a phase III, multicentre, double-blind trial. *Lancet* 2005: **366**: 1367-1374.
7. Abrams J R, Kelley S L, Hayes E *et al*. Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. *J Exp Med* 2000: **192**: 681-694.
8. Baker B S, Ovigne J M, Powles A V, Corcoran S, Fry L. Normal keratinocytes express Toll-like receptors (TLRs) 1, 2 and 5: modulation of TLR expression in chronic plaque psoriasis. *Br J Dermatol* 2003: **148**: 670-679.
9. de Jongh G J, Zeeuwen P L J M, Kucharekova M *et al*. High Expression Levels of Keratinocyte Antimicrobial Proteins in Psoriasis Compared with Atopic Dermatitis. *J Investig Dermatol* 2005: **125**: 1163-1173.
10. Gearing A J, Fincham N J, Bird C R *et al*. Cytokines in skin lesions of psoriasis. *Cytokine* 1990: **2**: 68-75.
11. Harder J, Schroder J M. Psoriatic scales: a promising source for the isolation of human skin-derived antimicrobial proteins. *J Leukoc Biol* 2005: **77**: 476-486.
12. Jablonska S. Immunological mechanisms in psoriasis: role of polymorphonuclear leucocytes. In: Farber EM, ed. *Psoriasis*. New York: Elsevier 2007: 1479-137.
13. Nickoloff B J, Wrone-Smith T, Bonish B, Porcelli S A. Response of murine and normal human skin to injection of allogeneic blood-derived psoriatic immunocytes: detection of T cells expressing receptors typically present on natural killer cells, including CD94, CD158, and CD161. *Arch Dermatol* 1999: **135**: 546-552.
14. Nickoloff B J. Keratinocytes regain momentum as instigators of cutaneous inflammation. *Trends Mol Med* 2006: **12**: 102-106.
15. Teunissen M B M. Langerhans cells and other skin dendritic cells. In: Bos JD, ed. *Skin immune system: cutane-*

ous immunology and clinical immunodermatology, 3rd edition. Boca Raton, Florida: CRC Press 2007: 123-182.

16. Zhou X, Krueger J G, Kao M C *et al*. Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol Genomics* 2003: **13**: 69-78.
17. Wu C, Li C, Wei L, Zheng Z. Innate immune modulation of keratinocytes by antikeratin 16 antibodies. *Exp Dermatol* 2008: **17**: 645-652.
18. Gottlieb A B, Chamian F, Masud S *et al*. TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. *J Immunol* 2005: **175**: 2721-2729.
19. Marble D J, Gordon K B, Nickoloff B J. Targerting TNF alpha rapidly reduces density of dendritc cells and macrophages in psoriatc plaques with restoration of epidermal keratinocyte differentation. *J Dermatol Sci* 2007: **48(2)**: 87-101.
20. Markham T, Mullan R, Golden-Mason L *et al*. Resolution of endothelial activation and down-regulation of Tie2 receptor in psoriatic skin after infliximab therapy. *J Am Acad Dermatol* 2006: **54(6)**: 1003-1012.
21. Van Lingen R G, Korver J E, Van de Kerkhof P C *et al*. Relevance of compartmentalization of T-cell subsets for clinical improvement in psoriasis: effect of immune-targeted antipsoriatic therapies. *Br J Dermatol* 2008: **159**: 91-96.
22. Bovenschen H J, Van de Kerkhof P C M, Gerritsen W J, Seyger M M B. The role of lesional T cells in recalcitrant psoriasis during infliximab therapy. *Eur J Dermatol* 2005: **15(6)**: 454-458.
23. Vincek V, Jacob S E, Nassiri M, Herbert L M, Nadji M, Kerdel F A. Infliximab monotheray in psoriasis: A case of rapid decline and clinical histological response. *Int J Dermatol* 2004: **43**: 303-308.
24. Nestle F O, Conrad C, Tun-Kyi A *et al*. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* 2005: **202**: 135-143.
25. Mahiques L, Pitarch G, Sanchez-Carazo J L, Perez-Ferriols A, Soriano C J, Alegre V. Analysis of lymphocyte populations in psoriatic plaques following inhibition of tumor necrosis factor alpha with etanercept. *Actas Dermosifiliogr* 2007: **98**: 539-544.
26. Zaba L C, Cardinale I, Gilleaudeau P *et al*. Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J Exp Med* 2007: **204**: 3183-3194.
27. Goedkoop A Y, Kraan M C, Picavet D I *et al*. Deactivation of endothelium and reduction in angiogenesis in psoriatic skin and synovium by low dose infliximab therapy in combination with stable methotrexate therapy: a prospective single-centre study. *Arthritis Res Ther* 2004: **6(4)**: R326-R334.
28. Goedkoop A Y, Kraan M C, Teunissen M B *et al*. Early effects of tumour necrosis factor alpha blockade on skin and synovial tissue in patients with active psoriasis and psoriatic arthritis. *Ann Rheum Dis* 2004: **63(7)**: 769-773.
29. Ogilvie A L, Luft M, Antoni C, Schuler G, Kalden J R, Lorenz H M. Leucocyte infiltration and mRNA expression of IL-20, IL-8 and TNF-R P60 in psoriatic skin is driven by TNF-alpha. *Int J Immunopathol Pharmacol* 2006: **19(2)**: 271-278.
30. Van der Laan N, De Leij L F, Buurman W, Timens W, Ten Duis H J. Tumor necrosis factor alpha (TNF alpha) in human skin: a comparison of different antibodies for immunohistochemisry. *Arch Dermatol Res* 2001: **293(5)**: 226-232.
31. Lan C CE, Wu, C S, Chen G S. Pretreatment tumour necrosis factor-α secretions from peripheral blood mononuclear cells of psoriatic patients do not correlate with rapid clinical response: urgent need for prognostc parameters. *J Eur Acad Dermatol Venereol* 2008: **22(3)**: 398-399.

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A PROSPECTIVE, RANDOMIZED, PLACEBO-CONTROLLED STUDY TO IDENTIFY BIOMARKERS ASSOCIATED WITH ACTIVE TREATMENT IN PSORIATIC ARTHRITIS: EFFECTS OF ADALIMUMAB TREATMENT ON LESIONAL AND NON-LESIONAL SKIN

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ABSTRACT

Objective: To determine which of the changes of several immunological markers in psoriatic skin correlates best with clinical response associated with effective therapy (adalimumab).

Methods: Twenty-four active PsA patients were randomized to receive adalimumab (n=12) or placebo (n=12) for 4 weeks. Lesional and non-lesional skin biopsies were obtained before and after 4 weeks of treatment from 22 patients with active skin lesions. Immunohistochemical analysis was performed to characterize several markers of innate immunity (CD68, CD161, elastase, TNF-α, BDCA-2) and T cells (CD3). Sections were analyzed by manual quantification by two independent observers. Statistical analysis was performed using covariance analysis.

Results: The mean Psoriasis and Severity Index (PASI) after 4 weeks of treatment was 2.61 points lower compared to placebo (95% CI -0.08 to 5.30, p=0.056). Paired pre-treatment and post-treatment skin samples were available for 18 patients. After applying a ranked analysis of covariance (ANCOVA) model to correct for baseline imbalances, a significant effect of treatment was observed on lesional dermal $CD161^+$ and $elastase^+$ cells. There was a median reduction of 6.9 cells/mm^2 for lesional dermal $CD161^+$ cells after adalimumab versus placebo treatment (p=0.0046). For $elastase^+$ cells there was a median reduction of 9.0 cells/mm^2 in lesional dermis after adalimumab versus placebo (p=0.024).

Conclusion: Adalimumab therapy in psoriasis lesions in PsA is associated with a reduction of dermal $CD161^+$ and $elastase^+$ cells, suggesting that these parameters could be used as biomarkers that are sensitive to change after active treatment in small proof of concept studies.

INTRODUCTION

Because the skin is a primary site for inflammation in psoriasis, and because this tissue is easy to obtain, serial skin biopsies are commonly used to evaluate the effects of novel treatments for psoriasis[1-5]. The increase in the development of a variety of new, targeted therapies clearly raises the need for sensitive biomarkers, which could be used for selection purposes during the development process.

Various hypotheses on psoriasis pathogenesis have been proposed over the years, varying from keratinocyte-centered, to T-cell mediated, to aggravation at the level of innate immunity[6;7]. The latter is based on the remarkable improvements seen in clinical trials with tumour necrosis factor (TNF)- α antagonists[8-11], together with the discovery of activation of several cellular elements and humoral components of the innate immune system in lesional and non-lesional psoriatic skin[12-21]. Recently, we showed reduction of different inflammatory cell types of the innate immune system in psoriatic skin during etanercept treatment[22].

The primairy objective of this study was to investigate the early changes in lesional and non-lesional psoriatic skin alongside of the clinical response, by using a known clinically effective therapy (i.e. adalimumab 40 mg subcutaneously every other week)[8;23;24], to identify sensitive biomarkers, in particular of the innate immune system, that may facilitate the planning of future studies with novel agents to treat psoriasis. Effect of adalimumab therapy on synovial tissue in these patients has been published elsewhere [25].

We assessed the cellular changes in the skin and clinical changes (PASI, BSA) at baseline and after 4 weeks of treatment with either adalimumab or placebo. The cellular changes were analyzed by immunohistochemical staining of cryostat sections from lesional and non-lesional psoriatic skin derived from psoriatic arthritis patients. We focused on the determination of numbers of infiltrating inflammatory cells of the innate immune system (CD68, CD161, elastase, BDCA-2 and TNF-α) and T cells (CD3).

PATIENTS AND METHODS

Patients

Patients with active psoriatic arthritis were enrolled into a 12 week randomized double-blind, placebo-controlled treatment period[25]. In all patients psoriatic arthritis was diagnosed at least 3 months prior and was considered to be moderate to severely active, as defined by ≥ 2 swollen and ≥ 2 tender joints. Furthermore, active cutaneous lesions of psoriasis had to be present or a documented history of psoriasis diagnosed by a dermatologist. Of the 24 patients included based on their psoriatic arthritis[25], 22 also had cutaneous lesions.

Patients were allowed to use concomitant methotrexate, which had to be stable for at least 28 days. Patients were not allowed to use any other disease-modifying

anti-rheumatic drugs (DMARDs) one month prior to baseline. Use of non-steroidal anti-inflammatory drugs was allowed, provided that the dose had been stable for at least 28 days. Parenteral, intra-articular or oral use of corticosteroids within 28 days before enrolment into the study was not allowed. Topical treatments for psoriasis were not allowed 14 days prior to baseline, with the exception of low potency (class I) topical steroids to be used on scalp, palms, groin and/or soles of feet only, and emollients. Other exclusion criteria were the use of any biological agent or investigational drug within the previous 6 months and having a history of tuberculosis or a malignancy in the past 10 years. Infection with HIV, hepatitis B or C virus was excluded via serological testing. Patients with another serious infection within 4 weeks before baseline, or a significant history of cardiac, renal, neurological or metabolic disease were excluded from the study. Female patients who were pregnant or breastfeeding were not allowed to enter the study.

Study protocol

This was a randomised, double-blind, placebo-controlled, single center study performed at the Academic Medical Center of the University of Amsterdam.

The study protocol was reviewed and approved by the medical ethical committee and all patients gave their written informed consent before enrolment. The study was conducted according to the Declaration of Helsinki principles.

Treatment

Patients were randomised to receive subcutaneous injections with either adalimumab 40 mg or matching placebo at baseline and day 15 in a 1:1 ratio.

Skin biopsies

At baseline and week 4 lesional and non-lesional punch biopsies of 4 mm were taken from 22 patients with skin lesions, preferentially from a non-sun-exposed area. Lesional biopsies were taken from the inside border of the same target psoriatic plaque, separated by at least 1 cm. The biopsy samples were randomly coded, snap-frozen in Tissue-Tek OCT compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands) by immersion in liquid nitrogen and stored at -80˚C until processing. Five-micrometer cryostat sections were cut and mounted on glass slides (Star Frost adhesive slides, Knittelgläser, Braunschweig, Germany), before being stored at -80˚C until immunohistochemical staining. For each staining three sections of each biopsy were analysed to minimize random variation.

Immunohistochemical analysis

Serial sections were stained with the following antibodies: FITC-conjugated anti-CD3 (BD Pharmingen, San Jose, CA, USA) to identify T cells, anti CD68 (clone EBM11; Dako, Glostrup, Denmark) to identify macrophages, anti-human neutrophil elastase

(Dako), anti-CD161 (BD Pharmingen) to stain for NK-T cells and Th17 cells, TNF-α (Monosan, Uden, the Netherlands) and FITC-conjugated anti-BDCA-2 (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) to identify plasmacytoid dendritic cells. A polyclonal rabbit anti human Von Willebrand Factor (VWF; Dako) antibody was used in double stainings with TNF-α to distinguish TNF-α expressing endothelial cells from other TNF-α positive cells. After rinsing with Tris Buffered Saline (TBS), all sections were further incubated with biotin-conjugated goat anti-mouse antibody and HRP-conjugated streptavidin (Dako) in case of elastase, CD68 and TNF-α which was amplified with the tyramide signal amplification (TSA) system (Perkin Elmer, MA, USA). In case of CD3 and BDCA-2 sections were incubated with rabbit anti-FITC (Dako) in 10% normal human serum (NHS) in TBS for 30 minutes. Following a wash step with TBS, sections were subsequently incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (Dako) in 1% Bovine Serum Albumine (BSA) in TBS for 30 min and in case of BDCA-2 staining the signal was amplified with the TSA system. For the CD161 staining, the sections were incubated with goat anti mouse immunoglobulins (GAM) in NHS and after the wash step with alkaline phosphatase anti alkaline phosphatase (APAAP) in BSA (both from Dako), for further amplification these steps were repeated. In the case of the TNF-α / VWF double stainings, TNF-α was labelled with AP-conjugated Streptavidin (Dako) after using the TSA system and colour development achieved with an AP staining-kit (Vector, Brunschwig Chemie, Amsterdam, The Netherlands). After this staining the sections were blocked with 10% normal mouse serum and incubated with HRP-labelled VWF. The colour development was achieved with Fast Red (Dako) for the CD161 staining and for the other stainings an amino-ethylcarbazole (AEC)-kit from Vector (Brunschwig Chemie, Amsterdam, The Netherlands) was used. Except for the TNF-α / VWF double staining, sections were counterstained with Mayer's haematoxylin (Merck, Darmstadt, Germany) and all stained sections were mounted with Kaiser's glycerol gelatine (Merck).

All sections were analyzed through manual quantification of the twenty high power fields per section. Manual quantification was done by two independent observers blinded for order, patient and clinical data. The epidermal and dermal regions were separately counted. Positive staining of CD3, CD68, CD161, BDCA-2, elastase and TNF-α was expressed as positive cells per millimeter squared.

Clinical evaluation

To evaluate the clinical response to the different treatments the Psoriasis Area and Severity Index (PASI) and the Body Surface Area (BSA) were assessed at baseline, week 4 and week 12.

Statistical analysis

SPSS 17.0 for Windows (SPSS, Chicago, Illinois, USA) was used for statistical analysis. Baseline characteristics between the two groups were compared using a

Student's t-test for normal distributed data and a Mann-Whitney U test for variables with a very skewed distribution. Correlations of changes in clinical parameters and immunohistochemical markers were analysed with Spearman rank correlation. Additionally, each of the end points was analysed using a analysis of covariance model (ANCOVA) after rank transformation to correct for baseline differences [26]

RESULTS

Clinical results

The baseline demographical and clinical features of the 22 patients with cutaneous lesions of the different treatment groups are specified in Table 1. There were no statistically significant differences with regards to the baseline demographical and clinical features between the two treatment groups.

The mean PASI score after 4 weeks of adalimumab treatment was 2.61 points lower compared to placebo (95% CI -0.08 to 5.30, p=0.056). The mean (SD) PASI decreased from 5.89 (4.25) to 4.01 (2.49) in the adalimumab group, whereas there was a slight increase in the placebo group from 4.72 (2.55) to 5.45 (4.05).

The mean BSA score after 4 weeks of adalimumab treatment was 1.43 points lower compared to placebo (95% CI -0.71 to 3.56, p=0.18). In the adalimumab group the mean BSA (SD) decreased from 4.88 (3.91) to 3.79 (3.81), whereas there was a slight increase in the placebo group from 3.26 (3.08) to 3.60 (3.53).

Table 1 Demographical and clinical features of 22 patients with psoriatic skin lesions in the different treatment groups

	Adalimumab (n=11)	Placebo (n=11)
Age, yrs	43.1 (21-61.1)	47.4 (25.3-78.4)
No. men/female	8/3	6/5
No. (%) currently receiving MTX	6 (54.5)	4 (36.4)
Dose MTX, mg/week	17.5 (10-25)	20 (15-25)
Duration PsO, yrs	6.0 (0.4-18.5)	7.4(1.9-18.2)
Duration PsA, yrs	11.1(0.1-27.7)	18.8(1.9-53.2)
Baseline PASI	5.9 (1.5-13.8)	4.7 (0.7-7.1)
Baseline BSA	4.9 (0.5-10.6)	3.3 (0.3-9.7)

Data are shown as means (range). MTX, methotrexate; PsO, psoriasis; PsA, psoriatic arthritis; PASI, Psoriasis Area and Severity Index; BSA, Body Surface Area

Table 2 Median values (range lower, range upper) for the lesional and non-lesional psoriatic skin biomarkers before treatment and median reduction(range lower, range upper) after 4 weeks of treatment in each group.

			Adalimumab		Placebo		ANCOVA
			baseline	reduction	baseline	reduction	p value
lesional	CD3	epidermis	23.5 (13.8-37.9)	14.7 (-36.3-34.3)	29.5 (16.9-42.3)	7.7 (-18.9-6.3)	0.92
		dermis	179.8 (105.8-299.8)	21.8 (-118.8-70.7)	308.3 (249.4-375.7)	-67.3 (-91.6-237.1)	0.51
	CD68	epidermis	0.8 (0.0-4.2)	-0.3 (-8.9-25.3)	0.2 (-4.0-2.2)	-0.2 (-2.1-5.7)	0.89
		dermis	102.0 (42.8-305.7)	19.2 (-95.1-41.9)	50.5 (21.2-171.5)	-11.7 (-43.9-58.0)	0.36
	CD161	epidermis	5.5 (1.8-12.5)	1.2 (-5.1-3.0)	2.0 (0.5-5.4)	-0.2 (-3.1-4.6)	0.83
		dermis	33.5 (14.8-44.4)	6.9 (-17.9-1.2)	23.7 (15.7-35.4)	-9.5 (-5.5-16.3)	0.046*
	elastase	epidermis	0.0 (-4.9-18.8)	0.5 (-21.0-6.5)	1.0 (-4.5-31.5)	-0.2 (-28.9-8.0)	0.73
		dermis	29.7 (-2.7-115.3)	9.0 (-100.6-12.5)	36.3 (-2.6-162.9)	-8.2 (-123.3-80.0)	0.024*
	BDCA-2	epidermis	1.8 (-0.3-7.4)	0.8 (-2.2-2.1)	0.8 (0.4-2.9)	-1.2 (-0.6-1.6)	0.31
		dermis	29.3 (15.9-51.3)	7.8 (-23.7-8.7)	33.2 (16.8-61.6)	5.8 (-37.0-23.6)	0.70
	TNF-α	epidermis	0.0 (-0.4-0.4)	-0.2 (-0.5-1.5)	0.0 (-0.04-0.3)	0.0 (-0.2-0.4)	0.35
		dermis	21.2 (11.0-32.7)	3.4 (-15.3-10.8)	19.7 (13.8-28.9)	-7.6 (-2.8-13.7)	0.31
Non-lesional	CD3	epidermis	0.8 (0.1-3.2)	-0.7 (-2.4-5.2)	2.4 (1.6-3.7)	0.6 (-2.2-2.1)	0.92
		dermis	44.2 (18.5-73.2)	-7.0 (-32.6-64.1)	45.4 (30.1-70.6)	-1.6 (-13.4-46.6)	0.96
	CD68	epidermis	0.5 (0.2-2.3)	-0.2 (-1.1-0.9)	0.7 (0.4-1.5)	0.3 (-1.1-0.6)	0.36
		dermis	27.7 (8.5-90.8)	-1.0 (-13.5-29.4)	20.4 (11.9-42.2)	-9.5 (-11.2-75.0)	0.084
	CD161	epidermis	1.2 (0.6-4.5)	0.3 (-2.4-0.8)	0.7 (0.3-1.6)	0.2 (-1.3-2.2)	0.58
		dermis	6.0 (3.1-14.9)	-2.7 (-0.4-13.3)	7.5 (6.0-11.4)	-3.2 (-3.3-11.5)	0.65
	elastase	epidermis	0.0 (-0.4-1.0)	0.0 (-1.0-0.4)	0.0 (-0.01-0.2)	0.0 (-0.1-0.4)	0.40
		dermis	2.3 (0.9-6.3)	0.5 (-2.6-0.9)	1.8 (-0.7-9.7)	-1.1 (-3.0-3.1)	0.16
	BDCA-2	epidermis	0.3 (0.1-3.3)	0.0 (-0.5-0.3)	1.2 (0.5-2.1)	-0.4 (-0.5-1.5)	0.22
		dermis	7.3 (4.7-11.2)	-3.3 (-1.2-14.4)	5.4 (1.9-17.3)	-0.6 (-5.7-16.4)	0.78
	TNF-α	epidermis	0.3 (0.2—1.2)	0.0 (-0.7-0.3)	0.3 (0.1-0.7)	0.1 (-0.4-0.6)	0.59
		dermis	6.2 (4.4-15.6)	0.5 (-4.3-2.1)	6.8 (5.7-10.4)	0.3 (-4.0-3.9)	0.93

CD3+ T cells, CD68+ macrophages, CD161+ natural killer T cells, elastase+ neutrophils, BDCA-2+ plasmacytoid dendritic cells and TNF-α + cells are provided as median (range lower, range upper) cells/mm2. After ANCOVA was applied to correct for baseline imbalances, the effect of treatment after 4 weeks was significant only for the reduction in the number of lesional dermal CD161+ (p=0.046) and elastase+ cells (p=0.024).

Immunohistochemical analysis

Of the 22 patients with cutaneous lesions, paired pretreatment and post-treatment lesional and non-lesional skin samples were available from 18 patients for analyses. In three patients no lesional samples could be obtained due to the localization of the psoriatic lesions (e.g. scalp, intra-auricular or anal cleft) and in one patient we could not dispose of the non-lesional sample at week 4. The remaining 18 paired lesional and non-lesional skin biopsies were analysed. The results of this analysis are shown in Table 2. The differences at baseline between the two treatment groups, as well as any reduction or increase of cell type after effective treatment, were not statistically significant. However, following adalimumab treatment in lesional skin almost all numbers of epidermal and dermal innate immunity markers decreased, with the exception of epidermal $CD68^+$ and TNF^+ cells. In non-lesional skin, adalimumab treatment decreased the number of epidermal $CD161^+$ cells and dermal $elastase^+$ and TNF^+ cells.

In the placebo group there was a reduction of lesional epidermal $CD3^+$ cells and dermal BDCA-2^+ cells. In non-lesional biopsies there was a reduction of $CD3^+$, $CD68^+$ and $CD161^+$ cells in the placebo group. Furthermore , TNF^+ cells were reduced in the epidermis as well as the dermis.

When ANCOVA was applied to correct for the imbalance at baseline, it turned out that the baseline measurement of several parameters had a strong effect on change.

The effect of treatment in lesional skin was significant for dermal $CD161^+$ and $elastase^+$ cells (p=0.046 and P=0.024, respectively). The effect of treatment in non-lesional skin was only significant for epidermal elastase (p=0.040).

Correlation between clinical improvement and changes in psoriatic skin lesional and non-lesional biomarkers

After applying a Spearman rank correlation, there was no statistically significant correlation between clinical improvement and changes in cellular markers. Yet, there was a trend towards a correlation between improvement in PASI and reduction of $elastase^+$ cells located in epidermis of lesional skin (rho = 0.423, p=0.071).

DISCUSSION

This placebo-controlled study with adalimumab was conducted to address the question which immunological markers in psoriatic skin could be used as a biomarker for clinical efficacy on the group level in relatively small studies of short duration.

In concordance with our recently published study in synovial tissue [25], almost all studied inflammatory cell types showed a trend towards a reduction of numbers in psoriatic skin, although not statistically significant. After applying a covariance analysis, a statistically significant effect of adalimumab was seen with regards to

reduction of lesional dermal CD161+ and elastase+ cells. Previously, we also reported a decline of CD161+ and elastase+ cells in psoriatic skin after 3 weeks of treatment with etanercept in psoriasis patients [22].

As concerns CD161, this marker is expressed among others by NK-T cells. Activation of NK-T cells results in prompt release of high levels of cytokines like INF-γ and TNF-α, and NK-T cells have mutual interactions with dendritic cells and keratinocytes, which are thought to be relevant in psoriasis [27;28]. Furthermore, CD161 is a cell surface marker associated with Th17 cells [29;30], which is a new type of T cell that plays a pivotal role in the pathogenesis of psoriasis [31].

Contrary to our result, another study with adalimumab in psoriasis did not show significant reduction of CD161+ cells in either the epidermis or dermis after 12 weeks of treatment [32]. However, only four patients were treated with adalimumab in this study and no data were shown regarding the clinical response of each individual patient.

Besides CD161+ cells, elastase + cells in lesional dermis were also reduced after 4 weeks of treatment with adalimumab. Elastase is a marker of neutrophils [33] and infiltration of neutrophils in the epidermis is one of the morphological characteristics of psoriasis [34]. Previous studies showed that elastase correlates well with skin induration [35] and disappears with successful therapy [36]. Furthermore, expression of dermal elastase correlates statistically significant to PASI [37]. Consistent with our results, a previous study showed a significant reduction of elastase+ cells after etanercept treatment [38].

In contrast to findings for the synovial tissue [25], we did not find a significant correlation between clinical improvement and changes in the cellular markers in the skin. This might be explained by the selection criteria for this study. Patients were primarily included based on the activity of their psoriatic arthritis, not on the activity of the skin lesions. It is known that the severity of the skin disease and the arthritis often do not correlate with each other[39]. Furthermore, 10 of the 22 patients were being treated with a stable dose of methotrexate for their arthritis, but this may have an impact on their skin disease as well. As a result, the PASI of the included patients in this study was relatively low, making it more difficult to evaluate the clinical efficacy, and even more difficult to evaluate a possible correlation between clinical efficacy and the reduction of expression of several inflammatory markers.

Despite these suboptimal conditions for evaluation of the skin, our study shows that changes in CD161+ and elastase+ cells of psoriatic dermis may be used as biomarkers to screen for effective therapies during early drug development. Future investigations on biomarkers in psoriatic skin are necessary in order to confirm our results in studies evaluating other mechanisms of action.

CD3+ T cells, CD68+ macrophages, CD161+ natural killer T cells, elastase+ neutrophils, BDCA-2+ plasmacytoid dendritic cells and TNF-α + cells are provided as median (range lower, range upper) cells/mm^2. After ANCOVA was applied to correct for baseline imbalances, the effect of treatment after 4 weeks was significant only for the reduction in the number of lesional dermal CD161+ (p=0.046) and elastase+ cells (p=0.024).

REFERENCE LIST

1. Goedkoop AY, de Rie MA, Picavet DI *et al.* Alefacept therapy reduces the effector T-cell population in lesional psoriatic epidermis. *Arch Dermatol Res* 2004; **295**: 465-73.
2. Goedkoop AY, Kraan MC, Teunissen MB *et al.* Early effects of tumour necrosis factor alpha blockade on skin and synovial tissue in patients with active psoriasis and psoriatic arthritis. *Ann Rheum Dis* 2004; **63**: 769-73.
3. Goedkoop AY, de Rie MA, Teunissen MB *et al.* Digital image analysis for the evaluation of the inflammatory infiltrate in psoriasis. *Arch Dermatol Res* 2005; **297**: 51-9.
4. Gottlieb AB, Chamian F, Masud S *et al.* TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. *J Immunol* 2005; **175**: 2721-9.
5. Van Lingen RG, Korver JE, Van de Kerkhof PC *et al.* Relevance of compartmentalization of T-cell subsets for clinical improvement in psoriasis: effect of immune-targeted antipsoriatic therapies. *Br J Dermatol* 2008; **159**: 91-6.
6. Bos JD, de Rie MA, Teunissen MB *et al.* Psoriasis: dysregulation of innate immunity. *Br J Dermatol* 2005; **152**: 1098-107.
7. Bos JD. Psoriasis, innate immunity, and gene pools. *J Am Acad Dermatol* 2007; **56**: 468-71.
8. Gordon KB, Langley RG, Leonardi C *et al.* Clinical response to adalimumab treatment in patients with moderate to severe psoriasis: Double-blind, randomized controlled trial and open-label extension study. *J Am Acad Dermatol* 2006; **55**: 598-606.
9. Leonardi CL, Powers JL, Matheson RT *et al.* Etanercept as monotherapy in patients with psoriasis. *N Engl J Med* 2003; **349**: 2014-22.
10. Reich K, Nestle FO, Papp K *et al.* Infliximab induction and maintenance therapy for moderate-to-severe psoriasis: a phase III, multicentre, double-blind trial. *The Lancet* 2005; **366**: 1367-74.
11. Tracey D, Klareskog L, Sasso EH *et al.* Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* 2008; **117**: 244-79.
12. Abrams JR, Kelley SL, Hayes E *et al.* Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. *J Exp Med* 2000; **192**: 681-94.
13. Baker BS, Ovigne JM, Powles AV *et al.* Normal keratinocytes express Toll-like receptors (TLRs) 1, 2 and 5: modulation of TLR expression in chronic plaque psoriasis. *Br J Dermatol* 2003; **148**: 670-9.
14. de Jongh GJ, Zeeuwen PLJM, Kucharekova M *et al.* High Expression Levels of Keratinocyte Antimicrobial Proteins in Psoriasis Compared with Atopic Dermatitis. *J Investig Dermatol* 2005; **125**: 1163-73.
15. Gearing AJ, Fincham NJ, Bird CR *et al.* Cytokines in skin lesions of psoriasis. *Cytokine* 1990; **2**: 68-75.
16. Harder J, Schroder JM. Psoriatic scales: a promising source for the isolation of human skin-derived antimicrobial proteins. *J Leukoc Biol* 2005; **77**: 476-86.
17. Jablonska S. Immunological mechanisms in psoriasis: role of polymorphonuclear leucocytes. In: *Psoriasis* (Farber,EM, Nall,L, Morhenn,V et al, eds). New York: Elsevier, 2007: 1479-137.
18. Nickoloff BJ, Wrone-Smith T, Bonish B *et al.* Response of murine and normal human skin to injection of allogeneic blood-derived psoriatic immunocytes: detection of T cells expressing receptors typically present on natural killer cells, including CD94, CD158, and CD161. *Arch Dermatol* 1999; **135**: 546-52.

19. Nickoloff BJ. Keratinocytes regain momentum as instigators of cutaneous inflammation. T*rends Mol Med* 2006; **12**: 102-6.
20. Teunissen MBM. Langerhans cells and other skin dendritic cells. In: *Skin Immune System: Cutaneous Immunology and Clinical Immunodermatology* (Bos,JD, ed), 3rd edn. Boca Raton, Fl: CRC Press, 2007: 123-82.
21. Zhou X, Krueger JG, Kao MC *et al.* Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol Genomics* 2003; **13**: 69-78.
22. de Groot M, Teunissen MB, Picavet DI *et al.* Reduction of different inflammatory cell types of the innate immune system in psoriatic skin during etanercept treatment. *Exp Dermatol* 2010; **19**: 754-6.
23. Menter A, Tyring SK, Gordon K *et al.* Adalimumab therapy for moderate to severe psoriasis: A randomized, controlled phase III trial. *J Am Acad Dermatol* 2008; **58**: 106-15.
24. Saurat JH, Stingl G, Dubertret L *et al.* Efficacy and safety results from the randomized controlled comparative study of adalimumab vs. methotrexate vs. placebo in patients with psoriasis (CHAMPION). *Br J Dermatol* 2008; **158**: 558-66.
25. van Kuijk AW, Gerlag DM, Vos K *et al.* A prospective, randomised, placebo-controlled study to identify biomarkers associated with active treatment in psoriatic arthritis: effects of adalimumab treatment on synovial tissue. *Ann Rheum Dis* 2009; **68**: 1303-9.
26. Conover WJ, Iman RL. Analysis of covariance using the rank transformation. *Biometrics* 1982; **38**: 715-24.
27. Bonish B, Jullien D, Dutronc Y *et al.* Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-gamma production by NK-T cells. *J Immunol* 2000; **165**: 4076-85.
28. Nickoloff BJ, Bonish B, Huang BB *et al.* Characterization of a T cell line bearing natural killer receptors and capable of creating psoriasis in a SCID mouse model system. *J Dermatol Sci* 2000; **24**: 212-25.
29. Cosmi L, De PR, Santarlasci V *et al.* Human interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. *J Exp Med* 2008; **205**: 1903-16.
30. Hueber W, Patel DD, Dryja T *et al.* Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. *Sci Transl Med* 2010; **2**: 52ra72.
31. Di CA, Di MP, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol* 2009; **129**: 1339-50.
32. Van Lingen RG, de Jong EM, Berends MA *et al.* Good clinical response to anti-psoriatic treatment with adalimumab and methotrexate does not inflict a direct effect on compartmentalization of T-cell subsets: A pilot study. *J Dermatol Treat* 2008; 1-6.
33. Ohlsson K, Olsson I, Spitznagel K. Localization of chymotrypsin-like cationic protein, collagenase and elastase in azurophil granules of human neutrophilic polymorphonuclear leukocytes. *Hoppe Seylers Z Physiol Chem* 1977; **358**: 361-6.
34. Meyer-Hoffert U, Wingertszahn J, Wiedow O. Human leukocyte elastase induces keratinocyte proliferation by epidermal growth factor receptor activation. *J Invest Dermatol* 2004; **123**: 338-45.
35. Wiedow O, Wiese F, Streit V *et al.* Lesional elastase activity in psoriasis, contact dermatitis, and atopic dermatitis. *J Invest Dermatol* 1992; **99**: 306-9.
36. Wiedow O, Wiese F, Christophers E. Lesional elastase activity in psoriasis. Diagnostic and prognostic significance. *Arch Dermatol Res* 1995; **287**: 632-5.
37. Chen GS, Wu TM, Yang SA *et al.* Quantitative assessments of physiological and biological parameters in psoriatic lesions and its correlations to

the clinical severity of psoriasis. Kaohsiung *J Med Sci* 2001; **17**: 408-18.

38. de Vlam K, Gottlieb AB, Fitzgerald O. Biological biomarkers in psoriatic disease. A review. *J Rheumatol* 2008; **35**: 1443-8.

39. Gottlieb A, Korman NJ, Gordon KB *et al.* Guidelines of care for the management of psoriasis and psoriatic arthritis: Section 2. Psoriatic arthritis: overview and guidelines of care for treatment with an emphasis on the biologics. *J Am Acad Dermatol* 2008; **58**: 851-64.

5b

A PROSPECTIVE, RANDOMIZED, PLACEBO-CONTROLLED STUDY TO IDENTIFY BIOMARKERS ASSOCIATED WITH ACTIVE TREATMENT IN PSORIATIC ARTHRITIS: EFFECTS OF ADALIMUMAB TREATMENT ON SYNOVIAL TISSUE

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ABSTRACT

Objective: To determine which of the changes in synovial tissue correlates best with clinical response associated with effective therapy (adalimumab) to facilitate the planning of future studies with therapeutic agents for psoriatic arthritis (PsA).

Methods: Twenty-four active PsA patients were randomized to receive adalimumab (n=12) or placebo (n=12) for 4 weeks. Synovial biopsies were obtained before and after 4 weeks of treatment. Immunohistochemical analysis was performed to characterize the cell infiltrate, expression of cytokines and matrix metalloproteinases (MMPs), and vascularity. Sections were analyzed by digital image analysis. Statistical analysis was performed using covariance analysis.

Results: The mean Disease Activity Score in 28 joints (DAS28) after 4 weeks was 1.92 units lower (95% confidence interval (CI) 1.07 - 2.77) after adalimumab therapy compared with placebo. Paired pre- and post-treatment synovial samples were available from 19 patients. Many cell types were reduced after adalimumab treatment compared to placebo. After applying a ranked analysis of covariance (ANCOVA) model to correct for baseline imbalances, a significant effect of treatment was observed on CD3-positive cells: there was a median reduction of 248 cells/mm^2 after adalimumab versus placebo treatment ($p = 0.035$). In addition, the expression of MMP-13 was significantly reduced after active treatment: the integrated optical density (IOD)/mm^2 was 18,190 lower after adalimumab treatment as compared to placebo ($p = 0.033$).

Conclusion: Adalimumab therapy in PsA is associated with a marked reduction in T cell infiltration and MMP-13 expression in synovial tissue, suggesting that these parameters could be used as biomarkers that are sensitive to change after active treatment in small proof of concept studies in PsA.

INTRODUCTION

Because the synovium is a primary site of inflammation, there is increasing interest in studying the synovial tissue (ST) of patients with rheumatoid arthritis (RA) and psoriatic arthritis (PsA). In addition to the use of synovial biopsies for diagnostic purposes [1;2] and pathogenetic studies [3;4], serial synovial biopsies have been used to evaluate the effects of novel treatments [5;6]. This approach has been proposed to screen for therapeutic effects of novel antirheumatic interventions [7].

The increase in the development of a variety of new, targeted therapies clearly raises the need for sensitive biomarkers, which could be used for selection purposes during the development process. In RA a decrease in synovial macrophages has been shown to correlate with clinical improvement [8-10]. No synovial changes were detectable with analysis of serial synovial samples from RA patients who received either placebo or ineffective treatment [9;11-13]. This suggests that analysis of serial biopsies could be used as a screening method to test new compounds requiring relatively small numbers of subjects. The absence of changes in the ST after treatment would suggest that the therapy is probably not effective.

To date, only a few studies have been conducted in PsA evaluating synovial changes after therapy. These studies were not placebo-controlled, with biopsies taken at different time points, and in part with variable results [6;14-18]. Based on the limited data available it was hypothesized that an early decrease in macrophages (or macrophage subsets), combined with decrease in vascular markers and/or adhesion molecules, which were observed in some of these studies, would best predict clinical response in PsA.

The primary objective of this study was, therefore, to investigate the early changes in the ST alongside clinical response, by using a known clinically effective therapy (i.e. adalimumab 40 mg subcutaneously every other week) [19], to identify sensitive biomarkers that may facilitate the planning of future studies with novel agents to treat PsA.

PATIENTS AND METHODS

Patients

Patients with PsA fulfilling the ClASsification of Psoriatic Arthritis (CASPAR) criteria for PsA [20;21], aged 18-80 years, were included into the study after written informed consent was obtained. Patients had to have active disease at time of enrollment, defined by the presence of at least 2 tender and 2 swollen joints out of the 68 joints for tenderness and 66 joints for swelling assessed. One of the swollen joints had to be a knee, ankle or wrist joint that was accessible for arthroscopy. Patients were allowed to use concomitant methotrexate, which had to be stable for at least 28 days. They were not allowed to use any other disease-modifying antirheumatic drugs

(DMARDs) 1 month prior to baseline. For leflunomide a 2-month washout period was required. Use of non-steroidal anti-inflammatory drugs was allowed, provided that the dose had been stable for at least 28 days. Parenteral, intra-articular or oral use of corticosteroids within 28 days before enrolment into the study was not allowed. Topical treatments for psoriasis were not allowed 14 days prior to baseline, with the exception of low potency (class I) topical steroids to be used on scalp, palms, groin and/or soles of feet only. Other exclusion criteria were the use of any biological agent or investigational drug within the previous 6 months, and having a history of tuberculosis or a malignancy in the past 10 years. Infection with HIV, hepatitis B or C virus was excluded via serologic testing. Patients with another serious infection within 4 weeks before baseline, or a significant history of cardiac, renal, neurological or metabolic disease were excluded from the study. Female patients who were pregnant or breastfeeding were not allowed to enter the study.

Study protocol

This was a randomized, double-blind, placebo-controlled, single center study performed at the Academic Medical Center of the University of Amsterdam. The study protocol was approved by the Medical Ethics Committee of the institute and all patients signed informed consent before start of the study. They had a complete medical history taken and underwent a full physical examination, including joint assessment by a rheumatologist and skin assessment by a dermatologist. Routine clinical chemistry, hematology and urinalysis assessments were performed, as well as a chest X-ray and purified protein derivative (PPD) skin test prior to baseline to exclude patients with (latent) tuberculosis. Eligible patients were included into the study within 2 weeks after screening, and were seen at baseline, week 4 and week 12.

Clinical assessment included a 68 joint count for tenderness and 66 joint count for swelling, a Psoriasis Area and Severity Index (PASI), a subject's visual analog score (VAS) for pain, ranging from 0 mm (no pain) to 100 mm (the worst possible pain), a subject's and investigator's global disease assessment, and a Health Assessment Questionnaire (HAQ) for functional (dis)ability. Furthermore, C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR) were determined. A blinded, independent assessor performed the clinical evaluation. The 28-joint Disease Activity Score (DAS28), which has been shown to discriminate between active drug and placebo in clinical trials in PsA, was chosen to monitor changes in clinical disease activity after therapy [22;23]. In addition, the American College of Rheumatology (ACR) criteria for improvement were assessed [24].

Treatment

Patients were randomized to receive subcutaneous injections with either adalimumab 40 mg or matching placebo at baseline (day 1) and day 15 in a 1:1 ratio. After the second arthroscopy all patients received adalimumab 40 mg every other week.

Arthroscopy

A needle arthroscopy of an actively inflamed joint (knee, ankle, or wrist) was performed under local anesthesia in all patients before treatment and 28 days after initiation of study medication from the same joint. The procedures for needle arthroscopy and tissue processing were performed as described previously in detail 25. In summary, to minimize sampling error biopsies were taken from 6 or more sites of the joint during each procedure 26;27. ST biopsy specimens were immediately embedded in TissueTek OCT (Miles Diagnostics, Elkhart, IN, USA), snap frozen by immersion in methylbutane (-80°C), and stored in liquid nitrogen until further processing. Before staining, coded ST samples were cut with a cryostat (5 mm), fixed with acetone, and endogenous protease activity was blocked with 0.3% hydrogen peroxide.

Immunohistochemical analysis

Serial sections were stained with the following antibodies: anti-CD3 (SK7, BD Biosciences, San Jose, CA, USA), anti-CD4 (SK3, BD Biosciences), anti-CD8 (C8/114B, Dako, Glostrup, Denmark), anti-CD15 (C3D-1, Dako), anti-CD22 (CLB-B-ly/1,6B11, Sanquin, Amsterdam, The Netherlands), anti-CD38 (HB-7, BD Biosciences), anti-CD55 (clone 67, Serotech, Oxford, UK), anti-CD68 (EMB11; Dako), and anti-CD163 (5C6 FAT, Bachem Peninsula Laboratories, San Carlos, CA, USA) to analyze the cell infiltrate. Antibodies against myeloid-related protein (MRP)8 (8-5C2, BMA Biomedicals, Augst, Switzerland) and MRP14 (S36.48, BMA Biomedicals) were used to detect infiltrating monocytes/macrophages in an early stage of differentiation.

For immunohistochemical analysis of cytokine expression, IL-1β (2D8; Immunokontact, Stockholm, Sweden), and IL-6 (B-E8, Invitrogen, Breda, The Netherlands) were stained. Furthermore, von Willebrand's factor (vWF; F8/86; Dako) was used as a marker to detect blood vessels. Additionally, the expression of metalloproteinase (MMP)-3 (MAB1339, Bio-Connect, Huissen, the Netherlands) and MMP-13 (VIIIA2, Oncogene, Cambridge, MA, USA) was determined.

Sections with non-assessable tissue, defined by the absence of an intimal lining layer, were excluded from analysis. Staining for cellular markers was performed using a three-step immunoperoxidase method as previously described [28]. For the determination of cytokine expression, biotinylated tyramine (BT) was used for amplification after incubation with the secondary antibody, and incubation with streptavidin- horseradish peroxidase (HRP) conjugate was followed by detection with aminoethylcarbazole (AEC), as previously described [29;30]. For control sections, the primary antibodies were omitted or irrelevant antibodies were applied.

Digital image analysis

After immunohistochemical staining, all sections were coded and randomly analyzed by computer-assisted digital image analysis (DIA). For all markers, 18 high-power fields were analyzed. CD68 and CD163 expression (macrophage markers) were analyzed

separately in the intimal lining layer and the synovial sublining. The images of the high-power fields were analyzed using the Qwin analysis system (Leica, Cambridge, UK), as described previously in detail [31;32].

Statistical analysis

SPSS 12.0.1 for Windows (SPSS, Chicago, IL, USA) was used for statistical analysis. Baseline characteristics between the two groups were compared using a Student's t-test for normal distributed data and a Mann-Whitney U test for variables with a very skewed distribution. Correlations of changes in clinical parameters and immunohistochemical markers were analyzed with Spearman rank correlation. Additionally, each of the endpoints was analyzed using an analysis of covariance model (ANCOVA) after rank transformation to correct for baseline differences [33]. The model included terms for treatment as a fixed effect and the baseline measurement as a covariate. The aim was to assess the treatment difference.

RESULTS

Demographic features

A total of 24 patients (15 males, 9 females) were included in the trial, 12 patients were randomized to receive adalimumab and 12 placebo treatment. The mean age was 42.8 (range 21-61) years for the patients in the adalimumab group and 47.2 (25-78) years in the placebo group (Table 1). The mean PsA disease duration was comparable in both groups. Concomitant methotrexate was used by 7 patients in the adalimumab group and 5 patients in the placebo group in a comparable dose (mean 18.2 mg/week and 19.0 mg/week, respectively). None of the patients had used TNF inhibitors in the past. Most patients (n=16) had polyarticular involvement according to the Moll and Wright classification [34], a minority had an oligoarticular phenotype (n=7) or predominantly distal interphalangeal involvement (n=1). Two of the patients with polyarticular disease also had axial involvement. The PsA phenotypes were not equally distributed among the two groups, with the placebo group harboring more polyarticular patients than the adalimumab group (10 versus 6). Despite this difference, the patient groups were comparable with regard to disease activity markers, such as CRP and DAS28.

All patients received the two injections with study medication according to the protocol. Overall study medication was well tolerated. No infections other than common cold were reported in the 4 weeks study period. One patient in the placebo group complained of more pain in her ankle after the first arthroscopy, but could undergo the second arthroscopy.

Table 1. Demographic and clinical features of the 24 patients with psoriatic arthritis (PsA) enrolled in the study

	Adalimumab (n=12)	Placebo (n=12)
age, years	42.8 (21-61)	47.2 (25-78)
no. men/women	9/3	6/6
no. (%) currently receiving MTX	7 (58)	5 (42)
dose MTX, mg/week	18.2 (10-25)	19.0 (15-25)
PsA disease duration, years	5.5 (0.4-14.1)	8.4 (1.9-18.2)
no. (%) RF positive	2 (17)	1 (8)
no. (%) anti-CCP positive	1 (8)	0
no. (%) erosive	7 (58)	5 (42)
ESR, mm/hour	24.2 (4-66)	22.4 (3-66)
CRP, mg/liter	19.9 (2.3-81.6)	9.9 (1.3-26.7)
DAS28 score	4.67 (3.0-5.78)	5.07 (2.21-6.83)
patient's global assessment of disease activity (VAS 0-100 mm)	73 (45-94)	62.8 (18-92)
patient's assessment of pain (VAS 0-100 mm)	72.8 (55-91)	67.4 (11-89)
PASI	5.89 (0-14.0)	4.72 (0-7.0)

All values are mean (range) except where indicated otherwise. MTX, methotrexate; RF, rheumatoid factor; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; VAS, visual analog scale; PASI, Psoriasis Area and Severity Index.

Clinical response

As anticipated, a markedly positive effect of adalimumab treatment was seen on the DAS28 [23], which was 1.92 units lower compared with placebo after 4 weeks of treatment (95% confidence interval (CI) 1.07 - 2.77, $p < 0.001$) (Figure 1). The mean (SD) DAS28 decreased from 4.67 (0.98) to 2.87 (1.27) one month after initiation of adalimumab therapy. This improvement was not seen in the placebo group, where the mean DAS28 was 5.07 (1.29) before versus 5.20 (1.31) after treatment. After 4 weeks of treatment, 11 of the adalimumab patients fulfilled the European League Against Rheumatism (EULAR) criteria for clinical response [23;35] (6 patients were good and 5 were moderate responders), versus 0 in the placebo group. Clinical improvement was sustained at week 12, when all 12 adalimumab treated patients fulfilled the EULAR criteria for clinical response (7 good and 5 moderate responders). Based on the 68 tender joint count and 66 swollen joint count an ACR20 response for improvement [24] was observed in 5 patients after 4 weeks of adalimumab treatment (of which 4 also fulfilled the ACR50 response criteria), but in none of the placebo treated patients. After 12 weeks of adalimumab treatment 10 patients fulfilled ACR20 criteria, of which 7 also met the ACR50 response criteria.

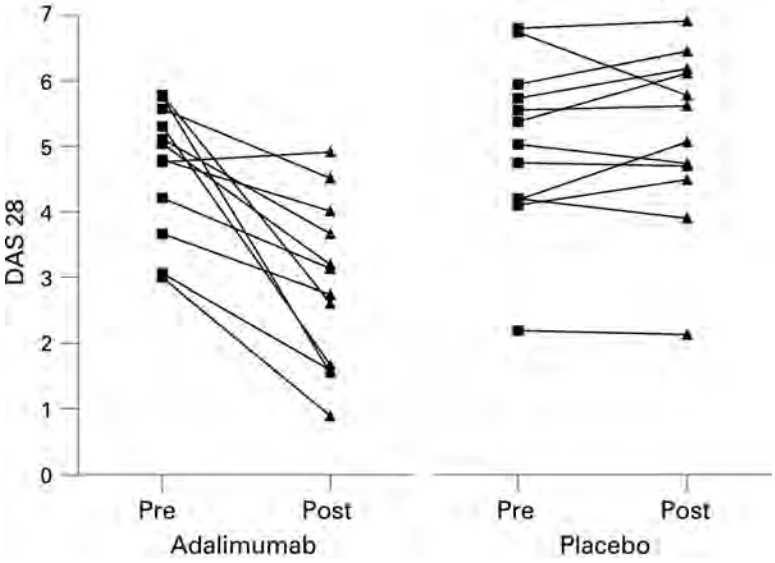

Figure 1 Effect of treatment with either adalimumab or placebo on the individual 28-joint Disease Activity Score (DAS28). A marked reduction is seen after adalimumab treatment for 4 weeks.

The mean PASI score after 4 weeks of adalimumab treatment was 2.61 points lower compared to placebo (95% CI -0.08 - 5.30, p = 0.056). The mean (SD) PASI decreased from 5.89 (4.25) to 4.01(2.49) in the adalimumab group, whereas there was a slight increase in the placebo group from 4.72 (2.55) to 5.45 (4.05).

At 4 weeks the ESR and CRP levels were respectively 58% (95% CI 30% to 86%, p = 0.001) and 57% (95% CI 29% to 84%, p < 0.001) lower after adalimumab treatment than after placebo treatment. The mean (SD) ESR was reduced from 24.2 (21.7) mm/h to 8.1 (7.5) mm/h after adalimumab treatment, and the mean (SD) CRP from 19.9 (25.7) mg/L to 2.1 (1.4) mg/L, whereas after placebo treatment there was no clear cut change: ESR from 22.4 (18.7) to 19.6 (11.9) mm/h and CRP from 9.9 (9.1) to 9.2 (10.0) mg/L, respectively.

Immunohistochemical analysis

Paired pretreatment and post-treatment synovial samples from 19 patients were available for analysis. The pretreatment ST biopsies of 3 patients did not contain an intimal lining layer, and therefore did not pass the quality control tests. Due to an unfortunate freezer accident ST biopsies of 2 other patients were lost. The remaining 19 paired ST samples were analyzed, 10 were in the adalimumab group and 9 in the placebo group. The results of this analysis are shown in Table 2.

Despite randomization, there were clear differences at baseline for several synovial markers between adalimumab and placebo groups, as shown in Table 2 (adalimumab and placebo before). Most of these baseline differences were not

Table 2. Median values (standard error (SE) of the median) for the synovial biomarkers before treatment and median reduction (SE of the median) after 4 weeks of treatment in each group and p value of the ranked analysis of covariance (ANCOVA) applied

	Adalimumab		Placebo		ANCOVA
	before	reduction	before	reduction	p value
CD68 total	1927 (133)	435 (197)	835 (516)	-534 (567)	0.31
CD68L	378 (28)	236 (61)	177 (140)	-419 (249)	0.35
CD68SL	1423 (262)	147 (59)	753 (628)	-184 (99)	0.23
CD163 total	1243 (208)	765 (309)	480 (178)	286 (409)	0.67
CD163L	426 (203)	167 (72)	68 (8)	39 (46)	0.15
CD163SL	1081 (155)	674 (265)	468 (206)	256 (389)	0.85
CD3	533 (36)	212 (230)	144 (66)	-36 (33)	0.035*
CD4	1130 (265)	386 (278)	184 (21)	-148 (589)	0.084
CD8	177 (103)	103 (72)	27 (7)	-4 (7)	0.12
CD15	35 (13)	22 (8)	13 (1)	12 (28)	0.42
CD22	306 (167)	210 (145)	44 (7)	20 (27)	0.89
CD38	15 (11)	4 (1)	4 (1)	1 (1)	0.83
CD55	660 (218)	85 (145)	681 (30)	230 (288)	0.60
VWF	137853 (42944)	47107 (25755)	87300 (8092)	-38191 (76306)	0.37
IL-1β	515740 (200575)	272189 (74412)	620179 (485911)	31219 (134893)	0.31
IL-6	169164 (181653)	39063 (49052)	38671 (12175)	-66403 (125364)	0.18
MRP8	1483 (1550)	1313 (1644)	396 (139)	-23 (233)	0.11
MRP14	3976 (1380)	2814 (2339)	1837 (1360)	489 (247)	0.82
MMP-3	247021 (101010)	138679 (99428)	17680 (1913)	138 (529)	0.64
MMP-13	25822 (15822)	9188 (8653)	14673 (10350)	-9002 (5469)	0.033*

$CD68^+$ macrophages (total), $CD68^+$ macrophages in the intimal lining layer (L) and in the synovial sublining (SL), $CD163^+$ cells (subset of macrophages) (total), $CD163^+$ cells in L and SL, $CD3^+$ T cells, $CD4^+$ and $CD8^+$ T cells, $CD15^+$ neutrophils, $CD22^+$ B cells, $CD38^+$ plasma cells, and $CD55^+$ fibroblast-like synoviocytes are provided as cells/mm^2. Expression of von Willebrand Factor (vWF), IL-1β, IL-6, myeloid related protein (MRP)-8, MRP-14, matrix metalloproteinase (MMP)-3, MMP13, are provided as median (SE of the median) integrated optical density (IOD)/mm^2. Differences before treatment between the groups were statistically significant for $CD163^+$ cells (total, L and SL), $CD8^+$ cells, and MMP-3 expression, which were higher in the adalimumab group. After ANCOVA was applied to correct for baseline imbalances, the effect of treatment after 4 weeks was significant only for the reduction in the number of CD3-positive cells (P=0.035) and MMP-13 expression (=0.033)*.

statistically significant except for the number of CD8-positive cells, CD163-positive cells, and MMP-3 expression, which were significantly higher in the adalimumab group. A marked reduction was observed in most cell types after active treatment. The effect after adalimumab was significant for CD3-positive T cells: there was a median decrease of 248 cells/mm^2 after adalimumab compared to placebo treatment. There was clear interindividual variability, which is a well known phenomenon in patients with various arthritides including PsA [4;36]. However, on the group level there was a marked decrease in CD3-positive T cells (standard error (SE) of the median: 212 (230) cells/mm^2) in the adalimumab group (Figure 2), whereas in the placebo group there was an increase (36 (33) cells/mm^2). A similar effect was observed for the two T cell subpopulations: there was a trend towards a reduction of CD4-positive cells (386 (278) cells/mm^2) after adalimumab compared to an increase (148 (589) cells/mm^2) after placebo treatment; and a reduction of CD8-positive cells (103 (72) cells/mm^2) after adalimumab versus a small increase (4 (7) cells/mm^2) after placebo treatment.

Following adalimumab treatment there also was a decrease in the number of CD163-positive cells (resident macrophages), and CD68-positive macrophages, especially in the synovial sublining, but these changes did not reach statistical significance, possibly due to the relatively small number of patients. Similar changes were observed for MRP-8 and MRP-14-positive macrophages (infiltrating monocytes/macrophages in an early stage of differentiation). There were also trends towards

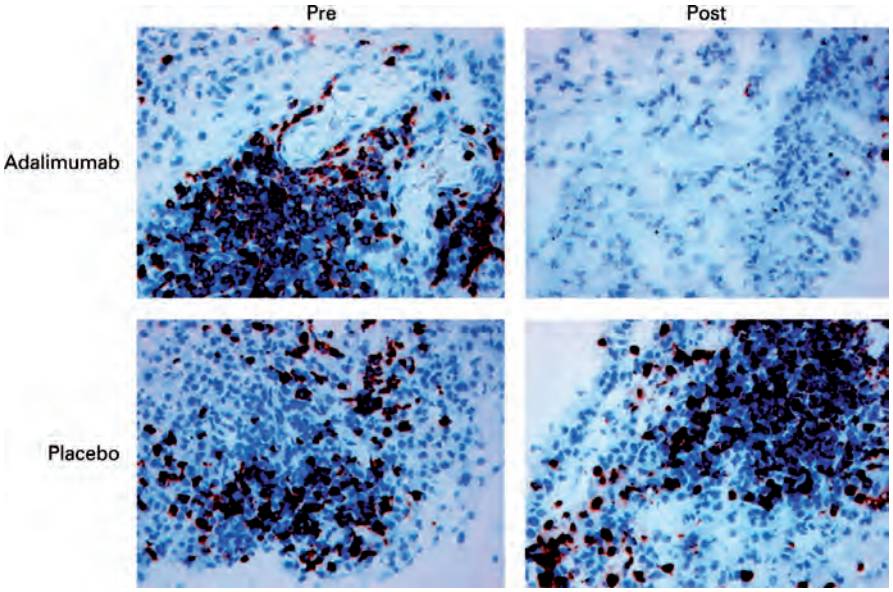

Figure 2 Representative photograph showing CD3$^+$ T cells (*red-brown*) in psoriatic arthritis synovial tissue before and after treatment with adalimumab (upper panel left and right) or placebo (lower panel left and right). Magnification x 200. A marked reduction of the number of CD3$^+$ T cells was observed after 4 weeks of adalimumab treatment.

decreased infiltration by CD22-positive B cells, CD38-positive plasma cells, and CD15-positive neutrophils after active treatment.

There was a trend towards reduction in the expression of the vascular marker vWF, and the pro-inflammatory cytokines IL-1 and IL-6 after treatment with adalimumab as compared to placebo, but the differences were not statistically significant. There was considerable reduction of expression of MMP-3 and MMP-13 in the adalimumab group as compared to placebo. The expression of MMP-13 was decreased with 9,188 (8,653) IOD/mm^2 (median (SE) of the median) after adalimumab treatment as compared to an increase of 9,002 (5,469) IOD/mm^2 after placebo. When ANCOVA was applied to correct for the imbalances at baseline, it turned out that the baseline measurement had the strongest effect on change in any parameter tested, but for reduction in the number of CD3-positive cells and MMP-13 expression the effect of treatment remained significant ($p = 0.035$ for CD3 positive cells, and $p = 0.033$ for expression of MMP-13).

Correlation between clinical improvement and changes in synovial biomarkers

For the cellular markers clinical improvement was strongly correlated with a decrease in CD3-positive T cells (Spearman rho = 0.644, $p = 0.003$), CD4-positive cells (rho = 0.649, $p = 0.003$), and MRP8-positive macrophages (rho = 0.561, $p = 0.012$) (Figure 3), but there was no statistically significant correlation with changes in other cell types. There was also a strong correlation between clinical improvement and the reduction of MMP-13 (rho = 0.619, $p = 0.005$) (Figure 3) and MMP-3 (rho = 0.560, $p = 0.013$).

DISCUSSION

This placebo-controlled study with adalimumab was conducted to address the question which features in PsA ST samples could be used as a biomarker for clinical efficacy on the group level in relatively small studies of short duration. The results presented here show that clinically effective adalimumab therapy is particularly associated with a marked reduction in CD3-positive T cell infiltration and expression of MMP-13 in the ST of PsA patients 4 weeks after initiation of treatment.

Previous work on synovial biomarkers in PsA patients has been mainly limited to open studies, mostly of longer duration. Open label treatment of 10 PsA patients with methotrexate resulted in a decrease in T cells and macrophages as well as reduced expression of IL-8, E-selectin, intercellular adhesion molecule-1 (ICAM-1), and MMP-3 after 6 to 12 months of treatment [14]. In another study 52 patients with peripheral arthritis due to spondyloarthritis were included; 16 of these had PsA [15]. The patients underwent synovial biopsy at baseline and 12 weeks following different treatment regimens (infliximab, etanercept, sulfasalazine or no DMARD).

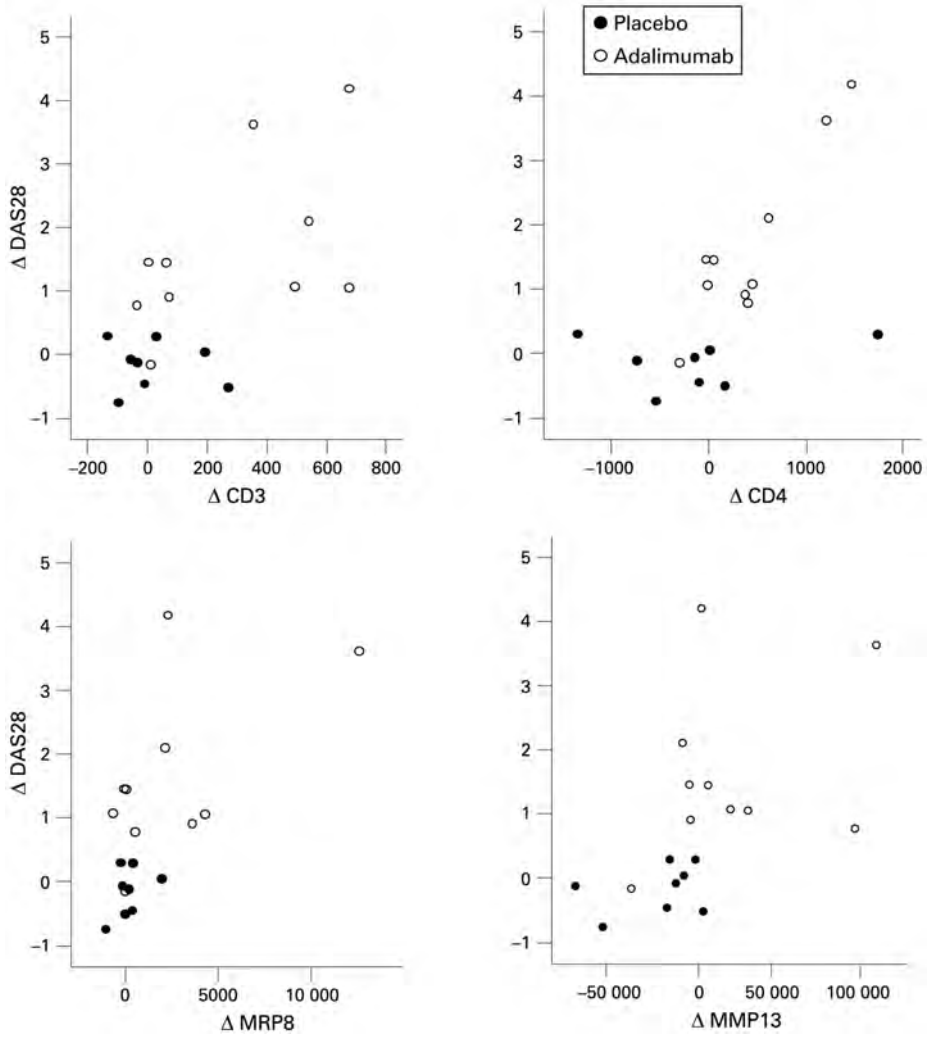

Figure 3 Scatter plots showing individual data points for the correlation between change in 28-joint Disease Activity Score (ΔDAS28, a positive value represents clinical improvement) on the Y-axis and change in biomarker (a positive value represents a reduction of the expression) on the X-axis after 4 weeks for: CD3-positive cells (rho = 0.644, p = 0.003), CD4-positive cells (rho = 0.649, p = 0.003), expression of MRP8 (rho = 561, p = 0.012) and MMP-13 (rho = 619, p = 0.005).

Clinical improvement correlated with a decrease in CD163-positive macrophages, polymorphonuclear cells (PMNs), and MMP-3 expression. A study on the effects of TNF blockade showed in 11 PsA patients, who were treated with infliximab (3 mg/kg) combined with stable methotrexate treatment, a significant reduction of ICAM-1, vWF and αvβ3 expression [17]. There was also a trend towards a reduction of both T cells and macrophages. Similarly, another study demonstrated a decrease in macrophage

numbers and vascular markers in the synovium of nine PsA patients 8 weeks after initiation of infliximab (5 mg/kg) treatment [18]. A study on the effects of alefacept revealed a significant decrease in T cell numbers after 4 weeks of treatment, and a significant reduction of both T cells and macrophages after 12 weeks of treatment in 11 PsA patients [6]. Finally, experimental treatment with IL-10 subcutaneously for 4 weeks resulted in decreased T cell and macrophage infiltration in the synovium [37].

The present study was designed to determine which synovial biomarkers are associated on the group level with active treatment, identical to the approach that we have previously described in RA patients [8]. In RA macrophage numbers were previously identified as synovial biomarkers associated with active treatment. These results have been confirmed and validated in various studies [9;10;38]. Of interest, although we did observe clear trends towards decreased numbers of macrophages and macrophage subsets after active treatment, analysis of covariance indicates that in PsA especially the number of CD3 positive T cells and the expression of MMP-13 could be used to screen for potentially active drugs in small proof of concept studies of short duration. The results presented here underscore the important role of T cells in the pathogenesis of PsA [39], consistent with the observation that specific targeting of T cells may result in clinical benefit in this disease [6;40].

A potential drawback of this study, although randomized, is the fact that there were baseline differences – in both clinical and synovial variables – between the adalimumab and placebo group despite randomisation, which is probably related to the relatively small number of patients. The ANCOVA model, however, includes the baseline value for each marker as a covariate, thereby correcting for these baseline imbalances [41]. Using this model to describe the relationship between clinical improvement and changes in ST, the effect of treatment was sustained for a reduction of T cells and MMP-13 expression. Another potential limitation of this exploratory study is related to the high number of synovial parameters tested, and the chance of erroneously reporting statistical significance in the context of multiple comparisons. The limited power of our study did not allow a conservative Bonferroni correction. Of importance, very recently other investigators have independently confirmed the specific decrease in $CD3^+$ T cells in the synovium after initiation of TNF blockade in patients with PsA [42]. Thus, it appears unlikely that the identification of $CD3^+$ T cells as a key synovial biomarker associated with active treatment can merely be explained by chance.

The reduction of synovial inflammation associated with clinical improvement of the joint and skin after adalimumab treatment is also consistent with previous reports showing a decrease in leukocyte numbers (including T cells and macrophages), as well as reduced vascularity and expression of pro-inflammatory cytokines and MMPs in the synovium of PsA patients after infliximab treatment [15-18]. The cellular changes might be explained in part by changes in cell migration due to reduced neoangiogenesis and reduced expression of adhesion molecules and chemokines, as suggested previously [14;16;17;43].

Analysis of molecular markers in synovial tissue is increasingly used in clinical trials on targeted therapies. With this approach, tissue specificity is not a problem and examination of serial biopsy samples can be used to monitor the response in individual patients and screen for interesting biological effects at the site of inflammation. This study clearly shows that changes in T cell numbers and the expression of MMP-13 in the synovium of PsA patients may be used as biomarkers to screen for effective therapies during early drug development. It can be anticipated that future developments will include the use of more extensive markers of synovial inflammation and joint degradation as well as the use of panels of biomarkers in serum and synovial tissue samples.

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REFERENCE LIST

1. Kraan MC, Haringman JJ, Post WJ *et al*. Immunohistological analysis of synovial tissue for differential diagnosis in early arthritis. *Rheumatology (Oxford)* 1999; **38**: 1074-80.
2. Baeten D, Steenbakkers PG, Rijnders AM *et al*. Detection of major histocompatibility complex/human cartilage gp-39 complexes in rheumatoid arthritis synovitis as a specific and independent histologic marker. *Arthritis Rheum* 2004; **50**: 444-51.
3. Tak PP, Bresnihan B. The pathogenesis and prevention of joint damage in rheumatoid arthritis: advances from synovial biopsy and tissue analysis. *Arthritis Rheum* 2000; **43**: 2619-33.
4. van Kuijk AW, Reinders-Blankert P, Smeets TJ *et al*. Detailed analysis of the cell infiltrate and the expression of mediators of synovial inflammation and joint destruction in the synovium of patients with psoriatic arthritis: implications for treatment. *Ann Rheum Dis* 2006; **65**: 1551-7.
5. Tak PP. Lessons learnt from the synovial tissue response to anti-rheumatic treatment. *Rheumatology (Oxford)* 2000; **39**: 817-20.
6. Kraan MC, van Kuijk AW, Dinant HJ *et al*. Alefacept treatment in psoriatic arthritis: reduction of the effector T cell population in peripheral blood and synovial tissue is associated with improvement of clinical signs of arthritis. *Arthritis Rheum* 2002; **46**: 2776-84.
7. Gerlag DM, Tak PP. Novel approaches for the treatment of rheumatoid arthritis: lessons from the evaluation of synovial biomarkers in clinical trials. *Best Pract Res Clin Rheumatol* 2008; **22**: 311-23.
8. Gerlag DM, Haringman JJ, Smeets TJ *et al*. Effects of oral prednisolone on biomarkers in synovial tissue and clinical improvement in rheumatoid arthritis. *Arthritis Rheum* 2004; **50**: 3783-91.
9. Wijbrandts CA, Vergunst CE, Haringman JJ *et al*. Absence of changes in the number of synovial sublining macrophages after ineffective treatment for rheumatoid arthritis: Implications for

use of synovial sublining macrophages as a biomarker. *Arthritis Rheum* 2007; **56**: 3869-71.

10. Haringman JJ, Gerlag DM, Zwinderman AH *et al*. Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005; **64**: 834-8.
11. Smeets TJ, Kraan MC, Versendaal J *et al*. Analysis of serial synovial biopsies in patients with rheumatoid arthritis: description of a control group without clinical improvement after treatment with interleukin 10 or placebo. *J Rheumatol* 1999; **26**: 2089-93.
12. Baeten D, Houbiers J, Kruithof E *et al*. Synovial inflammation does not change in the absence of effective treatment: implications for the use of synovial histopathology as biomarker in early phase clinical trials in rheumatoid arthritis. *Ann Rheum Dis* 2006; **65**: 990-7.
13. Cunnane G, Madigan A, Murphy E *et al*. The effects of treatment with interleukin-1 receptor antagonist on the inflamed synovial membrane in rheumatoid arthritis. *Rheumatology (Oxford)* 2001; **40**: 62-9.
14. Kane D, Gogarty M, O'leary J *et al*. Reduction of synovial sublining layer inflammation and proinflammatory cytokine expression in psoriatic arthritis treated with methotrexate. *Arthritis Rheum* 2004; **50**: 3286-95.
15. Kruithof E, De RL, Vandooren B *et al*. Identification of synovial biomarkers of response to experimental treatment in early-phase clinical trials in spondylarthritis. *Arthritis Rheum* 2006; **54**: 1795-804.
16. Goedkoop AY, Kraan MC, Teunissen MB *et al*. Early effects of tumour necrosis factor alpha blockade on skin and synovial tissue in patients with active psoriasis and psoriatic arthritis. *Ann Rheum Dis* 2004; **63**: 769-73.
17. Goedkoop AY, Kraan MC, Picavet DI *et al*. Deactivation of endothelium and reduction in angiogenesis in psoriatic skin and synovium by low dose infliximab therapy in combination with stable methotrexate therapy: a prospective single-centre study. *Arthritis Res Ther* 2004; **6**: R326-R334.
18. Canete JD, Pablos JL, Sanmarti R *et al*. Antiangiogenic effects of anti-tumor necrosis factor alpha therapy with infliximab in psoriatic arthritis. *Arthritis Rheum* 2004; **50**: 1636-41.
19. Mease PJ, Gladman DD, Ritchlin CT *et al*. Adalimumab for the treatment of patients with moderately to severely active psoriatic arthritis: results of a double-blind, randomized, placebo-controlled trial. *Arthritis Rheum* 2005; **52**: 3279-89.
20. Taylor W, Gladman D, Helliwell P *et al*. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum* 2006; **54**: 2665-73.
21. Chandran V, Schentag CT, Gladman DD. Sensitivity of the classification of psoriatic arthritis criteria in early psoriatic arthritis. *Arthritis Rheum* 2007; **57**: 1560-3.
22. Prevoo ML, van 't Hof MA, Kuper HH *et al*. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; **38**: 44-8.
23. Fransen J, Antoni C, Mease PJ *et al*. Performance of response criteria for assessing peripheral arthritis in patients with psoriatic arthritis: analysis of data from randomised controlled trials of two tumour necrosis factor inhibitors. *Ann Rheum Dis* 2006; **65**: 1373-8.
24. Felson DT, Anderson JJ, Boers M *et al*. American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum* 1995; **38**: 727-35.
25. Kraan MC, Reece RJ, Smeets TJ *et al*. Comparison of synovial tissues from the knee joints and the small joints of rheumatoid arthritis patients: Implications for pathogenesis and evaluation

of treatment. *Arthritis Rheum* 2002; **46**: 2034-8.

26. Dolhain RJ, Ter Haar NT, De Kuiper R *et al*. Distribution of T cells and signs of T-cell activation in the rheumatoid joint: implications for semiquantitative comparative histology. *Br J Rheumatol* 1998; **37**: 324-30.
27. Boyle DL, Rosengren S, Bugbee W *et al*. Quantitative biomarker analysis of synovial gene expression by real-time PCR. *Arthritis Res Ther* 2003; **5**: R352-R360.
28. Tak PP, van der Lubbe PA, Cauli A *et al*. Reduction of synovial inflammation after anti-CD4 monoclonal antibody treatment in early rheumatoid arthritis. *Arthritis Rheum* 1995; **38**: 1457-65.
29. Smeets TJ, Kraan MC, Galjaard S *et al*. Analysis of the cell infiltrate and expression of matrix metalloproteinases and granzyme B in paired synovial biopsy specimens from the cartilage-pannus junction in patients with RA. *Ann Rheum Dis* 2001; **60**: 561-5.
30. Smeets TJ, Barg EC, Kraan MC *et al*. Analysis of the cell infiltrate and expression of proinflammatory cytokines and matrix metalloproteinases in arthroscopic synovial biopsies: comparison with synovial samples from patients with end stage, destructive rheumatoid arthritis. *Ann Rheum Dis* 2003; **62**: 635-8.
31. Kraan MC, Smith MD, Weedon H *et al*. Measurement of cytokine and adhesion molecule expression in synovial tissue by digital image analysis. *Ann Rheum Dis* 2001; **60**: 296-8.
32. Haringman JJ, Vinkenoog M, Gerlag DM *et al*. Reliability of computerized image analysis for the evaluation of serial synovial biopsies in randomized controlled trials in rheumatoid arthritis. *Arthritis Res Ther* 2005; **7**: R862-R867.
33. Conover WJ, Iman RL. Analysis of covariance using the rank transformation. *Biometrics* 1982; **38**: 715-24.
34. Moll JM, Wright V. Psoriatic arthritis. *Semin Arthritis Rheum* 1973; **3**: 55-78.
35. van Gestel AM, Prevoo ML, van 't Hof MA *et al*. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. *Arthritis Rheum* 1996; **39**: 34-40.
36. Tak PP, Smeets TJ, Daha MR *et al*. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. *Arthritis Rheum* 1997; **40**: 217-25.
37. McInnes IB, Illei GG, Danning CL *et al*. IL-10 improves skin disease and modulates endothelial activation and leukocyte effector function in patients with psoriatic arthritis. *J Immunol* 2001; **167**: 4075-82.
38. Bresnihan B, Gerlag DM, Rooney T *et al*. Synovial macrophages as a biomarker of response to therapeutic intervention in rheumatoid arthritis: standardization and consistency across centers. *J Rheumatol* 2007; **34**: 620-2.
39. Choy E. T cells in psoriatic arthritis. *Curr Rheumatol Rep* 2007; **9**: 437-41.
40. Mease PJ, Gladman DD, Keystone EC. Alefacept in combination with methotrexate for the treatment of psoriatic arthritis: Results of a randomized, double-blind, placebo-controlled study. *Arthritis Rheum* 2006; **54**: 1638-45.
41. Vickers AJ, Altman DG. Statistics notes: Analysing controlled trials with baseline and follow up measurements. *BMJ* 2001; **323**: 1123-4.
42. Pontifex EK, Gogarty M, Gibbs A *et al*. Change in CD3 positive cell infiltration in psoriatic arthritis (PsA) synovial tissue correlates with change in DAS28 following initiation of biologic therapy. Ann.Rheum.Dis. 67 (Suppl II), 628. 2008. Ref Type: Abstract
43. Tracey D, Klareskog L, Sasso EH *et al*. Tumor necrosis factor antagonist mechanisms of action: A comprehensive review. *Pharmacol Ther* 2008; **117**: 244-79.

6

EXPRESSION OF THE CHEMOKINE RECEPTOR CCR5 IN PSORIASIS AND RESULTS OF A RANDOMIZED PLACEBO CONTROLLED TRIAL WITH A CCR5 INHIBITOR

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ABSTRACT

Several reports have indicated that the chemokine receptor CCR5 and its ligands, especially CCL5 (formerly known as RANTES), may play a role in the pathogenesis of psoriasis. The purpose of this investigation was to examine the expression of CCR5 and its ligands in chronic plaque psoriasis and to evaluate the clinical and immunohistochemical effect of a CCR5 receptor inhibitor. Immunohistochemical analysis showed low but significant increased total numbers of CCR5 positive cells in epidermis and dermis of lesional skin in comparison to non-lesional skin. However, relative expression of CCR5 proportional to the cells observed revealed that the difference between lesional and non-lesional skin was only statistically significant in the epidermis for CD3 positive cells and in the dermis for CD68 positive cells. Quantification of mRNA by reverse transcriptase-polymerase chain reaction (RT-PCR) only showed an increased expression of CCL5 (RANTES) in lesional skin.

A randomized placebo-controlled clinical trial in 32 psoriasis patients revealed no significant clinical effect and no changes at the immunohistochemical level comparing patients treated with placebo or a CCR5 inhibitor SCH351125. We conclude that although CCR5 expression is increased in psoriatic lesions, this receptor does not play a crucial role in the pathogenesis of psoriasis.

INTRODUCTION

Psoriasis is a chronic skin disease affecting approximately 2% to 3% of the population worldwide. Despite its common occurrence, the exact pathogenesis of psoriasis remains unclear and adaptations to the pathogenesis of this inflammatory disease are continuing [1-6]. Although the specific effector cells responsible for the inflammatory process in psoriasis are not known, reported beneficial effects of specific T cell targeted therapies, such as cyclosporine A, DAB389IL-2 and alefacept support a central role for T cells in the pathogenesis of psoriasis [7-13]. Further investigation on the immunophenotype and cytokine secretion patterns of T cells have indicated that specifically Th1-cells are involved in psoriasis [14-16].

In the many aspects encompassing T-cell homeostasis, the trafficking of T cells from blood to tissues is thought to be relevant in chronic inflammatory diseases such as psoriasis. Key factors in this migration are chemoattractant cytokine molecules known as chemokines and their receptors[17-23]. The predominant chemokine receptors expressed on Th1-cells are CCR5 and CXCR3 [24-30]. Besides its preferential expression on Th1 cells, CCR5 is also expressed on monocytes, macrophages, natural killer and dendritic cells: all thought to be significant elements in the pathogenesis of psoriasis [31-41].

The ligands of CCR5 (CCL3, CCL4 and CCL5 (formerly known as MIP1α, MIP1β and RANTES, respectively)) are highly expressed by keratinocytes in psoriatic tissue [42-45]. Furthermore, it has been demonstrated that the proinflammatory cytokines IFN-γ and TNF-α can induce the expression of these chemokines [43;44] and that treatment of psoriasis resulted in a significant decrease of CCL5, as well as a reduction of $CCR5^+$ T cells in the skin [44;46;47].

Several animal models resembling psoriasis have been developed, yet, none of these models imitates psoriasis completely, hence limiting their utility [48]. Investigations by Mack et al. showed a different expression pattern of CCR5 in mice and humans [40]. Additionally, different expression of a single amino acid in the CCR5 molecule between rhesus macaques and humans resulted in a different response to inhibitors of the receptor in the species [49]. Therefore, research on the expression of CCR5 in psoriasis, as well as clinical efficacy of a CCR5 inhibitor, is limited to humans. So far, the data available on the expression of CCR5 in psoriatic skin in humans are not univocal (varying from high to minimal) and was obtained with divergent methods in investigations in which CCR5 was never the main focus [41;46;47;50;51].

The primary purpose of this study was to determine the expression of CCR5 in its ligands in chronic plaque psoriasis *in situ* compared to non-lesional skin, through analysis by immunohistochemistry and quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). In order to examine the possibility that CCR5 plays a functional role in the pathogenesis of psoriasis, we also analysed clinical and immunohistochemical data obtained from lesional and non-lesional skin biopsies of psoriasis patients before and after treatment with a CCR5 inhibitor.

MATERIAL AND METHODS

Study design and patients

Lesional and non-lesional skin biopsies were obtained from 9 patients with moderate to severe chronic plaque psoriasis, defined by a Psoriasis Area Severity Index (PASI) ≥ 8. These skin biopsies were analysed by manual quantification of immunohistochemical double-staining and quantitative RT-PCR. In order to get insight in the possibility of a functional role of CCR5 in the pathogenesis of psoriasis, 34 patients, including the previous 9 patients, participated in an 8 week, randomized, placebo-controlled, parallel group, multi-centre, double-blind clinical trial in which patients received either 50 mg twice daily of the CCR5 inhibitor SCH351125 (23 patients) or matched placebo (11 patients) orally for 28 days, followed by a follow-up period of 4 weeks. During the follow-up period patients were only allowed to use emollients as treatment and on day 56 vital signs, PASI and blood were assessed. Patients were included at the dermatology outpatient departments of four academic hospitals from April 2004 until December 2004. At baseline and the last day of treatment (day 28), lesional biopsies were taken to evaluate the immunohistochemical effect of the CCR5 inhibitor. For this immunohistochemical evaluation, single- stained sections were analysed with digital image analysis, semi-quantitative analysis (SQA) and confocal scanning microscopy, and double-stained sections on baseline and day 28 were analysed by manual quantification. To evaluate the clinical effect of the CCR5 inhibitor the PASI was assessed at baseline, day 28 and day 56.

In all patients, psoriasis was diagnosed at least 12 months prior to enrolment and patients were not allowed to use systemic psoriasis treatment or phototherapy within 4 weeks of study entry. Only emollients were allowed as topical treatment. All other topical anti-psoriasis therapy (e.g. corticosteroids, vitamin D derivates, etc) had to be stopped 2 weeks before study entry. The protocol was reviewed and approved by the medical ethical committees of all participating centres and all patients gave their written informed consent before enrolment. The study was conducted according to the Declaration of Helsinki principles and is registered at the ISRCT register (http://www.controlled-trials.com/ISCRT14986467).

Biopsies

Four-millimeter biopsies were taken from the inside border of a target psoriatic plaque, preferentially from a non-sun-exposed area. Lesional biopsies from each patient were obtained from the same target lesion, separated by at least 1 cm. The biopsy samples were randomly coded, snap-frozen in Tissue-Tek OCT compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands) by immersion in liquid nitrogen and stored at -80˚C until processing. Five-micrometer cryostat sections were cut and mounted on glass slides (Star Frost adhesive slides, Knittelgläser, Braunschweig, Germany), before being stored at -80˚C until immunohistochemical staining. For each staining three sections of each biopsy were analysed to minimize random variation.

Immunohistochemistry

After fixation of the slides, the endogenous peroxide activity was inhibited with 0.1% sodium azide and 0.3% hydrogen peroxide in Tris-buffered saline (TBS), before incubating for 15 min with 10% normal goat serum in TBS. Next, the sections were incubated for 1 hour at room temperature (overnight at 4°C in the case of CCR5) with the primary antibody in 1% bovine serum albumin (BSA; Sigma-Chemical Co, St. Louis, MO, USA) in TBS. The following mouse anti-human monoclonal antibodies were used: FITC-conjugated anti-CD3 (BD Pharmingen, San Jose, CA, USA), FITC-conjugated-anti CD68 (clone EBM11; Dako), anti-human neutrophil elastase (Dako, Glostrup, Denmark), anti-cytokeratin 8.12 (keratin 16; Sigma, Saint Louis, MO, USA), anti-CD161 (NK-T cells; BD Pharmingen) and anti-CCR5 (CD195; BD Pharmingen). After rinsing with TBS, sections were further incubated with biotin-conjugated goat anti-mouse antibody (Dako) or, in case of CD3 and CD68 staining, with rabbit anti-FITC (Dako) in 10% normal human serum (NHS) in TBS for 30 minutes. Following a wash step with TBS, sections were subsequently incubated with horseradish peroxidase (HPR)-conjugated streptavidin (Dako) or, in case of CD3 staining, with HRP-conjugated goat anti-rabbit antibody (Dako), in 1% BSA in TBS for 30 minutes. In case of CCR5 staining an amplification step was performed with the TSA biotin system (Perkin Elmer, Boston, MA, USA). Sections were counterstained with Mayer's haematoxylin (Merck, Darmstadt, Germany) and mounted in Kaiser's glycerol gelatine (Merck). Skin sections were double stained with anti-CCR5 together with anti-CD3 or macrophage marker anti-CD68. The double stained sections were manually counted by two independent observers blinded for order, patient and clinical data. Using a 0.5x0.5 mm ocular grid and at 200x magnification, single red ($CCR5^+$), single blue ($CD3^+$ or $CD68^+$), and purple double-positive cells were counted in the entire section. The epidermal and dermal regions were separately counted. The results are expressed as the number of double-positive cells/mm^2.

RNA analysis

RNA was extracted from frozen skin biopsies using the RNeasy mini kit (Qiagen). RNA quantity was assessed by OD at 260 nm and RNA quality was analyzed by measuring the ratio of 28s and 18s rRNA using the Agilent 2100 bioanalyzer.

Quantitative PCR

Taqman primers and probes were designed with Primer Express software (ABI), and purchased from ABI. The sequences of the human primers and probes are available upon request. For the human skin tissue, quantitative PCR was carried out with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The PCR reactions were prepared using the components from the Invitrogen Platinum Quantitative RT-PCR One-Step kit and assembled according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The final concentrations of the primers and probe in the PCR reactions were 200 and 100 nM, respectively. The RT-PCR

reactions for each gene were performed in a single 384-well plate. Separate plates of the same RNAs were used to quantitate 18S RNA as an internal control for RNA quality, and a primer/probe set for the CD4 promotor was used to check the RNAs for genomic contamination. The PCR data was quantitated based on a standard curve generated using fourfold serial dilutions of the target genes. The fourfold dilutions began at 0.25 ng, and eight dilutions were used to generate the standard curve. This procedure provides an absolute quantitation of the amount of CCR5 mRNA in a given tissue. Data were analyzed using Sequence Detection Systems software version 1.7 (Applied Biosystems, Foster City, CA, USA).

Digital image analysis

Single stained sections were randomly coded and analyzed by computer-assisted image analysis as described previously in detail [52]. In short, images were acquired and analyzed using Syndia algorithm on a Qwin based analysis system (Leica, Cambridge, UK). Twenty high power fields per section were analyzed. Positive staining of cellular markers was expressed as positive cells/mm^2.

Semi-quantitative analysis (SQA)

For keratinocyte expression of K16 keratin, a semi-quantitative score was done by two independent observers, blinded for order, patient and clinical data, with a standard binocular light microscope (Olympus) at 200x magnification. The semi-quantitative score ranged from 0 to 4+. A score of 0 represented no expression, while a score of 4 represented abundant expression in all layers of the epidermis.

Confocal scanning microscope

Cryosections of 5μm on silanized slides were fixed with acetone and dried at room temperature, and stored at -80^{0}C. Sections were incubated in PBS-3% BSA for 30 minutes and washed with PBS before all steps. Next, monoclonal anti-human CCR-5 antibody (R&D Systems, MAB183, clone 45549.11), Texas red immunoglobulins diluted in PBS-3% BSA (1:100) was applied, followed by application of the primary monoclonal specific antibodies CD3 (clone SK7; BD Biosciences), CD4 (clone SK3; BD Biosciences), CD8 (clone DK25, DakoCytomation) or CD68 (clone KP1, DakoCytomation). Next, FITC-conjugated affinipure rabbit anti-mouse IgG (Jackson, A=492, E=520) diluted in PSB-3% BSA (1:200) was applied and sections were mounted in fluorescent mounting medium (DakoCytomation). Of each double stained slide three pictures were taken with an image definition of 1024/1024 pixels at a magnification of x 25.

Sample size calculation

The randomized placebo controlled clinical trial was targeted to randomize a total of 30 subjects (20 on active treatment and 10 on placebo). With this sample size,

the trials would be able to detect a difference of 38% in the response rate from the placebo group assuming a 0% response rate in the placebo group with 80% power at an alpha level of 0.05 (two-sided test).

Randomization

Randomization was stratified by sites. Each site was assigned a fixed number of subjects numbers; e.g. site 1 would get numbers 1 to 9 and so on. Once the physician of the study site would enroll a subject, the subject would be assigned the next available subject number assigned to the site, starting with the bottom of the list; e.g. the first subject enrolled in site 1 would get number 1, the second subject would get number 2, and so on. Treatments would be assigned in a active to placebo ratio of 2:1 according to a computer generated randomization schedule. No stratification based on age, sex or other characteristics was performed. Throughout the study both patient and treating physician were blinded to the group assignment

Statistical analysis

We used SPSS 12.0.1 for Windows (SPSS, Chicago, IL, USA) for the statistical analysis of clinical data. The Mann-Whitney test was used to compare lesional and non-lesional skin at baseline and to compare skin biopsies before and after treatment. All statistical tests were two-sided: P values less than 0.05 were considered significant. The results are expressed as median ± standard error of the mean. To evaluate the clinical effect of treatment with a CCR5 inhibitor, an intention to treat analysis was performed. Quantitative PCR data analysis was performed by two-sided t-test as implemented by Graphpad Prizm (version 4.0 Graphpad Software, San Diego, CA, USA). A *P* value less than 0.05 was considered significant.

RESULTS

Comparison of CCR5 expression in lesional versus non-lesional psoriatic skin

The expression of CCR5 in T cells (CD3) and macrophages (CD68) in lesional and non-lesional skin of nine psoriasis patients was compared at baseline. We observed a clear expression of CCR5 which was primarily present in the dermis. In absolute numbers approximately half of the T cells and half of the macrophages co-expressed CCR5 (Figure 1). The $CD3^+CCR5^+$ and $CD68^+CCR5^+$ double positive cells showed a low but statistically significant increased expression of CCR5 in epidermis and dermis of lesional skin in comparison to non-lesional skin, as shown in Figure 1. Focussing on the expression of CCR5 as the percentage of all T cells or macrophages present in the sections, the difference between lesional and non-lesional skin was only statistically significant in the epidermis for $CD3^+$ cells ($p< 0,05$) and in the dermis for $CD68^+$ cells ($p< 0,001$).

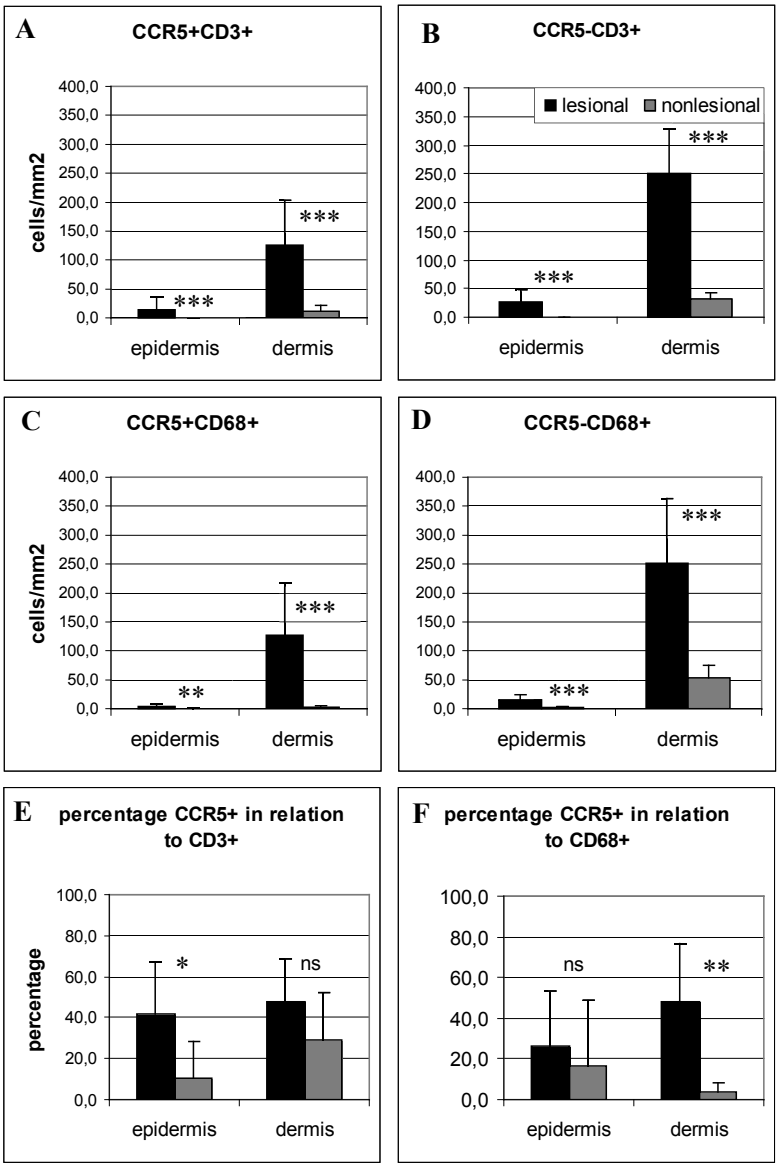


Fig. 1 Immunohistochemical analysis of CCR5 in lesional versus non-lesional skin. Data are shown as mean ± SD; ns, non-significant, * P< 0.05, ** P< 0.01, *** P< 0.001.

Quantitative RT-PCR analysis indicated no increased expression of mRNA for CCR5 and CCR5-ligand CCL4 (MIP-1β) in lesional skin (Figure 2), only the expression of CCR5-ligand CCL5 (RANTES) and IL-8 was significantly increased in lesional skin ($p<0,0001$ and $p<0,05$). The well-known enhanced expression of IL-8 in psoriatic skin was included as positive control.

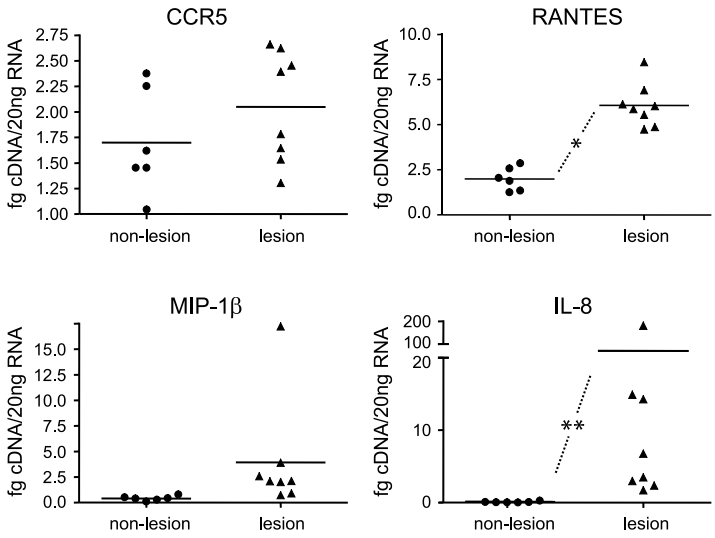


Fig. 2 mRNA analysis of CCR5 in lesional psoriatic skin in comparison with non-lesional skin. IL-8 is used as a control marker. * P<0.0001, ** P<0.05.

Lack of clinical efficacy of SCH351125, a CCR5 inhibitor

In total 34 patients were randomized, as shown in Figure 3. The demographical data of the patients are shown in Table 1. With regards to co-morbidities, one patient had hypercholesterolemia and one had obesity in the placebo group. In the SCH351125 group one patient also had obesity and two had a history of hypertension. After treatment with the CCR5 inhibitor there was no change in mean PASI in the SCH351125 group (n=23) (15.5± 3.8 at baseline, 15.4 ± 7.4 at day 28 (Figure 4A)). Three of the patients treated with SCH351125 (13%) attained an improvement of 50% or more compared to baseline (PASI 50 responders), showing improvements of 67%, 77% and 69%. In the placebo group (n=9) the mean PASI slightly decreased (14.2 ± 4.7 at baseline, 12.9 ± 3.7 at day 28). None of the patients treated with placebo showed an improvement of more than 50%. All changes observed were not statistically significant. In the follow-up period no changes in mean PASI were seen in either treatment groups.

In the treatment group four patients discontinued. One patient developed an erythrodermic eruption after 4 days of treatment, which was considered by the site physician as an serious adverse event (SAE). Two patients discontinued due to adverse events (AEs): one developed shingles in the n.trigeminus area of the right side of his face after 8 days of treatment and one patient discontinued due to hair loss after 21 days of treatment. One patient discontinued due to non-compliance. In the placebo group two patients discontinued due to AEs: both exacerbation of their psoriasis after two weeks of treatment.

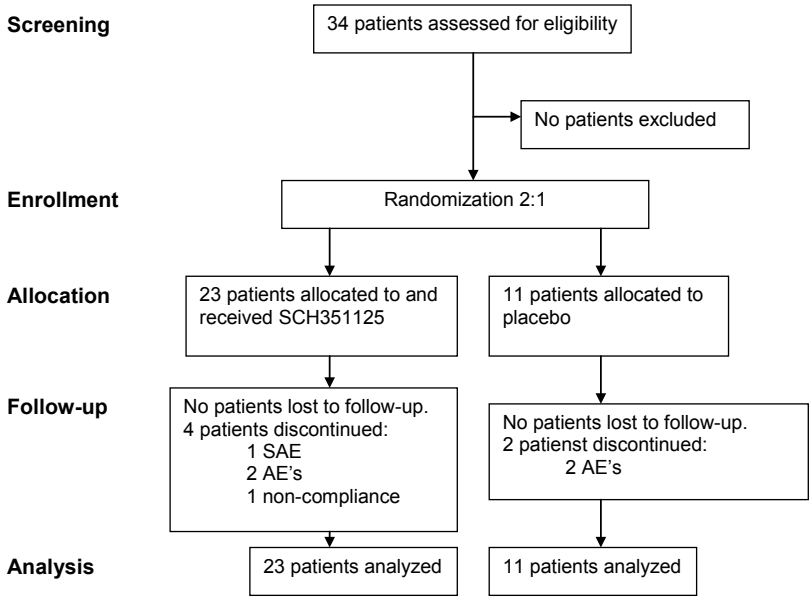


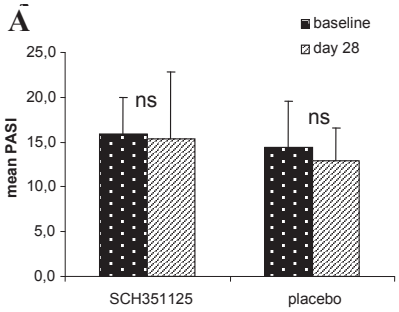
Fig. 3 Flow chart of randomized, placebo controlled clinical trial. SCH351125, CCR5 ligand inhibitor; SAE, serious adverse event; AE, adverse event.

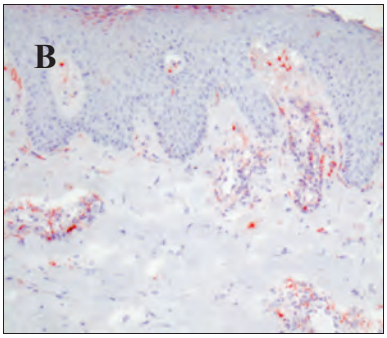
Table 1. Demographical data patients

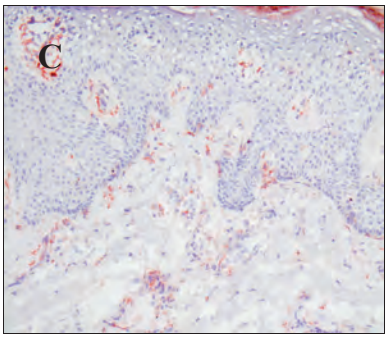
	Randomized clinical trial	
	placebo	SCH351125
Number	11	23
Male:female	7:4	18:5
Age (years)[1]	41.8 (10.2)	49.4 (14.3)
Duration of skin disease (years)[1]	20.6 (9.8)	19.8 (11.6)
Baseline PASI[1]	14.9 (4.7)	15.7 (4.3)

SCH351125, CCR5 ligand inhibitor; PASI, Psoriasis Area and Severity Index; BSA, Body Surface Area; [1]mean (±SD).

Fig. 4 Clinical and immunohistochemical respons after treatment with a CCR5 receptor inhibitor. In a randomized placebo controlled clinical trial 34 psoriasis patients were randomized for treatment with a CCR5 receptor inhibitor (SCH351125) or placebo for 28 days. Clinical efficacy was measured by Psoriasis Area and Severity Index (PASI) (**a**). Lesional skin biopsies were taken from all patients at baseline and day 28 to evaluate immunohistochemical effect. Immunohistochemical single staining of CCR5 of a patient treated with SCH351125 at baseline (**b**) and day 28 (**c**). Immunohistochemical markers in relation to clinical response (**d**). PASI 50 non-responder, improvement of PASI of less than fifty percent; PASI 50 responder, improvement of PASI of fifty or more percent; data are shown as mean ± SD; ns, non-significant; [1]P=0.05.







D

		PASI 50 non-responders		PASI 50 responders	
		Baseline	Day 28	Baseline	Day 28
Placebo	n	11	11	0	0
	CD3	105.9 ± 83.5	102.4 ± 81.0		
	CD161	17.3 ± 26.7	21.5 ± 32.4		
	elastase	91.5 ± 90.3	147.6 ± 179.7		
	K16	2.8 ± 0.3	2.7 ± 0.3		
	CCR5+CD3+ epidermis	7.5 ± 4.0	6.0 ± 4.2		
	CCR5+CD3+ dermis	89.8 ± 54.0	99.0 ± 80.9		
	CCR5+CD68+ epidermis	1.8 ± 1.9	1.2 ± 1.4		
	CCR5+CD68+ dermis	44.9 ± 29.0	31.8 ± 32.3		
SCH351125	n	18	18	3	3
	CD3	108.6 ± 68.0	113.9 ± 81.2	45.8 ± 32.5	56.5 ± 30.9
	CD161	10.3 ± 9.5	9.5 ± 13.2	9.1 ± 8.0	5.4 ± 7.0
	elastase	40.3 ± 40.8	52.6 ± 65.2	24.0 ± 3.9	4.4 ± 1.9[1]
	K16	3.6 ± 0.3	3.1 ± 0.2	3.3 ± 0.6	2.8 ± 0.3
	CCR5+CD3+ epidermis	13.4 ± 16.4	11.7 ± 15.4	7.2 ± 4.4	3.5 ± 1.0
	CCR5+CD3+ dermis	133.0 ± 82.5	116.9 ± 126.5	100.6 ± 39.0	43.4 ± 11.9[1]
	CCR5+CD68+ epidermis	2.2 ± 3.4	2.0 ± 2.1	1.7 ± 1.7	1.9 ± 1.0
	CCR5+CD68+ dermis	35.2 ± 33.2	32.5 ± 27.1	21.3 ± 7.6	16.7 ± 4.8

CCR5 expression before and after treatment with SCH351125

Immunohistochemical analysis of lesional tissue samples from the SCH351125 group and the placebo group revealed no statistically different expression of CCR5 between baseline and day 28 in both treatment groups, as illustrated by Figure 4B and C. When focusing on the markers CD3, CD68, CD161, elastase and K16 in relation to the clinical response, no statistically significant difference after 28 days of treatment with either SCH351125 or placebo was found (Figure 4D), except for elastase and dermal $CCR5^{+}CD3^{+}$ cells, which were statistically significantly lowered in the three PASI 50 responders treated with SCH351125. Additional data obtained by confocal scanning microscopy corresponded with the digital image and semi-quantitative analysis (data not shown).

DISCUSSION

The primary objective of this study was to explore the possibility of involvement of CCR5 in the pathogenesis of chronic plaque psoriasis. Therefore we determined the expression of CCR5 *in situ* at the protein and mRNA level by immunohistochemical analysis and, quantitative RT-PCR respectively. The total number of single positive ($CCR5^{+}$) and double positive ($CCR5^{+}CD3^{+}$ and $CCR5^{+}CD68^{+}$) cells in lesional psoriatic skin significantly outnumbered those in non-lesional skin. However, when expressed as percentage of CD3 or CD68 cells, the difference between lesional versus non-lesional expression was less clear. With the latter approach we found that the proportion of CCR5 expression was significantly higher in the epidermal $CD3^{+}$ cells and dermal $CD68^{+}$ cells only, when comparing lesional skin to non-lesional skin.

Analysis of CCR5 mRNA expression demonstrated a slight, though not significant, increased expression of CCR5 in lesional psoriatic skin, perhaps due to the small numbers of patients. In line with earlier observations we found that the mRNA expression for CCL5 [42;44] and IL-8 [53-57] was significantly higher expressed in the lesional samples. However, in contrast to previous research [58] the expression of CCL4 was not increased in lesional psoriatic skin.

In summary, our results do not provide a clear answer to our objective of determining whether the percentage of CCR5 expressing cells is similar in lesional and non-lesional skin, or if this percentage is increased in lesional skin.

To assess any possible functional participation of CCR5 in the development or maintenance of psoriatic plaques we investigated the clinical response to treatment with a CCR5 inhibitor as well as the effect of this drug on the inflamed skin *in situ*. The randomized placebo-controlled clinical trial revealed no significant clinical effect and changes at the immunohistochemical level between patients treated with placebo or the CCR5 inhibitor. The specific type and dose of the CCR5 inhibitor used in this clinical trial has proven its efficacy previously *in vitro*, *in vivo* and clinical studies with other diseases in which CCR5 is known to play a pivotal or significant role [59-62].

However, only 3 patients treated with the CCR5 inhibitor demonstrated a clinical improvement of 50% or more. It cannot be excluded that this low and not statistically significant number of patients is due to a spontaneous improvement, reflecting the unpredictability of psoriasis. Surprisingly, the immunohistochemical markers analysed in the skin biopsies of the so-called PASI 50 responders, did only partially correspond with the clinical response. Of all immunohistochemical markers only elastase and dermal $CCR5^{+}CD3^{+}$ showed a significant decline after 28 days of treatment. Notably, the expression of CD3, a marker known to correspond well with the clinical severity as measured by PASI, was increased after treatment with SCH351125 in two out of the 3 responding patients (Fig. 4D). In addition, their baseline expression of CD3 was lower in comparison with the other patients while their PASI was similar. This inconsistency could be due to the low number of patients or suggests an individual difference in CD3 kinetics.

The increased expression of CCR5 observed by immunohistochemistry and the increased mRNA expression of CCR5-ligand CCL5 in lesional psoriatic skin may suggest an involvement of this receptor and ligand in psoriasis. However, our clinical trial with an effective CCR5 inhibitor unequivocally demonstrated that this is not the case. Previous research has shown increased expression of several chemokines and chemokine receptors in psoriasis, [54;63-66] indicating that multiple receptors may participate in regulating T cell recruitment to the inflamed skin. Furthermore, CCL5 is known to also bind with CCR1 and CCR3, whereas CCL4 is solely connected to CCR5. Given this complexity of interactions between chemokines and chemokine receptors, it is not unlikely that blocking a single chemokine receptor (i.e. CCR5) would have been insufficient in diminishing the inflammatory process.

According to Homey, chemokine antagonistic approaches to impede with the inflammatory process may perhaps be preventive rather than therapeutic [38]. Chemokines and their receptors play an essential role in the trafficking of T cells to all kinds of tissue, including the skin. Yet, once leucocytes have entered the target organ and underwent activation processes, impairment of recruitment of pathogenic T cells is likely to be less effective in reducing the clinical symptoms. When combined with a successful eradicative treatment of the T cells, chemokine antagonists could perhaps be promising candidates for prevention of acute flares, prolongation of lesion-free interval and therefore provide optimized long-term management of patients suffering from chronically relapsing inflammatory skin disease such as psoriasis. So, although CCR5 does not seem to be a key chemokine receptor in the pathogenesis of psoriasis, further efforts are needed to unravel the complete set of chemokines and chemokine receptors significant in the recruitment of inflammatory cells in psoriasis, and may help to identify crucial molecules, as demonstrated previously by TNF in various immune mediated inflammatory diseases.

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REFERENCE LIST

1. Barker JN. The pathophysiology of psoriasis. *Lancet* 1991; **338**: 227-30.
2. Bos JD, de Rie MA, Teunissen MB *et al*. Psoriasis: dysregulation of innate immunity. *Br J Dermatol* 2005; **152**: 1098-107.
3. Lebwohl M. Psoriasis. *Lancet* 2003; **361**: 1197-204.
4. Nickoloff BJ, Nestle FO. Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. *J Clin Invest* 2004; **113**: 1664-75.
5. Schon MP, Boehncke WH. Psoriasis. *N Engl J Med* 2005; **352**: 1899-912.
6. Barker JN. The pathophysiology of psoriasis. *Lancet* 1991; **338**: 227-30.
7. Bata-Csorgo Z, Hammerberg C, Voorhees JJ *et al*. Intralesional T-lymphocyte activation as a mediator of psoriatic epidermal hyperplasia. *J Invest Dermatol* 1995; **105**: 89S-94S.
8. Bos JD, Hulsebosch HJ, Krieg SR *et al*. Immunocompetent cells in psoriasis. In situ immunophenotyping by monoclonal antibodies. *Arch Dermatol Res* 1983; **275**: 181-9.
9. Ferenczi K, Burack L, Pope M *et al*. CD69, HLA-DR and the IL-2R identify persistently activated T cells in psoriasis vulgaris lesional skin: blood and skin comparisons by flow cytometry. *J Autoimmun* 2000; **14**: 63-78.
10. Baker BS, Griffiths CE, Lambert S *et al*. The effects of cyclosporin A on T lymphocyte and dendritic cell subpopulations in psoriasis. *Br J Dermatol* 1987; **116**: 503-10.
11. Ellis CN, Krueger GG. Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. *N Engl J Med* 2001; **345**: 248-55.
12. Gottlieb AB, Grossman RM, Khandke L *et al*. Studies of the effect of cyclosporine in psoriasis in vivo: combined effects on activated T lymphocytes and epidermal regenerative maturation. *J Invest Dermatol* 1992; **98**: 302-9.
13. Gottlieb SL, Gilleaudeau P, Johnson R *et al*. Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nat Med* 1995; **1**: 442-7.
14. Lew W, Bowcock AM, Krueger JG. Psoriasis vulgaris: cutaneous lymphoid tissue supports T-cell activation and "Type 1" inflammatory gene expression. *Trends Immunol* 2004; **25**: 295-305.
15. Piskin G, Sylva-Steenland RM, Bos JD *et al*. T cells in psoriatic lesional skin that survive conventional therapy with NB-UVB radiation display reduced IFN-gamma expression. *Arch Dermatol Res* 2004; **295**: 509-16.
16. Austin LM, Ozawa M, Kikuchi T *et al*. The majority of epidermal T cells in Psoriasis vulgaris lesions can produce type 1 cytokines, interferon-gamma, interleukin-2, and tumor necrosis factor-alpha, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. *J Invest Dermatol* 1999; **113**: 752-9.
17. Kunkel EJ, Butcher EC. Chemokines and the tissue-specific migration of lymphocytes. *Immunity* 2002; **16**: 1-4.
18. Loetscher P, Seitz M, Baggiolini M *et al*. Interleukin-2 regulates CC chemokine receptor expression and chemotactic responsiveness in T lymphocytes. *J Exp Med* 1996; **184**: 569-77.

19. Murdoch C, Finn A. Chemokine receptors and their role in inflammation and infectious diseases. *Blood* 2000; **95**: 3032-43.
20. Murphy PM, Baggiolini M, Charo IF *et al*. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev* 2000; **52**: 145-76.
21. Thelen M. Dancing to the tune of chemokines. *Nat Immunol* 2001; **2**: 129-34.
22. Wymann MP, Zvelebil M, Laffargue M. Phosphoinositide 3-kinase signalling--which way to target? *Trends Pharmacol Sci* 2003; **24**: 366-76.
23. Yoshie O, Imai T, Nomiyama H. Chemokines in immunity. *Adv Immunol* 2001; **78**: 57-110.
24. Bonecchi R, Bianchi G, Bordignon PP *et al*. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998; **187**: 129-34.
25. Loetscher P, Uguccioni M, Bordoli L *et al*. CCR5 is characteristic of Th1 lymphocytes. *Nature* 1998; **391**: 344-5.
26. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol* 2000; **18**: 593-620.
27. Kunkel EJ, Boisvert J, Murphy K *et al*. Expression of the chemokine receptors CCR4, CCR5, and CXCR3 by human tissue-infiltrating lymphocytes. *Am J Pathol* 2002; **160**: 347-55.
28. Qin S, Rottman JB, Myers P *et al*. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J Clin Invest* 1998; **101**: 746-54.
29. Loetscher P, Moser B, Baggiolini M. Chemokines and their receptors in lymphocyte traffic and HIV infection. *Adv Immunol* 2000; **74**: 127-80.
30. Bos JD, de Rie MA, Teunissen MB *et al*. Psoriasis: dysregulation of innate immunity. *Br J Dermatol* 2005; **152**: 1098-107.
31. Bonish B, Jullien D, Dutronc Y *et al*. Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-gamma production by NK-T cells. *J Immunol* 2000; **165**: 4076-85.
32. Cameron AL, Kirby B, Fei W *et al*. Natural killer and natural killer-T cells in psoriasis. *Arch Dermatol Res* 2002; **294**: 363-9.
33. Cameron AL, Kirby B, Griffiths CE. Circulating natural killer cells in psoriasis. *Br J Dermatol* 2003; **149**: 160-4.
34. Nickoloff BJ, Wrone-Smith T, Bonish B *et al*. Response of murine and normal human skin to injection of allogeneic blood-derived psoriatic immunocytes: detection of T cells expressing receptors typically present on natural killer cells, including CD94, CD158, and CD161. *Arch Dermatol* 1999; **135**: 546-52.
35. Nickoloff BJ, Bonish B, Huang BB *et al*. Characterization of a T cell line bearing natural killer receptors and capable of creating psoriasis in a SCID mouse model system. *J Dermatol Sci* 2000; **24**: 212-25.
36. Sozzani S, Allavena P, Vecchi A *et al*. Chemokines and dendritic cell traffic. *J Clin Immunol* 2000; **20**: 151-60.
37. Ottaviani C, Nasorri F, Bedini C *et al*. CD56brightCD16(-) NK cells accumulate in psoriatic skin in response to CXCL10 and CCL5 and exacerbate skin inflammation. *Eur J Immunol* 2006; **36**: 118-28.
38. Homey B. Chemokines and chemokine receptors as targets in the therapy of psoriasis. *Curr Drug Targets Inflamm Allergy* 2004; **3**: 169-74.
39. Fraziano M, Cappelli G, Santucci M *et al*. Expression of CCR5 is increased in human monocyte-derived macrophages and alveolar macrophages in the course of in vivo and in vitro Mycobacterium tuberculosis infection. *AIDS Res Hum Retroviruses* 1999; **15**: 869-74.
40. Mack M, Cihak J, Simonis C *et al*. Expression and Characterization of the Chemokine Receptors CCR2 and

CCR5 in Mice. *J Immunol* 2001; **166**: 4697-704.

41. Homey B, Dieu-Nosjean MC, Wiesenborn A *et al*. Up-regulation of macrophage inflammatory protein-3 alpha/CCL20 and CC chemokine receptor 6 in psoriasis. *J Immunol* 2000; **164**: 6621-32.
42. Raychaudhuri SP, Jiang WY, Farber EM *et al*. Upregulation of RANTES in psoriatic keratinocytes: a possible pathogenic mechanism for psoriasis. *Acta Derm Venereol* 1999; **79**: 9-11.
43. Giustizieri ML, Mascia F, Frezzolini A *et al*. Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. *J Allergy Clin Immunol* 2001; **107**: 871-7.
44. Fukuoka M, Ogino Y, Sato H *et al*. RANTES expression in psoriatic skin, and regulation of RANTES and IL-8 production in cultured epidermal keratinocytes by active vitamin D3 (tacalcitol). *Br J Dermatol* 1998; **138**: 63-70.
45. Nomura I, Gao B, Boguniewicz M *et al*. Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol* 2003; **112**: 1195-202.
46. Ghoreschi K, Thomas P, Breit S *et al*. Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease. *Nat Med* 2003; **9**: 40-6.
47. Uchida T, Suto H, Ra C *et al*. Preferential expression of Th2-type chemokine and its receptor in atopic dermatitis. *Int Immunol* 2002; **14**: 1431-8.
48. Schon MP. Animal models of psoriasis - what can we learn from them? *J Invest Dermatol* 1999; **112**: 405-10.
49. Billick E, Seibert C, Pugach P *et al*. The differential sensitivity of human and rhesus macaque CCR5 to small-molecule inhibitors of human immunodeficiency virus type 1 entry is explained by a single amino acid difference and suggests a mechanism of action for these inhibitors. *J Virol* 2004; **78**: 4134-44.
50. Rottman JB, Smith TL, Ganley KG *et al*. Potential role of the chemokine receptors CXCR3, CCR4, and the integrin alphaEbeta7 in the pathogenesis of psoriasis vulgaris. *Lab Invest* 2001; **81**: 335-47.
51. Vestergaard C, Deleuran M, Gesser B *et al*. Expression of the T-helper 2-specific chemokine receptor CCR4 on CCR10-positive lymphocytes in atopic dermatitis skin but not in psoriasis skin. *Br J Dermatol* 2003; **149**: 457-63.
52. Goedkoop AY, de Rie MA, Teunissen MB *et al*. Digital image analysis for the evaluation of the inflammatory infiltrate in psoriasis. *Arch Dermatol Res* 2005; **297**: 51-9.
53. Arican O, Aral M, Sasmaz S *et al*. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators Inflamm* 2005; **2005**: 273-9.
54. Gillitzer R, Ritter U, Spandau U *et al*. Differential expression of GRO-alpha and IL-8 mRNA in psoriasis: a model for neutrophil migration and accumulation in vivo. *J Invest Dermatol* 1996; **107**: 778-82.
55. Pietrzak A, Lecewicz-Torun B, Koziol-Montewka M. Plasma level of IL-8 in patients with psoriasis and its correlation with psoriasis area and severity index and the clinical type of the disease. *Ann Univ Mariae Curie Sklodowska [Med]* 2000; **55**: 261-7.
56. Schroder JM, Christophers E. Identification of C5ades arg and an anionic neutrophil-activating peptide (ANAP) in psoriatic scales. *J Invest Dermatol* 1986; **87**: 53-8.
57. Flisiak I, Klepacki A, Chodynicka B. Plasma and scales levels of interleukin 18 in comparison with other possible clinical and laboratory biomarkers of psoriasis activity. *Biomarkers* 2006; **11**: 194-200.
58. Nomura I, Gao B, Boguniewicz M *et al*. Distinct patterns of gene expression

in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol* 2003; **112**: 1195-202.

59. Palani A, Shapiro S, Clader JW *et al*. Biological evaluation and interconversion studies of rotamers of SCH 351125, an orally bioavailable CCR5 antagonist. *Bioorganic & Medicinal Chemistry Letters* 2003; **13**: 705-8.
60. Stephenson J. Researchers Explore New Anti-HIV Agents. *JAMA* 2002; **287**: 1635-7.
61. Strizki JM, Xu S, Wagner NE *et al*. SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection in vitro and in vivo. *Proc Natl Acad Sci U S A* 2001; **98**: 12718-23.
62. Vierboom MP, Zavodny PJ, Chou CC *et al*. Inhibition of the development of collagen-induced arthritis in rhesus monkeys by a small molecular weight antagonist of CCR5. *Arthritis Rheum* 2005; **52**: 627-36.
63. Flier J, Boorsma DM, van Beek PJ *et al*. Differential expression of CXCR3 targeting chemokines CXCL10, CXCL9, and CXCL11 in different types of skin inflammation. *J Pathol* 2001; **194**: 398-405.
64. Homey B, Dieu-Nosjean MC, Wiesenborn A *et al*. Up-regulation of macrophage inflammatory protein-3 alpha/CCL20 and CC chemokine receptor 6 in psoriasis. *J Immunol* 2000; **164**: 6621-32.
65. Kulke R, Bornscheuer E, Schluter C *et al*. The CXC receptor 2 is overexpressed in psoriatic epidermis. *J Invest Dermatol* 1998; **110**: 90-4.
66. Rottman JB, Smith TL, Ganley KG *et al*. Potential role of the chemokine receptors CXCR3, CCR4, and the integrin alphaEbeta7 in the pathogenesis of psoriasis vulgaris. *Lab Invest* 2001; **81**: 335-47.

7

EXPRESSION OF CHEMOKINE RECEPTOR CXCR3 BY LYMPHOCYTES AND PLASMACYTOID DENDRITIC CELLS IN HUMAN PSORIATIC LESIONS

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ABSTRACT

In psoriasis, leukocytes that infiltrate skin lesions have been shown to be involved in the pathogenesis of this disease. Previous investigations reporting the presence of CXCR3$^+$ T lymphocytes in psoriatic lesional skin have suggested a role of this receptor in the recruitment of T cells into the lesion. The purpose of this study was to quantify the mRNA levels of CXCR3 and to perform a systematic analysis of the cell populations that express CXCR3 in human lesional and non-lesional psoriatic biopsies. We showed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) that the mRNA levels of CXCR3 and its ligands, CXCL9-11, were significantly elevated in psoriatic lesions, as compared to non-lesional samples. Serial cryostat sections of psoriasis skin biopsies were evaluated by immunohistochemistry. The number of CXCR3$^+$ cells was low in non-lesional tissues. Quantitative image analysis demonstrated significant increases in the number of both epidermal and dermal CXCR3$^+$ cells in lesional compared to non-lesional biopsies. The majority of CXCR3$^+$ cells were located in the dermis of the lesional skin and 74% were demonstrated to be CD3$^+$ T lymphocytes. A small number of CXCR3$^+$ cells were CD68$^+$ myeloid cells. In addition, we found that nearly all BDCA-2$^+$ plasmacytoid dendritic cells in the psoriatic biopsies were CXCR3$^+$. These findings support and extend prior reports suggesting the potential role for CXCR3 in the pathophysiology of plaque psoriasis, by mediating the recruitment of plasmacytoid dendritic cells and T cells into the developing lesions.

INTRODUCTION

Psoriasis is an inflammatory skin disease with unique histopathological characteristics including the formation of rete peg morphology of the epidermis, thickening of epidermal layers, parakeratosis, increased vascularization and marked leukocyte infiltration into the dermis. The majority of the cellular infiltrates are mononuclear T lymphocytes. Other cell types, such as neutrophils, macrophages, dendritic cells and natural killer cells are among the infiltrating leukocytes. It was reported that the presence of infiltrating T lymphocytes and macrophages in the dermis preceded any significant epidermal changes[1]. The role of T lymphocytes in the maintenance of psoriasis was originally suggested by the efficacy of cyclosporine[2] and a lymphocyte-specific toxin[3]. Moreover biological agents that either stimulate T lymphocyte apoptosis or block T lymphocyte activation, co-stimulation and migration have demonstrated efficacy and gained approval as therapeutics for psoriasis (for review, see [4]). In xenograft models, injection of activated lymphocytes[5;6] or expansion of local resident T lymphocytes[7] was required to induce psoriasis-like phenotype in pre-symptomatic non-lesional human skin.

Both $CD4^+$ and $CD8^+$ T lymphocytes are present among the infiltrating T cells in psoriasis, although the role of each T lymphocyte subset in the pathogenesis of psoriasis is not well understood. It is thought that $CD4^+$ T cells are essential for the induction of psoriasis based on the ability of purified $CD4^+$, but not $CD8^+$, T lymphocytes to induce psoriatic lesions in the SCID-hu xenograft model[8]. However, $CD8^+$ T cells are the prevalent infiltrating T cells present in psoriatic epidermis[9] and have been speculated to be responsible for the activation of psoriatic keratinocytes. Both $CD4^+$ and $CD8^+$ T cells in psoriasis express Th1 cytokines such as interferon-γ (IFN-γ), interleukin (IL)-2 and tumor necrosis factor (TNF)-α. Based on this cytokine profile, psoriasis was initially considered to be a T-helper type 1 (Th1)-mediated inflammatory skin disease. Moreover, recent evidence also suggests an important contribution by Th17 cells in the development of psoriatic disease[10-14].

In addition to T lymphocytes, dendritic cells (DCs) are also among the prominent cellular aggregates in the dermis of psoriatic lesions. There are two major subsets of dermal DCs in psoriatic skin, myeloid (mDCs) and the plasmacytoid DCs (pDCs). mDCs are $CD11c^+$ while pDCs are $CD11c^-$/BDCA-2^+/$CD123^+$. mDCs are the predominant DCs in psoriatic tissue, found in both the epidermis and dermis, and express high levels of proinflammatory molecules such as TNF-α, IL-23 and IL-20[12;15;16], which may activate T lymphocytes and keratinocytes. In contrast, activated pDCs are present in lower numbers but express high levels of interferon-α (IFN-α). In contrast to normal human skin of healthy subjects, pDCs are present in normal-appearing skin of psoriasis patients, and considerable numbers of these cells can be present in lesional skin[17]. pDCs express intracellular toll-like receptor (TLR)7 and TLR9. These receptors recognize viral or microbial nucleic acids. The exacerbation of psoriasis in patients treated with TLR7 agonist imiquimod for other conditions was accompanied by a

large infiltrates of pDCs and a massive induction of type I IFN activity[18]. Very recently, a report demonstrated a unique pathway of pDC activation and its induction to secrete high levels of IFN-α by the binding of self-DNA in complex with an antimicrobial peptide LL37 over expressed in psoriatic skin[19]. These data suggest an upstream role of pDCs in the induction of the psoriatic lesion.

The role of chemokines and their receptors in the trafficking of various subtypes of leukocytes has been well documented. Chemokine receptor CXCR3 is known to be highly expressed by activated Th1 lymphocytes[20] and has been suggested to be one of the major chemokine receptors responsible for their recruitment to inflamed sites *in vivo*[21]. In addition, it can also be expressed by natural killer (NK) cell, B cells, pDCs and mDCs[22-28]. The presence of infiltrating CXCR3$^+$ T cells in psoriasis as well as other inflammatory skin disorders has been reported[29-34]. The cognate ligands of CXCR3, CXCL9 (MIG), CXCL10 (IP-10) and CXCL11 (ITAC) are induced by IFN-γ and have been shown to be expressed by inflammatory cells and/or keratinocytes in psoriatic skin and lesional skin of other inflammatory skin diseases[30;35;36]. Despite its documented role in the trafficking of activated T cells *in vitro* and the reported presence of CXCR3 T lymphocytes in the lesional psoriatic skin, a systematic analysis of CXCR3 expression in lesional psoriatic versus non-lesional skin biopsies has not been performed previously. In order to fully appreciate its potential role in the pathogenesis of this inflammatory skin disease, we have extensively examined the level and pattern of CXCR3 on infiltrating leukocytes in psoriatic lesions obtained from patients with extensive clinical histories. Here we report the leukocyte populations expressing CXCR3 in the psoriatic lesions and the results of our quantification.

METHODS

Skin biopsies

The human inflammatory skin disease panels were obtained from two independent clinical trials. One panel consists of lesional skin biopsies from 35 patients with moderate to severe chronic plaque psoriasis, defined by a Psoriasis Area Severity Index (PASI) ≥ 8. Skin samples from this collection were used in both quantitative PCR and immunohistochemistry. In 12 out of these 35 patients non-lesional skin biopsies were obtained. Four millimeter biopsies were taken from the inside border of a target psoriatic plaque, preferentially from a non-sun-exposed area. Lesional biopsies from each patient were obtained from the same target lesion, separated by at least 1 cm. The biopsy samples were randomly coded, either directly snap frozen in liquid nitrogen or snap-frozen in Tissue-Tek OCT compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands) by immersion in liquid nitrogen and stored at -80˚C until processing. In all patients, psoriasis was diagnosed at least 12 months prior to enrolment and patients were not allowed to use systemic psoriasis treatment or phototherapy within 4 weeks of study entry. Only emollients were allowed as

topical treatment. All other topical anti-psoriasis therapy (e.g. corticosteroids, vitamin D derivates, etc) was stopped 2 weeks before study initiation. The protocol was reviewed and approved by the medical ethical committees of all participating centres and all patients gave their written informed consent before enrolment. The study was conducted according to the Declaration of Helsinki principles. Tissues from this panel were used in quantitative PCR and immunohistochemistry studies described in this report.

A second panel of skin biopsies was also analyzed by quantitative PCR for the expression of CXCR3 ligands. This panel includes 35 normal skin samples (15 from autopsy donors and 20 from normal donors in clinical trial setting; see below), 24 non-lesional psoriasis skin samples, 25 lesional psoriasis skin samples, 30 non-lesional atopic dermatitis skin samples, and 30 lesional atopic dermatitis skin samples. All non-lesional and lesional patient samples were ranked by severity using either the PASI score or EASI (eczema area and severity index) score. For psoriasis patients, the PASI scores were in the range of 9-20.75. For atopic dermatitis patients, the EASI scores were in the range of 1.85-35.95. Two 4 millimeter punch biopsies were taken from each patient. Samples were obtained in a clinical trial setting at Stanford University Dermatology Department[37]. Autopsy donor materials were obtained from Zoion Diagnostics (Hawthorne, NY, USA).

RNA isolation and amplification

Total RNA was isolated from skin by pulverization of tissue in a dry ice encased metal mortal and pestle, followed by extraction using the RNeasy fibrous kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. Total RNA (5 μg) was subjected to treatment with DNase (Roche Molecular Biochemicals, Indianapolis, IN, USA) according to manufacturer's instructions to eliminate possible genomic DNA contamination. RNA quantity was assessed by OD at 260 nm and RNA quality was analyzed by measuring the ratio of 28S and 18S rRNA using the Agilent 2100 bioanalyzer (Agilent Technologies, Germany). DNase-treated total RNA was reverse-transcribed using Superscript II (Invitrogen) according to manufacturer's instructions.

Quantitative PCR

TaqMan primers and probes were designed with PrimerExpress software (ABI), and purchased from ABI (Applied Biosystems, Foster City, CA, USA). The PCR reactions were prepared using the components from the iScript Custom One-Step RT-PCR Kit with ROX and assembled according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). The fluorogenic probes were labeled with 6-carboxyfluorescein (6FAM) as the reporter and 6-carboxy-4,7,2,7'-tetramethylrhodamine (TAMRA) as a quencher. Real-time quantitative PCR was performed using either of two methods. In the first method, each 10 μl PCR reaction contained 20 ng of total RNA in a TAQMAN™ real-time quantitative PCR reaction on an ABI 7900 sequence detection system. The final concentrations of the primers in the PCR reactions were at 200 nM and the probe at

100 nM respectively. The RT-PCR reactions were performed in triplicate in a 384-well plate. An eukaryotic 18S rRNA endogenous control probe/primer set (ABI) was used as an internal control for RNA quality, and a primer/probe set for the CD4 promoter was used to check the RNAs for genomic DNA contamination. The PCR data was quantified based on a 12-point standard curve generated using 4-fold serial dilutions of a cDNA containing the gene of interest. The fourfold dilutions began at 20,000 fg. This procedure provides an absolute quantification of the amounts of CXCR3, CXCL9, CXCL10 and CXCL11 mRNA in a given sample.

In the second method, 10 ng of cDNA from each sample was used. Two unlabelled primers at either 400 nM or 900 nM each were used with 250 nM of FAM-labelled probe (Applied Biosystems, Foster City, CA, USA) in a TAQMAN™ real-time quantitative PCR reaction on an ABI 7000, 7300 or 7700 sequence detection system. Ubiquitin levels were measured in a separate reaction and used to normalize the data by the Δ-Δ cycle threshold (Ct) method. (Using the mean cycle threshold value for ubiquitin and the gene of interests for each sample, the equation 1.8 e (Ct ubiquitin minus Ct gene of interest) x 10^4 was used to obtain the normalized values.) Measurement of Ct values for ubiquitin was also used as a secondary measurement of RNA/cDNA quality and samples were deemed acceptable if they were at a Ct of 23 or less. High quality RNA generally leads to ubiquitin Ct values between 17 and 23 for 10 ng of input cDNA (McClanahan, unpublished data). The absence of genomic DNA contamination was confirmed using primers that recognize a region of genomic DNA. Samples with Ct values for genomic DNA of 35-40 were considered acceptable for analysis. The Δ-Δ Ct method described above results in normalized expression values relative to the housekeeping gene ubiquitin. Normalized values less than 1.0 are considered to be at the limit of detection for this method and were considered to be negative for analysis. Kruskal-Wallis statistical analysis was performed on log transformed data (median method).

Immunohistochemistry (IHC)

Acetone-fixed fresh frozen sections of skin biopsies at 5 μm thickness were incubated with primary antibodies for 1 hour at room temperature. Following washes, the bound primary antibodies were detected by incubation with either biotinylated donkey anti-rabbit or horse anti-mouse for 30 min followed by incubation with an alkaline phosphatase kit, Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA). Visualization of the IHC signal was accomplished by incubation with Liquid Permanent Red from Dako Cytomation (Carpinteria, CA, USA). After immunostaining tissue sections were counterstained with hematoxylin. Antibodies used were anti-CD8, anti-CD3, anti-CD68 (Dako Cytomation, Carpinteria, CA, USA), anti-CXCR3 (BD Biosciences, San Diego, CA, USA), Cytokeratin 16 (K16, Novacastra Lab., New Castle, UK), anti-BDCA-2 (Miltenyi Biotec, Auburn, CA, USA) and anti-CD123 (BioLegend, San Diego, CA, USA). Detection of anti-BDCA-2 was amplified using Tyramide Signal Amplification (TSA) kit from Perkin Elmer Life Sciences (Boston, MA, USA) according

to the manufacturer's directions. A total of 45 fresh frozen skin biopsies with 11 paired (lesional and non-lesional) tissue blocks were included in the final IHC analysis. Adjacent sections were used to compare the distribution patterns of T cells and $CXCR3^+$ cells.

The co-localization of cell type markers and CXCR3 was evaluated by double immunofluorescent (IFC) staining. Alexa Fluor 488 linked secondary antibodies, Alexa Fluor 488 linked streptavidin, Cy3 linked streptavidin and fluorescein (FITC)- or Cy3-linked TSA kits were used to detect the binding of primary antibodies. Sometimes both primary antibodies in the double staining were raised in the same species (such as CD8 vs. CXCR3 or CXCR3 vs. BDCA-2). In this situation, the first primary antibody was carefully titered to a concentration that would not be detected by the conditions used to detect the second primary antibody under the experimental condition. Detection of the binding of the first antibody was then amplified with a TSA kit while no TSA amplification was used to detect the binding of the second antibody. The reliability of this approach to avoid artificial co-localization generated from cross reactivity was evident by the co-existence of single and double positive signal of each marker, and was further confirmed by the lack of double positive cells when eliminating the second primary antibody in the staining protocol.

Quantification of IHC data

Single stained sections were randomly coded and analyzed by computer-assisted image analysis as described previously in detail[38]. Briefly, images were acquired and analyzed using Syndia algorithm on a Qwin based analysis system (Leica, Cambridge, U.K.). Five random, non-overlapping, high power fields (100X) per section were analyzed.

The percentage of $CXCR3^+$ T cells in the psoriatic lesions were analysed by double IFC staining and counted manually. Six random, non-overlapping, high power fields (200X) per section were counted twice. The sum of the total cell number was calculated for the epidermis and dermis compartments separately of the six fields and used to calculate the percentage of $CXCR3^+CD3^+$ in either the $CXCR3^+$ or $CD3^+$ cell populations.

RESULTS

Up-regulated expression of CXCR3 and its ligands in psoriatic lesional skin

To evaluate the expression of CXCR3 in the psoriatic lesions of moderate to severe psoriasis patients in comparison to non-lesional skin, the steady-state mRNA levels of CXCR3 and its ligands were analyzed by real-time RT-PCR. Thirty lesional and twelve non-lesional skin biopsies were included in the analysis. As shown in Figure 1, CXCR3 expression was significantly up-regulated in the lesions as compared to non-lesional biopsies. The same samples were also analyzed for the expression of its cognate

ligands, CXCL9, CXCL10 and CXCL11. A significant increase of the mRNA levels of all three ligands in psoriatic lesions was observed as compared to non-lesional samples (Figure 1).

In contrast to our findings, it was previously reported that the message levels of CXCR3 ligands were not significant upregulated in psoriatic lesional skin as compared to normal human skin or non-lesional skin[39]. To confirm our initial observation, a second panel of inflammatory skin biopsies which was obtained independently was analyzed for the expression of CXCL9 and CXCL10. This panel of samples includes skin biopsies from healthy donors and donors from patients with psoriasis and atopic dermatitis. As shown in Figure 2, significant increases in the expression of both ligands were observed in the lesional tissues from each patient population compared with normal skin or the corresponding non-lesional skin samples of each disease. In addition, the message levels of both ligands were also significantly elevated in non-lesional skin biopsies from both disease populations compared with normal skin.

Similar distribution patterns of T lymphocytes and CXCR3$^+$ cells in psoriatic skin

To qualify the lesional versus non-lesional biopsies for further analysis, the histopathology of the biopsies was first examined for epidermis morphology and

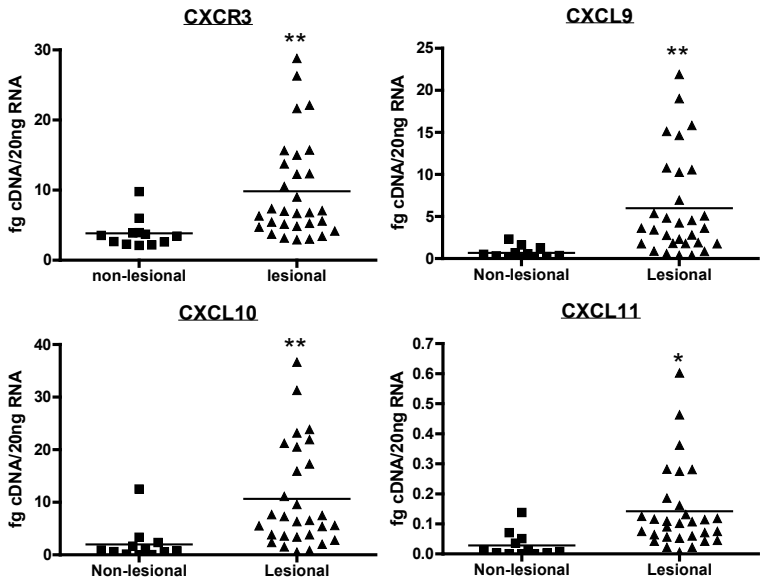

Figure 1 Real-time RT-PCR analysis of mRNA of CXCR3 and its ligands, CXCL9, CXCL10 and CXCL11, in psoriatic skin biopsies. The message levels of CXCR3 and its ligands from 12 non-lesional and 30 psoriatic lesional skin biopsies were evaluated by RT-PCR. **p< 0.01; *p< 0.05 versus non-lesional biopsies.

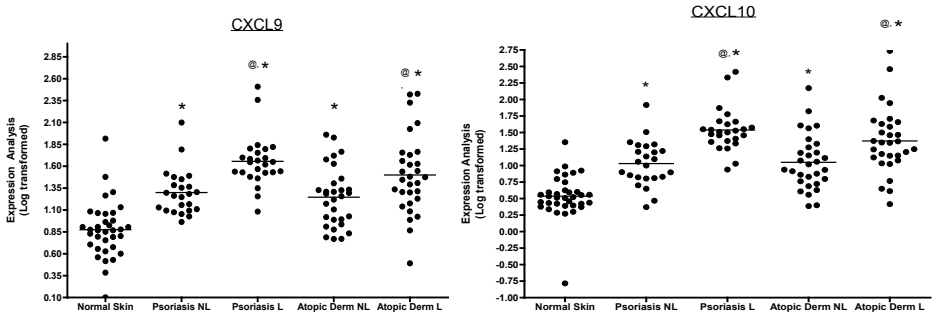

Figure 2 Real-time RT-PCR analysis of mRNA of CXCR3 ligands, CXCL9 and CXCL10, in psoriatic and atopic dermatitis skin biopsies. The message levels of CXCL9 and CXCL10 were evaluated by RT-PCR in 35 normal skin samples, 24 non-lesional psoriasis skin samples, 25 lesional psoriasis skin samples, 30 non-lesional atopic dermatitis skin samples and 30 lesional atopic dermatitis skin samples. CXCL9 and CXCL10 mRNA levels were normalized against the ubiquitin expression level and log transformed as described in Methods. *p< 0.01 versus normal skin; @p< 0.05 versus respective non-lesional biopsies.

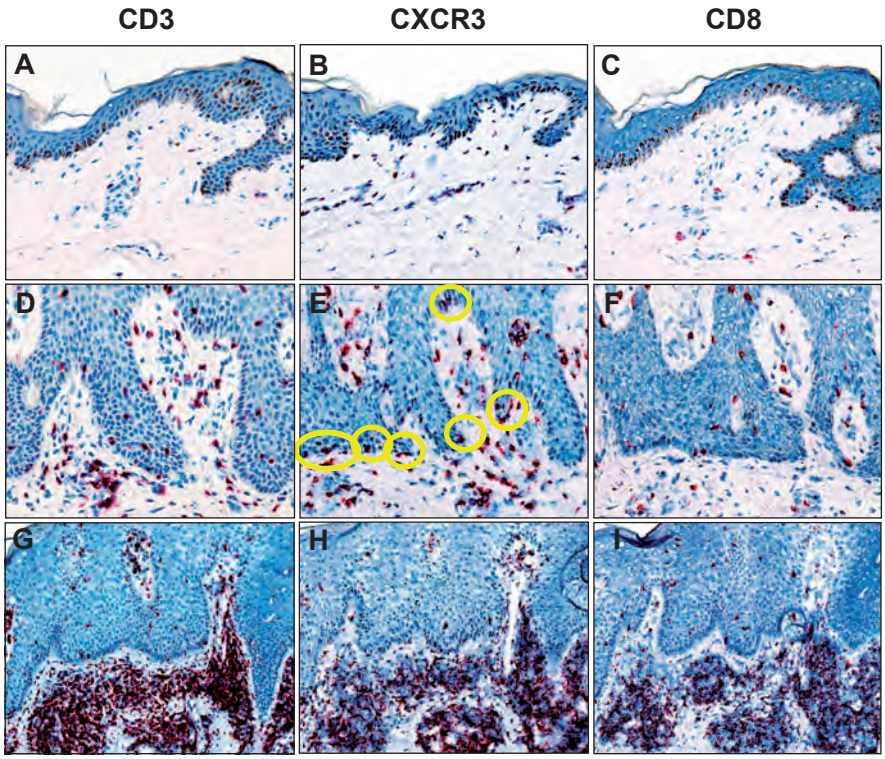

Figure 3 The distribution patterns of CD8+, CD3+, and CXCR3+ positive cells in psoriatic skin biopsies. Representative images of CD3 (A, D, G), CXCR3 (B, E, H) and CD8 (C, F, I) stained sections, showing the presence and distribution patterns of these cells in non-lesional skin biopsies (A-C) and psoriatic plaque lesions (D-I). All images are at the same magnification, 100x. Positively stained cells are in *red*. *Yellow arrowheads* in (E) highlight the CXCR3+ cells located at the basal keratinocyte layer.

thickness followed by staining with anti-cytokeratin 16 (K16) for the proliferation/ differentiation of keratinocytes. All non-lesional skin biopsies resembled normal skin histologically and were completely devoid of K16 staining, whereas all lesional tissue samples showed intense K16 staining (data not shown) and demonstrated histopathological characteristics of psoriasis, including rete elongation and thickening of epidermis (Figure 3). These observations are consistent with previous reports regarding the pattern of K16 expression[40].

Adjacent skin sections were used to compare the distribution pattern of $CXCR3^+$ cells to those of $CD3^+$ and $CD8^+$ T lymphocytes. Owing to the expression of CD4 by cell populations other than T lymphocytes[41], we excluded this cell marker in our analysis of T lymphocyte expression. In the non-lesional skin biopsies, small numbers of $CD3^+$ and $CD8^+$ T lymphocytes were detected and were mainly present in the dermis and occasionally in the epidermis (Figure 3). In psoriatic lesions, the majority of $CD3^+$ lymphocytes were found in the dermis, while $CD8^+$ cells appeared to be evenly distributed in the dermis and epidermis or slightly more prevalent in the epidermis. In most samples, the T lymphocytes had a scattered distribution, but in a small number of lesional biopsies, we observed large massive infiltrates of $CD3^+$ as well as $CD8^+$ T lymphocytes in the dermis (Figure 3G-I). The distribution pattern of dermal $CXCR3^+$ cells was similar to that of $CD3^+$ T cells (compare Figure 3E versus 3D; and 3H versus 3G) in the psoriatic lesions. There appeared to be fewer $CXCR3^+$ cells in the lesional epidermis as compared to those of $CD8^+$ and $CD3^+$ T lymphocytes (Figure 3D-F), indicating that the frequency of $CXCR3^+$ T cells in the epidermis is lower than in the dermis. Interestingly, the majority of epidermal $CXCR3^+$ cells in psoriatic lesions were frequently located at the basal keratinocyte layer as shown in Figure 3E.

Quantification of T lymphocytes and CXCR3+ cells by digital image analysis

To quantify the numbers of $CD3^+$, $CD8^+$, and $CXCR3^+$ cells in the skin biopsies, single positive cells were counted using a computer-aided image analysis program. Consistent with our visual observation, all three cell populations were significantly increased in both epidermis and dermis of lesional biopsies compared to the non-lesional samples (Table 1). Overall, there was a mean 6.9-fold increase of $CD3^+$ T lymphocytes in the lesional biopsies as compared to those in non-lesional tissue. The increase in CD3+ positive cells in lesional compared to non-lesional tissue was proportionally greater in the dermis than the epidermis (Table 2). The numbers of $CD8^+$ T lymphocytes also were increased in lesional compared to non-lesional tissue and they were more evenly distributed between epidermis and dermis in both lesional and non-lesional biopsies (Table 1 and 2). There was a very large fold increase in the numbers of epidermal $CXCR3^+$ cells in lesional compared to non-lesinal tissue (Table 1), and this large increase was heavily skewed towards the dermis over the epidermis (Table 2). These data indicate the selective accumulation of $CXCR3^+$ leukocytes associated with lesional tissues in psoriasis patients, especially in the dermis.

Table 1 Fold increase in cell numbers in the lesional skin biopsies

	CD3e[a]	CD3d[b]	CD8e	CD8d	CXCR3e	CXCR3d
Folds (L / NL)	4.4	8.6	3.1	3.4	15.4	6.8

The difference in cell numbers between NL and L is statistically significant for each cell marker. L, lesional skin biopsies; NL, non-lesional skin biopsies; [a], e stands for epidermis; [b], d stands for dermis.

Table 2 Fold increase of each cell population in dermis as compared to epidermis

	CD3 (NL)	CD3 (L)	CD8 (NL)	CD8 (L)	CXCR3 (NL)	CXCR3 (L)
Folds (dermis / epidermis)	1.5	2.8	1.4	1.6	21.4	9.5

The difference in cell numbers between dermis and epidermis is statistically significant for each cell marker in lesional skin samples and CXCR3 in non-lesional skin samples. L, lesional skin biopsy; NL, non-lesional skin biopsy.

Double immunofluorescent staining of cellular infiltrates in the lesional plaques

To identify the subtypes of $CXCR3^+$ cells in the psoriatic lesions, we performed double immunofluorescent staining with cell surface markers in combination with anti-CXCR3 antibody. As shown in Figure 4A, the majority of $CXCR3^+$ cells are clearly $CD3^+$ T lymphocytes. The proportion of $CXCR3^+$ T cells in the lesional biopsies was further quantified by manual counting of five subjects. Our analysis indicated that 71.3 ± 2.2% of $CXCR3^+$ cells co-stain as $CD3^+$ T lymphocytes, while 54.3 ± 3.7 % of $CD3^+$ T cells co-express CXCR3. Interestingly, while the percentage of $CXCR3^+$ T cells in the $CXCR3^+$ cell population remains constant in both dermis (71.4% ± 2.1%) and epidermis (71.1% ± 3.6%) of the psoriatic lesions, the frequency of epidermal $CXCR3^+$ T lymphocytes (30.7 ± 5.2%) was significantly lower that of the dermis (59.5 ± 4.5%, $p<0.003$). Among the $CXCR3^+$ T lymphocytes in the lesional skin, a subset of these also expresses the CD8 marker (Figure 4B). $CD8^+CXCR3^+$ T cells were also found in the epidermis of psoriatic lesions albeit at a much lower frequency than $CD3^+CXCR3^+$.

To further identify additional subsets of $CXCR3^+$ cells, we investigated the co-expression of the myeloid marker CD68 and the pDC marker BDCA-2. Although high numbers of $CD68^+$ cells were present in lesional skin, only a small percentage of these were $CXCR3^+$ (Figure 4C). We next examined the expression of CXCR3 by pDCs and found that nearly all BDCA-2^+ cells were $CXCR3^+$. In addition, the pDCs appeared to express the highest levels of this receptor (Figure 4D). To ensure that this observation did not result from a technical artifact, we validated the identity of the pDCs and the expression patterns of BDCA-2 and CXCR3 by analyzing the adjacent sections for the expression of CD3 versus BDCA-2 and CXCR3 versus CD3. As shown on

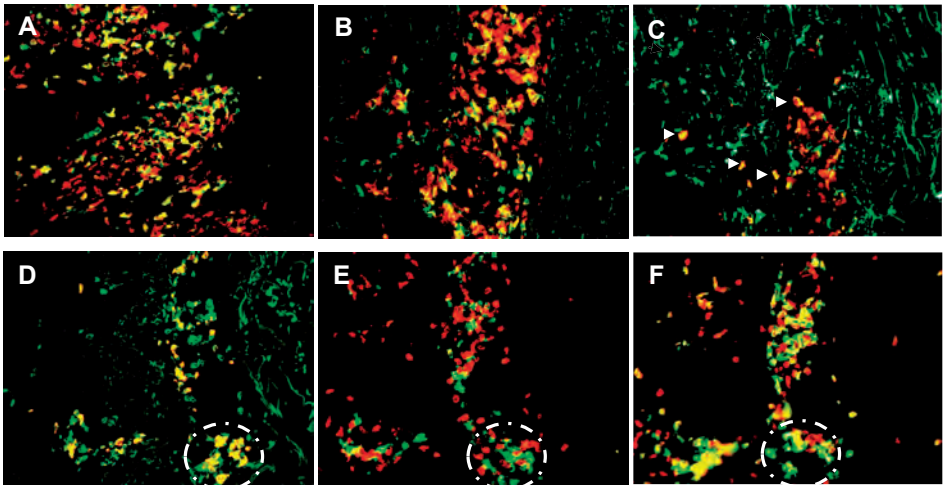

Figure 4 Expression of CXCR3 by $CD3^+$, $CD8^+$ T lymphocytes, $CD68^+$ myeloid cells and BDCA-2^+ pDCs in psoriatic lesional skin. Representative double-stained sections showing expression of CXCR3 by $CD3^+$ T lymphocytes (A, F), $CD8^+$ T lymphocytes (B), $CD68^+$ myeloid cells (C) and BDCA-2^+ pDCs (D). (A), CD3, *red*, CXCR3, *green*; (B), CXCR3, *red*, CD8, *green*; (C), CXCR3, *red*, CD68, *green*; (D), BDCA-2, *red*, CXCR3, *green*; (E), CD3, *red*, BDCA-2, *green*; (F), CD3, *red*, CXCR3, *green*. Double-positive cells are revealed as *yellow*. All images are at the same magnification, 100x. *White arrowheads* in (C) point to CD68/CXCR3 double-positive cells. (D-F), *white circles* illustrate that the BDCA-2/CXCR3 double-positive cells in (D) are not $CD3^+$ T lymphocytes (E) and neither are they CD3/CXCR3 double-positive (F).

Figure 4D – 4F, the $CXCR3^+BDCA\text{-}2^+$ cells were in fact $CD3^-$. The identity of $CXCR3^+$ pDCs was also confirmed by their expression of CD123 (data not shown). Thus, both T lymphocytes and pDCs appear to be the predominant infiltrating leukocyte populations expressing CXCR3 in human psoriatic lesions.

DISCUSSION

CXCR3 has been suggested to be one of the major chemokine receptors responsible for the trafficking of T lymphocytes to the psoriatic dermis[30]. Intraepidermal T lymphocytes that expressed cutaneous lymphocyte antigen (CLA) and $\alpha E\beta 7$ integrin have been demonstrated to selectively express CXCR3 and these data suggest that CXCR3 may also be responsible for T cell homing to the psoriatic epidermis[30]. However, a thorough analysis of CXCR3 expression in human psoriatic lesions has been lacking, which has limited the understanding of the role of this receptor in the etiology of this disease. In this study, we have performed a systematic analysis of the expression and pattern of CXCR3 in these tissues, and the identification of the cell types that express this receptor. We have demonstrated a significant increase of mRNA levels of CXCR3 and its cognate ligands in the psoriatic lesions as compared to non-lesional

skin from the same patients. In addition, using a distinct panel of biopsy samples, we have confirmed our observation for CXCL9 and CXCL10 expression and extended the study to include another inflammatory skin disease, atopic dermatitis. Our data contradicts a previous report by Meller et al.[39], which showed a lack of significant difference in the levels of these chemokines by RT-PCR between normal skin and the lesional skin biopsies from psoriatic or atopic dermatitis patients. Transcript levels of CXCR3 ligands in psoriatic lesions were relatively low and within similar ranges in both studies (see Figure 1 in this report and Figure 2 in[39]). Only patients with moderate to severe psoriasis were included in our study. It is likely that the difference between lesional skin and normal skin could be obscured if skin samples from milder disease or a wider range of disease scores were included in a study. Furthermore, differences in current or prior use of medications by the patient populations included in a study could also contribute to the variations in experimental results. The low expression levels of CXCR3 ligands detected in our study is consistent with previous observations made with in situ hybridization[35], microarray analysis or immunohistochemistry[34]. In spite of the low message levels observed a significant increase of CXCL9 protein in lesional skin from psoriasis and atopic dermatitis patients compared to normal skin has been reported[34]. Given the high affinity of these ligands to CXCR3[42], a relatively small but significant increase in their levels can still be envisioned to play a role in the etiology of the disease.

The elevation of CXCR3 messages in psoriatic lesions correlates with the marked increase in the number of $CXCR3^+$ leukocytes observed *in situ*. Most of the $CXCR3^+$ cells are located in the dermis of both non-lesional skin and psoriatic lesions. More than 50% of $CD3^+$ T lymphocytes present in lesional biopsies were $CXCR3^+$ and a subset of these $CXCR3^+$ T lymphocytes belonged to the CD8 subpopulation. In the epidermis, we also observed $CXCR3^+CD8^+$ T cells but their absolute numbers were relatively small. The numbers of $CXCR3^+$ cells were dramatically increased in both the epidermis (15-fold) and dermis (7-fold) of lesional tissues as compared to paired non-lesional samples. Interestingly, we also confirmed the prior observation that epidermal $CXCR3^+$ cells are located in the basal keratinocyte layer[30]. Based on these findings, our data support and extend the hypothesis that CXCR3 likely plays an important role in the recruitment of T lymphocytes into the dermis of psoriatic lesions. It is intriguing that despite high levels of CXCL9 and CXCL10 shown to be expressed by psoriatic keratinocytes[30;36] and the large numbers of $CXCR3^+$ T lymphocytes present in the lesional dermis, there was only a low frequency of T cells in the epidermis which expressed CXCR3 by our analysis. This observation suggests that this chemokine receptor may not be essential for the migration of T cells into the epidermis in psoriatic tissues and that the T lymphocytes that migrated into the epidermis were predominantly $CXCR3^-$ cells. Thus, other mechanisms, such as those involving $\alpha 1\beta 1$ integrin, may be more integral for the accumulation of epidermal T cells and the development of psoriasis[43]. Alternatively, we cannot rule out the possibility that the apparent low numbers of $CXCR3^+$ cells in the epidermis may have resulted from the internalization of this receptor following exposure to high levels of its cognate

ligands present in the tissues as the cells migrated into this anatomical site, as has been previously reported in the case of the asthmatic lung[44;45]. This possibility may also account for the predominant localization of epidermal CXCR3$^+$ T cells detected at the basal layer of the lesional epidermis.

In addition to T cells, we found that nearly all BDCA-2$^+$ pDCs express high levels of CXCR3. Consistent with previous reports we also observed higher numbers of pDCs in biopsies taken from lesional skin compared to non-lesional skin. It is relevant that pDCs are believed to be the major type I IFN-α producing cells in vivo in response to viral and certain microbial infections, and that accumulating data suggest that pDCs and type I IFN pathways play an integral role in the early events of the immunopathogenesis of psoriasis. For example, locally activated IFN-α signaling pathway has been reported in psoriatic skins with no alteration in IFN-α sensitivity[46;47]. Psoriatic T cells have increased sensitivity to IFN-α which resulted in prolonged activation of several STAT transcription factors and increased IFN-γ production[48], a cytokine known to induce the expression of CXCR3 ligands. In a xenograft model in AKG-129 mice, blocking IFN-α signaling or inhibiting the ability of pDCs to produce IFN-α prevented the T cell-dependent development of psoriasis[17]. Enhanced type I interferon signaling and recruitment of CXCR3-expressing cells into the skin has been demonstrated following treatment with the TLR7-agonist imiquimod[32]. CXCR3 ligands have been shown to cooperate with CXCL12 to induce migration of pDC[28]. CXCR3-dependent pDC migration had also been shown in response to immobilized CXCR3 ligands[24]. Very recently another chemokine, chemerin, has been described to play a critical role for the recruitment of pDC into psoriatic lesion [49] and its combination with CXCL10 and CXCL12 leads to a significantly higher migratory response of this cell type than the response to each individual chemokine[50]. Based on the data described here, along with the putative collaborative role of these three distinct molecules in mediating pDC migration, it is conceivable that interfering with one arm of this process, such as selective CXCR3 antagonism, could directly impact pDC migration into the skin of psoriatic patients. Together with its role in the trafficking of activated T lymphocytes into the dermis of psoriatic skin, CXCR3 represents a highly attractive target for the treatment of this disease.

Interestingly, a selective CXCR3 antagonist, T0906487, was found to be ineffective in a 28 day clinical psoriasis trial[51]. Pharmacodynamics responses were highly variable in all treatment groups in this study, and this was suggested to be due to the highly variable pharmacokinetics. The possibility also exists that the timeframe of this study was insufficient to optimally inhibit T cell-dependent activity in this disease, and that a longer (eg. 12 weeks study) may be preferable. Based on our observation that more than 40% of the T cells in the psoriatic lesions are CXCR3$^-$, the potential for redundancies among chemokine-dependent pathways in this disease may also be considered. With other CXCR3 antagonists currently in development[52;53] the therapeutic value of targeting CXCR3 in psoriasis will likely be conclusively defined with these emerging reagents.

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REFERENCE LIST

1. Bjerke JR, Krogh HK, Matre R. Characterization of mononuclear cell infiltrates in psoriatic lesions. *J Invest Dermatol* 1978; **71**: 340-3.
2. Ellis CN, Fradin MS, Messana JM *et al*. Cyclosporine for plaque-type psoriasis. Results of a multidose, double-blind trial. *N Engl J Med* 1991; **324**: 277-84.
3. Gottlieb SL, Gilleaudeau P, Johnson R *et al*. Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nat Med* 1995; **1**: 442-7.
4. Gottlieb AB. Psoriasis: emerging therapeutic strategies. *Nat Rev Drug Discov* 2005; **4**: 19-34.
5. Gilhar A, David M, Ullmann Y *et al*. T-lymphocyte dependence of psoriatic pathology in human psoriatic skin grafted to SCID mice. *J Invest Dermatol* 1997; **109**: 283-8.
6. Wrone-Smith T, Nickoloff BJ. Dermal injection of immunocytes induces psoriasis. *J Clin Invest* 1996; **98**: 1878-87.
7. Boyman O, Hefti HP, Conrad C *et al*. Spontaneous development of psoriasis in a new animal model shows an essential role for resident T cells and tumor necrosis factor-alpha. *J Exp Med* 2004; **199**: 731-6.
8. Nickoloff BJ, Wrone-Smith T. Injection of pre-psoriatic skin with CD4+ T cells induces psoriasis. *Am J Pathol* 1999; **155**: 145-58.
9. Bovenschen HJ, Seyger MM, Van de Kerkhof PC. Plaque psoriasis vs. atopic dermatitis and lichen planus: a comparison for lesional T-cell subsets, epidermal proliferation and differentiation. *Br J Dermatol* 2005; **153**: 72-8.
10. Langrish CL, Chen Y, Blumenschein WM *et al*. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005; **201**: 233-40.
11. Li J, Li D, Tan Z. The expression of interleukin-17, interferon-gamma, and macrophage inflammatory protein-3 alpha mRNA in patients with psoriasis vulgaris. *J Huazhong Univ Sci Technolog Med Sci* 2004; **24**: 294-6.
12. Lowes MA, Chamian F, Abello MV *et al*. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci U S A* 2005; **102**: 19057-62.
13. Prinz JC, Gross B, Vollmer S *et al*. T cell clones from psoriasis skin lesions can promote keratinocyte proliferation in vitro via secreted products. *Eur J Immunol* 1994; **24**: 593-8.
14. Teunissen MB, Koomen CW, de Waal MR *et al*. Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J Invest Dermatol* 1998; **111**: 645-9.
15. Oppmann B, Lesley R, Blom B *et al*. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 2000; **13**: 715-25.
16. Piskin G, Sylva-Steenland RM, Bos JD *et al*. In vitro and in situ expression of IL-23 by keratinocytes in healthy

skin and psoriasis lesions: enhanced expression in psoriatic skin. *J Immunol* 2006; **176**: 1908-15.

17. Nestle FO, Conrad C, Tun-Kyi A *et al*. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* 2005; **202**: 135-43.
18. Gilliet M, Conrad C, Geiges M *et al*. Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. *Arch Dermatol* 2004; **140**: 1490-5.
19. Lande R, Gregorio J, Facchinetti V *et al*. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* 2007; **449**: 564-9.
20. Yamamoto J, Adachi Y, Onoue Y *et al*. Differential expression of the chemokine receptors by the Th1- and Th2-type effector populations within circulating CD4+ T cells. *J Leukoc Biol* 2000; **68**: 568-74.
21. Kunkel EJ, Boisvert J, Murphy K *et al*. Expression of the chemokine receptors CCR4, CCR5, and CXCR3 by human tissue-infiltrating lymphocytes. *Am J Pathol* 2002; **160**: 347-55.
22. Garcia-Lopez MA, Sanchez-Madrid F, Rodriguez-Frade JM *et al*. CXCR3 chemokine receptor distribution in normal and inflamed tissues: expression on activated lymphocytes, endothelial cells, and dendritic cells. *Lab Invest* 2001; **81**: 409-18.
23. Jones D, Benjamin RJ, Shahsafaei A *et al*. The chemokine receptor CXCR3 is expressed in a subset of B-cell lymphomas and is a marker of B-cell chronic lymphocytic leukemia. *Blood* 2000; **95**: 627-32.
24. Kohrgruber N, Groger M, Meraner P *et al*. Plasmacytoid dendritic cell recruitment by immobilized CXCR3 ligands. *J Immunol* 2004; **173**: 6592-602.
25. Muehlinghaus G, Cigliano L, Huehn S *et al*. Regulation of CXCR3 and CXCR4 expression during terminal differentiation of memory B cells into plasma cells. *Blood* 2005; **105**: 3965-71.
26. Ocana E, Delgado-Perez L, Campos-Caro A *et al*. The prognostic role of CXCR3 expression by chronic lymphocytic leukemia B cells. *Haematologica* 2007; **92**: 349-56.
27. Qin S, Rottman JB, Myers P *et al*. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J Clin Invest* 1998; **101**: 746-54.
28. Vanbervliet B, Bendriss-Vermare N, Massacrier C *et al*. The inducible CXCR3 ligands control plasmacytoid dendritic cell responsiveness to the constitutive chemokine stromal cell-derived factor 1 (SDF-1)/CXCL12. *J Exp Med* 2003; **198**: 823-30.
29. Iijima W, Ohtani H, Nakayama T *et al*. Infiltrating CD8+ T cells in oral lichen planus predominantly express CCR5 and CXCR3 and carry respective chemokine ligands RANTES/CCL5 and IP-10/CXCL10 in their cytolytic granules: a potential self-recruiting mechanism. *Am J Pathol* 2003; **163**: 261-8.
30. Rottman JB, Smith TL, Ganley KG *et al*. Potential role of the chemokine receptors CXCR3, CCR4, and the integrin alphaEbeta7 in the pathogenesis of psoriasis vulgaris. *Lab Invest* 2001; **81**: 335-47.
31. Teraki Y, Miyake A, Takebayashi R *et al*. Homing receptor and chemokine receptor on intraepidermal T cells in psoriasis vulgaris. *Clin Exp Dermatol* 2004; **29**: 658-63.
32. Wenzel J, Uerlich M, Haller O *et al*. Enhanced type I interferon signaling and recruitment of chemokine receptor CXCR3-expressing lymphocytes into the skin following treatment with the TLR7-agonist imiquimod. *J Cutan Pathol* 2005; **32**: 257-62.
33. Wenzel J, Wiechert A, Merkel C *et al*. IP10/CXCL10-CXCR3 interaction: a potential self-recruiting mechanism for cytotoxic lymphocytes in lichen sclerosus atrophicans. *Acta Derm Venereol* 2007; **87**: 112-7.
34. Wenzel J, Peters B, Zahn S *et al*. Gene expression profiling of lichen planus

reflects CXCL9+-mediated inflammation and distinguishes this disease from atopic dermatitis and psoriasis. *J Invest Dermatol* 2008; **128**: 67-78.

35. Flier J, Boorsma DM, van Beek PJ *et al*. Differential expression of CXCR3 targeting chemokines CXCL10, CXCL9, and CXCL11 in different types of skin inflammation. *J Pathol* 2001; **194**: 398-405.
36. Gottlieb AB, Luster AD, Posnett DN *et al*. Detection of a gamma interferon-induced protein IP-10 in psoriatic plaques. *J Exp Med* 1988; **168**: 941-8.
37. Chan JR, Blumenschein W, Murphy E *et al*. IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. *J Exp Med* 2006; **203**: 2577-87.
38. Goedkoop AY, de Rie MA, Teunissen MB *et al*. Digital image analysis for the evaluation of the inflammatory infiltrate in psoriasis. *Arch Dermatol Res* 2005; **297**: 51-9.
39. Meller S, Winterberg F, Gilliet M *et al*. Ultraviolet radiation-induced injury, chemokines, and leukocyte recruitment: An amplification cycle triggering cutaneous lupus erythematosus. *Arthritis Rheum* 2005; **52**: 1504-16.
40. Leigh IM, Navsaria H, Purkis PE *et al*. Keratins (K16 and K17) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro. *Br J Dermatol* 1995; **133**: 501-11.
41. Kazazi F, Mathijs JM, Foley P *et al*. Variations in CD4 expression by human monocytes and macrophages and their relationships to infection with the human immunodeficiency virus. *J Gen Virol* 1989; **70 (Pt 10)**: 2661-72.
42. Cox MA, Jenh CH, Gonsiorek W *et al*. Human interferon-inducible 10-kDa protein and human interferon-inducible T cell alpha chemoattractant are allotopic ligands for human CXCR3: differential binding to receptor states. *Mol Pharmacol* 2001; **59**: 707-15.
43. Conrad C, Boyman O, Tonel G *et al*. Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nat Med* 2007; **13**: 836-42.
44. Kallinich T, Schmidt S, Hamelmann E *et al*. Chemokine-receptor expression on T cells in lung compartments of challenged asthmatic patients. *Clin Exp Allergy* 2005; **35**: 26-33.
45. Thomas SY, Banerji A, Medoff BD *et al*. Multiple chemokine receptors, including CCR6 and CXCR3, regulate antigen-induced T cell homing to the human asthmatic airway. *J Immunol* 2007; **179**: 1901-12.
46. Schmid P, Itin P, Cox D *et al*. The type I interferon system is locally activated in psoriatic lesions. *J Interferon Res* 1994; **14**: 229-34.
47. van der Fits L, van der Wel LI, Laman JD *et al*. In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-alpha sensitivity is unaltered. *J Invest Dermatol* 2004; **122**: 51-60.
48. Eriksen KW, Lovato P, Skov L *et al*. Increased Sensitivity to Interferon-alpha in Psoriatic T Cells. *J Invest Dermatol* 2005; **125**: 936-44.
49. Albanesi C, Scarponi C, Pallotta S *et al*. Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment. *J Exp Med* 2009; **206**: 249-58.
50. Skrzeczynska-Moncznik J, Wawro K, Stefanska A *et al*. Potential role of chemerin in recruitment of plasmacytoid dendritic cells to diseased skin. *Biochem Biophys Res Commun* 2009; **380**: 323-7.
51. Berry K, Friedrich M, Kersey K *et al*. Evaluation of T0906487, a CXCR3 antagonist, in a phase 2 psoriasis trial. In: Inflammation research association annual meeting 2004.
52. Horuk R. Chemokine receptor antagonists: overcoming developmental hurdles. *Nat Rev Drug Discov* 2009; **8**: 23-33.
53. Wijtmans M, Verzijl D, Leurs R *et al*. Towards small-molecule CXCR3 ligands with clinical potential. *Chem Med Chem* 2008; **3**: 861-72.

8

SUMMARY AND CONCLUSIONS
SAMENVATTING EN CONCLUSIES

SUMMARY OF CHAPTERS

The first part of this thesis considers the clinical effects and/or the effects on different leukocyte subsets *in situ* of several registered biological treatments for psoriasis. The last two chapters consider possible treatment targets for psoriasis patients.

Chapter 1 is a general introduction on psoriasis and describes the lines of knowledge on its pathogenesis. The development of treatments for psoriasis is summarized, which almost paralleled the increasing knowledge of this skin disease.

In **Chapter 2a** a case-report on a clinical side-effect is described of a psoriasis patient treated with efalizumab. **Chapter 2b** is a written response to a comment made in the discussion of another case-report, which referred to the case-report mentioned in 2a.

When etanercept and efalizumab became available for routine use in severe psoriasis in the Netherlands, we hypothesized that efficacy results would be less than those obtained in published phase 2 and 3 studies because resistance to all conventional therapies as a reimbursement condition would select for more resistant cases and inclusion would be more restricted to severe cases (higher PASI). Furthermore, we hypothesized that efficacy would be less in obese patients due to the possible role of adipose tissue in TNF-α homeostasis. In Chapter 3 we present the efficacy and safety results of a retrospective analysis of 50 patients treated with etanercept 25 or 50 mg twice weekly, and we compared it with data from published trials. Additionally we related the clinical effect to the Body Mass Index, for adipose tissue is thought to have a possible role in TNF-α homeostasis. Based on the literature 30% and 49% of the patients treated with respectively twice weekly 25 mg and 50 mg should have achieved PASI 75. PASI 90 should have been achieved in 10% and 21% in patients treated with respectively twice weekly 25 mg and 50 mg. Our data showed that 15,4% in the 2 x25 mg group and 17% in the 2x50 mg group achieved PASI 75. PASI 90 was only attained in 7.7% in patients treated with 25 mg and 11.1% after treatment with 50 mg. In contrary to our hypothesis the mean initial PASI was comparable to the mean PASI mentioned in the phase 2 and 3 clinical trials. In contrast to earlier reports on etanercept, fatigue was a often mentioned side-effect. High Body Mass Index, indicating overweight or obesity, was found both in patients with little efficacy and in patients achieving PASI 75 or better. We concluded that use of etanercept in real practice gives impressive results, but these are generally less favorable as those published in clinical trial reports. This is probably due to the stringent conditions for reimbursement, which select for more treatment-resistant patients. Fatigue as side-effect of etanercept should be an issue for further investigation. Finally, the Body Mass Index does not seem to influence the patients' response to etanercept, although further investigations would be needed to confirm this.

To investigate whether specific markers for innate immunity would diminish with successful treatment in psoriasis, we analyzed lesional and non-lesional skin biopsies taken from 6 patients with moderate to severe psoriasis during 12 weeks of treatment with etanercept in correlation with the clinical response, as described in Chapter 4.

In the clinical responders (PASI reduction > 50%), all markers (CD3, CD68, CD161, elastase, BDCA-2, TNF-α) showed a decline during treatment, indicating a pivotal role for innate immunity in the pathogenesis of psoriasis.

In **Chapter 5a** we describe the results of a prospective, randomized, placebo-controlled study in which we determined which of the changes of several immunological markers in psoriatic skin correlates best with clinical response associated with effective therapy (adalimumab). Therefore, twenty-four active PsA patients were randomized to receive adalimumab (n=12) or placebo (n=12) for 4 weeks. We obtained lesional and non-lesional skin biopsies before and after 4 weeks of treatment from 22 patients with active skin lesions. Immunohistochemical analysis was performed to characterize several markers of innate immunity (CD68, CD161, elastase, TNF-α, BDCA-2) and T cells (CD3). A significant effect of treatment was observed on lesional dermal $CD161^+$ and $elastase^+$ cells. We concluded that adalimumab therapy in psoriasis lesions in psoriatic arthritis patients is associated with a reduction of dermal $CD161^+$ and $elastase^+$ cells, suggesting that these parameters could be used as biomarkers that are sensitive to change after active treatment in small proof of concept studies.

The results of this study in synovial tissue are described **Chapter 5b**. Immunohistochemical analysis was performed to characterise the cell infiltrate, expression of cytokines, MMPs and vascularity. Many cell types were reduced after adalimumab treatment compared to placebo. A significant effect of treatment was observed on $CD3^+$ cells (T cells), and on the expression of MMP13; both were significantly reduced after active treatment. This suggest that these parameters, a reduction in synovial expression of CD3 and MMP13 following treatment, could be used as synovial biomarkers that are sensitive to change after active treatment in psoriatic arthritis.

In **Chapter 6** we describe the expression of the chemokine receptor CCR5 and its ligands in lesional and non-lesional psoriatic skin. In addition, we present the clinical and immunohistochemical results of a randomized placebo controlled trial with a CCR5 inhibitor.

Immunohistochemical analysis showed low but significant increased total numbers of $CCR5^+$ cells in epidermis and dermis of lesional skin in comparison to non-lesional skin. However, relative expression of CCR5 proportional to the cells observed revealed that the difference between lesional and non-lesional skin was only statistically significant in the epidermis for $CD3^+$ cells and in the dermis for $CD68^+$ cells. Quantification of mRNA by reverse transcriptase-polymerase chain reaction (RT-PCR) only showed an increased expression of CCL5 (RANTES) in lesional skin. A randomized placebo-controlled clinical trial in 32 psoriasis patients revealed no significant clinical effect and no changes at the immunohistochemical level comparing patients treated with placebo or a CCR5 inhibitor SCH351125. We concluded that although CCR5 expression is increased in psoriatic lesions, this receptor does not play a crucial role in the pathogenesis of psoriasis.

In **Chapter 7** we describe the expression of CXCR3 and its ligands in lesional and non-lesional psoriatic skin. RT-PCR showed that the mRNA levels of CXCR3

and its ligands, CXCL9-11, were elevated in psoriatic lesions, as compared to non-lesional samples. Serial cryostat sections of psoriasis skin biopsies were evaluated by immunohistochemistry. The number of $CXCR3^+$ cells was low in nonlesional tissues. Quantitative image analysis demonstrated significant increases in the number of both epidermal and dermal $CXCR3^+$ cells in lesional compared to non-lesional biopsies. The majority of $CXCR3^+$ cells were located in the dermis of the lesional skin and 74% were demonstrated to be $CD3^+$ T lymphocytes. A small number of $CXCR3^+$ cells were $CD68^+$ myeloid cells. In addition, we found that nearly all BDCA-2^+ plasmacytoid dendritic cells in the psoriatic biopsies were $CXCR3^+$. These findings support and extend prior reports suggesting the potential role for CXCR3 in the pathophysiology of plaque psoriasis, by mediating the recruitment of plasmacytoid dendritic cells and T cells into the developing lesions.

SAMENVATTING VAN DE HOOFDSTUKKEN

Het eerste gedeelte van dit proefschrift behandelt de klinische effecten en/of de *in situ* effecten op verschillende leukocyten subsets van verschillende geregistreerde behandelingen met biologicals van psoriasis patiënten. De laatste twee hoofdstukken gaan over mogelijke aangrijpingspunten voor nieuwe behandelingen van psoriasis patiënten.

Hoofdstuk 1 is een algemene introductie en beschrijft de ontwikkeling van nieuwe inzichten in de pathogenese van psoriasis. Daarnaast wordt een samenvatting gegeven van de ontwikkeling van de verschillende nieuwe behandelingen van psoriasis, die bijna parallel loopt met de toename van kennis over psoriasis.

In **Hoofdstuk 2a** wordt een case report beschreven van een psoriasis patiënt die behandeld werd met efalizumab. **Hoofdstuk 2b** betreft een schriftelijke reactie op commentaar dat was ontstaan mede naar aanleiding van publicatie van dit case report van hoofdstuk 2a.

Toen etanercept en efalizumab in Nederland beschikbaar kwamen voor reguliere behandeling van patiënten met psoriasis, werd verondersteld dat de werkzaamheid in de dagelijkse praktijk minder zou zijn dan die van de gepubliceerde fase 2 en 3 studies. Deze gedachte was gestoeld op het feit dat als gevolg van de strikte vergoedingscriteria die worden gehanteerd, in de dagelijkse praktijk patiënten met ernstigere en meer therapie-resistente psoriasis zouden worden behandeld, dan in de registratie studies. Verder werd verondersteld dat de werkzaamheid in obese patiënten minder zou zijn, vanwege de mogelijke rol van vetweefsel in de homeostase van TNF-α. In **Hoofdstuk 3** beschrijven we de resultaten betreffende werkzaamheid en veiligheid van een retrospectieve analyse van 50 patiënten die werden behandeld met tweemaal per week 25 of 50 mg etanercept. Deze data zijn vergeleken met de resultaten van gepubliceerde klinische trials. Daarnaast hebben we het klinische effect afgezet tegen de Body Mass Index, aangezien gedacht wordt dat vetweefsel een mogelijke rol speelt in de homeostase van TNF-α. Volgens de gepubliceerde data van de klinische trials zou 30% en 49% van de patiënten die respectievelijk met tweemaal per week 25 en 50 mg etanercept behandeld wordt een verbetering van de PASI van 75% moeten hebben. Een verbetering van 90% van de PASI zou bereikt moeten worden in 10% en 21 % van de patiënten die respectievelijk met tweemaal per week 25 en 50 mg etanercept behandeld wordt. Onze data toonde dat 15.4% in de tweemaal per week 25 mg groep en 17% in de tweemaal per week 50 mg groep een verbetering van de PASI van 75% behaalde. Een verbetering van 90% van de PASI werd slechts door 7.7% in de groep behandeld met 25 mg behaald en door 11.1% in de groep behandeld met 50 mg. In tegenstelling tot onze hypothese, was de gemiddelde PASI op baseline vergelijkbaar aan de baseline PASI in de fase 2 en 3 klinische trials. Moeheid was een vaak gemelde bijwerking, hoewel deze niet als bijwerking in de eerdere studies werd genoemd. Een hoge Body Mass Index, wat overgewicht of obesitas aangeeft, werd zowel gezien bij patiënten met weinig effect van etanercept, als in patiënten die 75% of meer verbetering van de PASI lieten zien.

De conclusie van onze analyse is dat behandeling met etanercept in de dagelijkse praktijk indrukwekkende resultaten geeft, maar dat deze resultaten over het algemeen iets minder zijn, dan die van de gepubliceerde klinische trials. De oorzaak hiervoor is waarschijnlijk het hanteren van strikte vergoedingscriteria, die ervoor zorgen dat er therapie-resistentere patiënten mee worden behandeld. Moeheid als bijwerking van etanercept zou een onderwerp van verder onderzoek moeten zijn. Tenslotte lijkt de Body Mass Index geen invloed te hebben op de werkzaamheid van etanercept, hoewel verder onderzoek hiernaar nodig is om dit te bevestigen.

Om te onderzoeken of specifieke markers van de aangeboren immuniteit af zouden nemen door een effectieve behandeling van psoriasis, analyseerden we lesionale en niet-lesionale huidbiopten, die afgenomen waren van 6 patiënten met matig tot ernstige psoriasis die 12 weken behandeld waren met etanercept en vergeleken de uitkomsten met de klinische respons, zoals beschreven in **Hoofdstuk 4**. In de klinische responders (verbetering van PASI met > 50 %), toonden alle markers (CD3, CD68, CD161, elastase, BDCA-2, TNF-α) een daling tijdens de behandeling, wat wijst op een cruciale rol voor de aangeboren immuniteit in de pathogenese van psoriasis.

In **Hoofdstuk 5a** beschijven we de resultaten van een prospectieve, gerandomiseerde, placebo-gecontroleerde studie, waarin we hebben gekeken welke van de veranderingen van verschillende immunologische markers in psoriasis huid het beste correleert met een klinische respons op behandeling met adalimumab. Vierentwintig patiënten met actieve artritis psoriatica werden gerandomiseerd naar een behandeling met adalimumab (n=12) of placebo (n=12) voor de duur van 4 weken.. We namen lesionale en niet-lesionale biopten af op baseline en na 4 weken behandeling. Immunohistochemische analyse werd uitgevoerd om verschillende markers van de aangeboren immuniteit inclusief het aantal T cellen (CD3), te karakteriseren (CD68, CD161, elastase, TNF-α, BDCA-2). Een significant effect van de behandeling werd gezien op lesionale dermale $CD161^+$ en $elastase^+$ cellen. We concludeerden dat een behandeling met adalimumab van artritis psoriatica patiënten gepaard gaat met een afname in de psoriasis laesies van de huid van deze $CD161^+$ en $elastase^+$ cellen. Dit suggereert dat deze parameters gebruikt zouden kunnen worden als biomarkers voor het evalueren van het therapeutische effect in 'proof of concept' studies.

De resultaten van deze studie naar de effecten van adalimumab op synovium weefsel zijn beschreven in **Hoofdstuk 5b**. In deze studie werd een immunohistochemische analyse gedaan om het celinfiltraat, de expressie van cytokines, MMP's en vasculariteit te karakteriseren. Veel celtypen namen in aantal af na behandeling met adalimumab in vergelijking met placebo. Een significant effect van de behandeling werd gezien op het aantal $CD3^+$ cellen (T cellen) en op de expressie van MMP13; beide waren significant afgenomen na actieve behandeling. Dit suggereert dat deze parameters (synoviaal gelokaliseerde CD3 en MMP13) zouden kunnen worden gebruikt als synoviale biomarkers voor evaluatie van behandeling van patiënten met artritis psoriatica.

In **Hoofdstuk 6** beschrijven we de expressie van de chemokine receptor CCR5 en de bijbehorende liganden in lesionale en niet-lesionale psoriasis huid.

Daarnaast presenteren we de klinische en immunohistochemische resultaten van een gerandomiseerde, placebo-gecontroleerde trial met een CCR5 remmer. Immunohistochemische analyse toonde een licht verhoogd, maar significant, aantal $CCR5^+$ cellen in de epidermis en dermis van lesionale huid in vergelijking met niet-lesionale huid. Echter, het verschil in relatieve expressie van CCR5 tussen lesionale en niet-lesionale huid was alleen statisch significant voor het aantal $CD3^+$ cellen in de epidermis en $CD68^+$ cellen in de dermis. Bepaling van mRNA (RT-PCR) liet alleen een toename zien van expressie van CCL5 (RANTES) in lesionale huid. Een gerandomiseerd, placebo-gecontroleerde klinische trial met 32 psoriasis patiënten toonde geen significant klinisch effect en geen veranderingen op immunohistochemische vlak bij vergelijking van patiënten die werden behandeld met placebo of een CCR5 remmer (SCH351125). We concludeerden dat, hoewel CCR5 expressie verhoogd is in psoriasis lesies, deze receptor geen cruciale rol speelt in de pathogenese van psoriasis.

In **Hoofdstuk 7** beschrijven we de expressie van CXCR3 en de bijbehorende liganden in lesionale en niet-lesionale psoriasis huid. Bepaling middels RT-PCR toonde aan dat de mRNA waarden van CXCR3 en de liganden CXC9-11 verhoogd waren in psoriasis lesies in vergelijking met niet-lesionale lesies. Seriële cryostat secties van psoriasis huidbiopten werden geëvalueerd door middel van immunohistochemie. Het aantal $CXCR3^+$ cellen was laag in niet-lesionaal huid. Digitale analyse van de biopten (zgn. quantitative image analyse) toonde significante toenames van zowel epidermale en dermale $CXCR3^+$ cellen in lesionale huid, in vergelijking met niet-lesionale huid. Het merendeel van de $CXCR3^+$ cellen was gelokaliseerd in de dermis van de lesionale huid. Bovendien bleken 74% van deze $CXCR3^+$ cellen $CD3^+$ T lymfocyten te zijn. Een klein aantal van de $CXCR3^+$ cellen was $CD68^+$, hetgeen duidt op myeloide oorsprong. Daarnaast vonden we dat bijna alle $BDCA2^+$ plasmacytoide dendritische cellen in de psoriasis biopten $CXCR3^+$ waren. Deze bevindingen bevestigen eerdere onderzoeken, die suggereerden dat CXCR3 een centrale rol te speelt in het aantrekken van plasmacytoide dendritische cellen en T cellen naar zich ontwikkelende psoriasis lesies. Dit betekent dat CXCR3 een belangrijke rol speelt in de pathogenese van psoriasis vulgaris.

9 GENERAL DISCUSSION

CURRENT BIOLOGICS

Without a doubt, the arrival of biologics has meant a big step forward for the treatment of psoriasis patients with moderate to severe psoriasis. Randomized clinical trials as well as prospective[1;2] and retrospective cohort studies on biological treatment in daily practice, like the study presented in Chapter 3 with etanercept[3], clearly show great clinical improvement of psoriasis in patients treated with biologics. Furthermore, a large proportion of patients treated with biologics indicate a substantial improvement on health-related quality of life[4].

However, although the clinical improvement by some these of immunosuppressive biologicals is impressive, the situation is still not optimal. There are still some serious side-effects, like drug eruptions as presented in Chapter 2, and long-term safety of biological is a concern. Biologics are associated with increased infections (tuberculosis, aspergillosis, etc), malignancies (lymphoma), haematological disorders and demyelating disorders (multiple sclerosis) [5;6]. The formation of autoantibodies and antibodies against biologic drugs themselves can occur causing impaired treatment outcome or even giving rise to certain side effects [7-10]. Also, with regards to the TNFα antagonists, there is a growing number of reports mentioning the paradoxical onset or worsening of psoriatic skin lesions during treatment with TNF inhibitors[11;12]. Besides side-effects and long term safety, the high costs are another factor limiting the widespread use of biologics. The average medication costs for a treatment with a biologic per year are € 12 000[13] and in case of non-optimal response requiring a double dosage, costs rise accordingly. In the future however, generic biologics will become available, making the costs less excessive.

As to clinical improvement, it is questionable whether new therapeuticals will be able to exceed the current clinical advances with regard to improvement of PASI. Ustekinumab and infliximab are already able to reduce PASI with 80% and the difference between 80% and 95% PASI improvement is almost negligible.

In clinical practice these new biological drugs have been remarkably well tolerated, but we have relative short-term safety data, for some biologics (like ustekinumab) even shorter than others (like etanercept), and need to continue to monitor these patients for long-term safety. National registries are of the utmost importance for collecting these data. Information from these registries will help us to guide strategies for long-term disease management of psoriasis.

FUTURE DIRECTIONS

Despite numerous recent advances in the treatment of psoriasis, it remains still a very recalcitrant disease to treat. For example, we cannot predict who is likely to respond well to a particular therapy and who will not. There are two major areas where we can expect to see further developments that may profoundly affect our ability to

help psoriasis patients in the future. First, continued advances in the understanding of the complex immunologic pathways that contribute to the chronic inflammatory state in psoriasis will certainly lead to the discovery of new targets and development of additional interventions. Second, personalization of treatment based on genetic and demographic characteristics may lead to better and safer outcomes in patients.

With regards to continued advances in the understanding of the complex immunological pathways that contribute to psoriasis lesions, the discovery that certain chemokines and their ligands that are upregulated in psoriasis (Chapter 6 and 7) may be a potential therapeutical target. Currently, several chemokine inhibitors are approved by the FDA for the treatment of several diseases[14]. In 2007 maraviroc, a CCR5 inhibitor, was approved for prevention of HIV and in 2008 a CXCR4 antagonist was approved for hematopoietic stem cell mobilization. Furthermore, recent result of a phase III trial with a CCR9 inhibitor for Crohn's disease are promising. Novel chemokine antagonist-based strategies to interfere with skin inflammation are rather preventive than therapeutic[15]. Chemokine antagonist could be excellent tools to impair the recruitment of pathogenic leukocyte subsets to the skin. Once leukocytes have entered the skin and underwent activation processes, chemokine antagonist are supposed to be less effective. In combination with established drugs, such as methotrexate, cyclosporine, phototherapy or biologicals, however, chemokine antagonist could be promising candidates for prevention of acute flares, prolongation of lesion-free interval and therefore provide optimized long-term management of this chronically relapsing disease. Chemokine antagonist could be an effective additional treatment in patients with specific phenotypes of psoriasis, like pustular psoriasis, where accumulation of neutrophils causes the specific phenotypic features.

Evolving insight in the pathogenesis of psoriasis has led and will lead to the development of new treatment options. In order to proof its clinical efficacy and safety, large numbers of patients have to be tested. Yet, large randomized controlled trials will become more difficult to perform: fewer psoriasis patients match up to the proposed inclusion criteria, whereas the number of candidate therapeuticals is ever-growing. Moreover, due to availability of effective treatment for a large number of psoriasis patients, their willingness to participate in clinical trials diminishes. In psoriasis only limited data on biomarkers are available[16;17]. Therefore, research regarding identification of biomarkers (Chapter 4-5) that could be used for prediction of the clinical response to treatment is necessary, enabling quick assessment of the efficacy of new treatment modalities in a small number of patients.

With regards to personalized treatment, pharmacogenomics may play a role in the future for psoriasis patients. Gene expression profiles could predict response to therapy or risk for side effects, preventing unnecessary periods of ineffective treatments (and thereby frustrations in patients and physicians) and consequently unnecessary high health care costs. Also, new targets could be identified that may lead to specific treatments. Several psoriasis-associated heritable loci have been identified, and the increased expression of several genes has been identified, but a

marker that predicts therapeutic response has yet to be identified. And perhaps, with the increasing knowledge of responsible genes in psoriasis, one day psoriasis may actually be cured by means of gene therapy.

Until then, we need to continue the research and keep the therapeutic pipeline active and productive.

REFERENCE LIST

1. Driessen RJ, Berends MA, Boezeman JB *et al*. Psoriasis treatment with etanercept and efalizumab: clinical strategies influencing treatment outcome. *Br J Dermatol* 2008; **158**: 1098-106.
2. Driessen RJ, Boezeman JB, Van de Kerkhof PC *et al*. Three-year registry data on biological treatment for psoriasis: the influence of patient characteristics on treatment outcome. *Br J Dermatol* 2009; **160**: 670-5.
3. de Groot M, Appelman M, Spuls PI *et al*. Initial experience with routine administration of etanercept in psoriasis. *Br J Dermatol* 2006; **155**: 808-14.
4. Reich K, Sinclair R, Roberts G *et al*. Comparative effects of biological therapies on the severity of skin symptoms and health-related quality of life in patients with plaque-type psoriasis: a meta-analysis. *Curr Med Res Opin* 2008; **24**: 1237-54.
5. Menter A, Griffiths CE. Current and future management of psoriasis. *Lancet* 2007; **370**: 272-84.
6. Bongartz T, Sutton AJ, Sweeting MJ *et al*. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* 2006; **295**: 2275-85.
7. Cheifetz A, Mayer L. Monoclonal antibodies, immunogenicity, and associated infusion reactions. *Mt Sinai J Med* 2005; **72**: 250-6.
8. Wolbink GJ, Vis M, Lems W *et al*. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; **54**: 711-5.
9. Baert F, Noman M, Vermeire S *et al*. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003; **348**: 601-8.
10. Lecluse LL, Driessen RJ, Spuls PI *et al*. Extent and clinical consequences of antibody formation against adalimumab in patients with plaque psoriasis. *Arch Dermatol* 2010; **146**: 127-32.
11. Ko JM, Gottlieb AB, Kerbleski JF. Induction and exacerbation of psoriasis with TNF-blockade therapy: a review and analysis of 127 cases. *J Dermatolog Treat* 2009; **20**: 100-8.
12. Seneschal J, Milpied B, Vergier B *et al*. Cytokine imbalance with increased production of interferon-alpha in psoriasiform eruptions associated with antitumour necrosis factor-alpha treatments. *Br J Dermatol* 2009; **161**: 1081-8.
13. Farmacotherapeutisch kompas 2009.
14. Proudfoot AE, Power CA, Schwarz MK. Anti-chemokine small molecule drugs: a promising future? *Expert Opin Investig Drugs* 2010; **19**: 345-55.
15. Homey B. Chemokines and chemokine receptors as targets in the therapy of psoriasis. *Curr Drug Targets Inflamm Allergy* 2004; **3**: 169-74.
16. de Vlam K, Gottlieb AB, Fitzgerald O. Biological biomarkers in psoriatic disease. A review. *J Rheumatol* 2008; **35**: 1443-8.
17. Rashmi R, Rao KS, Basavaraj KH. A comprehensive review of biomarkers in psoriasis. *Clin Exp Dermatol* 2009; **34**: 658-63.

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BIBLIOGRAFIE
CURRICULUM VITAE
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BIBLIOGRAFIE

van 't Oost L**,** de Groot M**,** Meinardi MMHM, Holleman J. Koude-urticaria: klinische verschijnselen, diagnostiek en behandelingsheden. *Ned Tijdschr Allergie* 2003; **5**: 202-208.

de Groot M, de Rie MA, Bos JD. Dermatitis during efalizumab treatment in a patient with psoriasis vulgaris. *Br J Dermatol* 2005; **153**: 843-845.

de Groot M, Appelman M, Spuls PhI, de Rie MA, Bos JD. Initial experience with routine administration of etanercept in psoriasis. *Br J Dermatol* 2006; **155**: 808-814.

de Groot M, Teunissen MBM, Ortonne JP, Lambert JR, Naeyaert JM, Picavet DI, Arreaza MG, Simon JS, Kraan M, Bos JD, de Rie MA. Expression of the chemokine receptor CCR5 in psoriasis and results of a randomized placebo controlled trial with a CCR5 inhibitor. *Arch Dermatol Res* 2007; **299**: 305-313.

Teunissen MBM, Piskin G, Res PCJM, de Groot M, Picavet DI, de Rie MA, Bos JD. The state of the art in the immunopathogenesis of psoriasis. *G Ital Dermatol Venereol* 2007; **142**: 229-242.

de Groot M, de Rie MA, Bos JD. DRESS syndrome caused by efalizumab: comment. *Clin Exp Dermatol* 2008; **34**: 413-414.

van Kuijk AWR, Gerlag DM, Vos K, Wolbink G, de Groot M, de Rie MA, Zwinderman AH, Dijkmans BAC, Tak PP. A prospective, randomized, placebo-controlled study to identify biomarkers associated with active treatment in psoriatic arthritis: effects of adalimumab treatment on synovial tissue. *Ann Rheum Dis* 2009; **68**: 1303-1309.

Lecluse LLA , de Groot M, Bos JD, Spuls PI. Experience with biologics for psoriasis in daily practice: switching is worth a try. *Br J Dermatol* 2009; **161(4)**: 948-51.

Chen SC, de Groot M, Kinsley D, Laverty M, McClanahan T, Arreaza M, Gustafson EL. Teunissen MBM, de Rie MA, Fine JS, Kraan M. Expression of chemokine receptor CXCR3 by lymphocytes and plasmacytoid dendritic cells in human psoriatic lesions. *Arch Dermatol Res* 2010; **302**: 113-123.

van Kuijk AWR, de Groot M, Stapel SO, Dijkmans BAC, Wolbink GJ, Tak PP. Relationship between the clinical response to adalimumab treatment and serum levels of adalimumab and anti-adalimumab antibodies in patients with psoriatic arthritis. *Ann Rheum Dis* 2010; **69**: 624-625.

de Groot M, Teunissen MBM, Picavet DI, de Rie MA, Bos JD. Reduction of inflammatory cell types of the innate immune system in psoriatic skin during etanercept. *Experimental Dermatology* 2010; **19**: 754-756.

CURRICULUM VITAE

Marjan de Groot werd geboren op 7 januari 1978 te Leeuwarden als eerste kind in een gezin met 2 dochters. Na de basisschool behaalde ze haar gymnasiumdiploma aan het Alberdink Thijm college te Hilversum. Aansluitend startte ze in 1996 met de studie geneeskunde aan de Vrije Universiteit te Amsterdam, die ze in 2003 cum laude afrondde. Na een coschap dermatologie in het OLVG en een keuze-coschap dermatologie op de polikliniek dermatologie in het AMC was de liefde voor de dermatologie geboren. In 2003 startte ze haar werkzaamheden bij de polikliniek dermatologie in het AMC als arts-onderzoeker. In deze hoedanigheid was ze betrokken bij verschillende klinische trials met biologicals voor psoriasis, die de basis vormden van haar proefschrift "Innovative therapies and new targets in psoriasis". In 2005 was zij een van de initiatiefnemers van het biological spreekuur voor psoriasis patiënten. Daarnaast was ze oprichtster van een database voor het verzamelen van lange termijn gegevens van systemische therapieën voor psoriasis. In oktober 2006 begon ze haar opleiding dermatologie in het AMC onder begeleiding van professor dr. J.D. Bos, die ze verwacht af te ronden in februari 2012. Sinds 2009 werkt zij via de Werkgroep Psoriasis mee aan het samenstellen van de nieuwe richtlijn psoriasis voor de Nederlandse Vereniging van Dermatologie en Venereologie (NVDV).

Marjan de Groot is getrouwd met Peter Jurriaans en samen hebben zij een zoon, Hugo (2009). Eind juni 2011 verwachten ze hun tweede kindje.

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