BJD
British Journal of Dermatology

The effect of adalimumab on key drivers in the pathogenesis of psoriasis*

A.G.M. Hendriks, H.M.J. van der Velden, E.A.W. Wolberink, M.M.B. Seyger, J. Schalkwijk, P.L.J.M. Zeeuwen, E.M.G.J. de Jong, M.C. Pasch, P.E.J. van Erp and P.C.M. van de Kerkhof

Department of Dermatology, Radboud University Medical Center, PO Box 9101, 6500 HB Nijmegen, the Netherlands

Correspondence
Anke G.M. Hendriks.
E-mail: Anke.GM.Hendriks@radboudumc.nl

Accepted for publication
23 October 2013

Funding sources
This study was funded by Abbott.

Conflicts of interest
See Appendix for details.

*Plain language summary available online

DOI 10.1111/bjd.12705

Summary

Background The use of recently introduced biologics targeting specific immune mechanisms has identified crucial steps in the pathogenesis of psoriasis. Studying the dynamics of changes of these target mechanisms in sequential skin biopsies during treatment with biologics may reveal potential biomarkers. Correlation between clinical parameters and the expression of specific genes during treatments may identify markers indicative of treatment response.

Objectives This observational open-label study aimed to provide an overview of important cell biological changes in lesional skin during treatment with adalimumab, and their relationship to clinical improvement.

Methods Ten patients with moderate-to-severe plaque psoriasis were included and treated with adalimumab for 16 weeks. At baseline, and after 10 days and 16 weeks of treatment clinical scores were assessed and biopsies were taken to examine gene expression at the mRNA and protein level.

Results The expression of marker genes for innate immunity, and epidermal differentiation and proliferation was rapidly restored to normal levels, whereas genes of the adaptive immune system showed a delayed decrease. The static and dynamic course of CD1a+ Langerhans cells and Ki67+ nuclei showed a significant strong correlation to the Psoriasis Area and Severity Index score. No correlation between interleukin-17 expression and clinical scores was found.

Conclusions The innate immune system is affected during adalimumab treatment well before the changes in the adaptive immune system become apparent. We may speculate that the addition of a treatment with an early effect on adaptive immunity to adalimumab may result in superior effectiveness compared with monotherapies.

What's already known about this topic?

- Recently introduced biologics targeting specific immune mechanisms have identified crucial steps in the pathogenesis of psoriasis.

What does this study add?

- During adalimumab treatment markers of epidermal differentiation, proliferation and the innate immune system revert rapidly to normal, well before changes in the adaptive immune system become apparent.

Psoriasis is a chronic disease affecting about 2% of the Caucasian population. Over the years, various hypotheses on the pathogenesis of psoriasis have been proposed. At first, psoriasis was considered a keratinocyte-centred disorder with epidermal hyperplasia as the most prominent clinical and histological feature.[1] From the 1980s onwards, a role for the immune system in its pathogenesis was suggested, based on the presence of large numbers of immune cells within psoriatic lesions.[2–4]

This hypothesis was supported by the beneficial therapeutic effect of systemic drugs targeting the immune system.[5,6] Further exploration of the role of the immune system revealed an important involvement of T cells. Until recently, psoriasis has been considered mainly to be a T helper (Th) 1 cell-mediated process,[7] but today it is thought to be a complex multifactorial disease, with involvement of the adaptive immune system, with an important role for Th1 and Th17 cells,[8,9] the innate immune system, with a significant role for polymorphonuclear leucocytes (PMNs), and for the skin barrier function of the epidermis.[10]

Targeting tumour necrosis factor (TNF)-α is an important approach in the treatment of patients with severe psoriasis. TNF-α is a naturally occurring cytokine that is involved in normal inflammatory and immune responses. Elevated levels of TNF-α play an important role within inflammatory diseases, such as psoriasis. Numerous clinical trials have demonstrated that the treatment of psoriasis with anti-TNF-α agents has an impressive clinical efficacy,[11–13] which indicates the actual relevance of TNF-α in its pathogenesis. One of the current anti-TNF-α agents for psoriasis treatment is adalimumab. It is a recombinant human immunoglobulin (IgG1) monoclonal antibody containing only human peptide sequences. Adalimumab is produced by recombinant DNA technology and binds with high affinity and specificity to soluble TNF-α.[14] It neutralizes the biological function of this cytokine by blocking its interaction with the cell surface TNF-α receptors. Adalimumab modulates biological responses that are induced or regulated by TNF-α, including changes in the expression of adhesion molecules, which are responsible for leucocyte migration.[14] Recently, a few studies have been conducted to investigate the mechanism of action by which adalimumab leads to a clinical improvement of psoriasis, focusing on different components thought to play an important role in the pathogenesis of psoriasis. Various authors have investigated the effect of anti-TNF-α on different parts of the immune system. Marble *et al.*[15] reported a rapid restoration of keratinocyte differentiation and a significant reduction in the number of dendritic cells, followed by the reduction of macrophages and T cells. In another study, the rapid restoration of the epidermal Langerhans cell density within the psoriatic lesions was described.[16] In addition, the investigation by Soegaard-Madsen *et al.*[17] demonstrated an association between neutralization of TNF-α due to adalimumab treatment and the inhibition of p38 mitogen-activated protein kinase (MAPK). Subsequently, a reduction in the p38 MAPK phosphorylation and a decrease in the expression of p38 MAPK-regulated genes, such as *IL8* and *IL17C*, were shown.[18]

In the present study a broad range of key aspects of the pathogenesis of psoriasis was examined, including markers of the epidermal compartment (genes involved in epidermal proliferation and differentiation), and markers for both the innate immune system (including neutrophils, epithelial proteinase inhibitors and antimicrobial peptides), and the adaptive immune system [including Th1 cells and interferon (IFN)-γ], and some key cytokines [including interleukin (IL)-17, IL-8 and TNF-α], which have been shown to be relevant therapeutic targets. These markers were studied both at the mRNA and the protein level. The aim of this comprehensive study was to elucidate the dynamics of a broad range of cell biological changes, which have been suggested to be crucial for psoriasis, in lesional skin during the treatment with adalimumab. In particular, we investigated the dynamics of adalimumab-induced changes within one single study population. In addition, the correlation between clinical and cell biological changes was studied.

Material and methods

Patients

Ten patients (five women and five men; age 42–73 years) with moderate-to-severe chronic plaque psoriasis were enrolled in the present observational open-label study. All experiments were approved by the local medical ethics committee prior to the study and were conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO). Written informed consent was given by all patients before inclusion. We included patients who were aged ≥ 18 years, and had moderate-to-severe plaque psoriasis as defined by a Psoriasis Area and Severity Index (PASI) score ≥ 10 or a PASI score ≥ 8 in combination with a Skindex (instrument to measure the effects of skin diseases on patients' health-related quality of life[19]) total score > 35 at the baseline visit. The patients had failed, were intolerant to or had contraindications to methotrexate, phototherapy and cyclosporin. Patients with a known history of an allergic reaction or significant hypersensitivity to the constituents of adalimumab were excluded. Other exclusion criteria were: non-plaque psoriasis, recent infections, HIV or hepatitis B or C positivity, active tuberculosis, a history of malignancies (other than successfully treated non-metastatic cutaneous squamous cell or basal cell carcinoma, or cervical carcinoma *in situ*), and female patients who were pregnant or breastfeeding, or considered becoming pregnant during the study or within 5 months of the last dose of adalimumab. Prior to enrolment no systemic antipsoriatic treatment or phototherapy was used for 4 weeks. Participants were allowed to use topical antipsoriatic therapy until the baseline visit. One patient was withdrawn at week 12 because his psoriasis worsened despite treatment. Therefore, the samples collected from this patient were not taken into account during analysis; his data were only used in calculating the correlation between clinical and cell biological changes.

Study procedures

An initial dose of 80 mg adalimumab was given subcutaneously, followed by 40 mg adalimumab subcutaneously every other week starting 1 week after the initial dose. Treatment duration was 16 weeks. One representative psoriasis lesion, the 'target lesion', was defined at the baseline visit. At each

visit, PASI and body surface area (BSA) scores were assessed and clinical photographs were taken. Before treatment, after 10 days and 4, 8, 12 and 16 weeks of treatment, SUM scores (scoring the erythema, infiltration and desquamation, but without considering the area) were assessed from the target lesion. At baseline and after 10 days and 16 weeks of treatment, two punch biopsies (diameter 4 mm) were taken from the target lesion after local anaesthesia (xylocaine 2%/adrenaline 1 : 200 000). One biopsy was stored in Trizol (Invitrogen, Carlsbad, CA, U.S.A.) for quantitative polymerase chain reactions (qPCR) and the other biopsy was formalin-fixed, embedded in paraffin and sectioned at 4 μm.

Immunohistochemistry

After cutting, the paraffin-embedded sections were deparaffinized and rehydrated. Staining procedures were performed as described before.[20] In short, after blocking endogenous peroxidase activity with 2% H_2O_2 in phosphate-buffered saline, sections were incubated with 1% bovine serum albumin (BSA). Antigen retrieval steps were used for keratin 10 (K10) staining (0·1% trypsin solution; pH 7·8, 10 min at 37 °C), for keratin 16 (K16) and Ki67 staining (citrate buffer; pH 6·0, 10 min at 100 °C) and IL-17 staining (Tris/ethylenediamine tetra-acetic acid buffer; pH 9·0, 10 min at 100 °C). Incubation with the primary antibodies (Table 1) dissolved in 1% BSA occurred overnight at room temperature. Visualization of the primary antibody was achieved with peroxidase-based EnVision™ kits (Dako, Glostrup, Denmark), 3,3′-diaminobenzidine tetrahydrochloride solution (Sigma-Aldrich, St Louis, MO, U.S.A.) and counterstaining with Mayer's haematoxylin (Sigma-Aldrich). Substitution with 1% BSA served as a negative control.

Histological quantification of cells and surface area

All microscopic evaluations were performed by the same observer (A.G.M.H.) and repeated on two different occasions per specimen. Whole-skin sections were assessed as described before[20] using ImageJ 1.38× software (National Institutes of Health; http://rsb.info.nih.gov/ij/). In short, with respect to the epidermis, an area without the stratum corneum, across the whole section was measured. The region of interest of the dermal compartment was defined as the surface from the basement membrane down 350 μm across the whole section. For analysis of CD1a, the epidermal surface area, and for analysis of CD3, T-Bet (a Th1-specific T box transcription factor), IL-17 and elastase, the dermal surface area was measured and positive cells were counted and expressed as number of positive cells per mm^2 epidermis and dermis, respectively. Cells positive for Ki67 were counted and expressed as positive cells per mm length of basement membrane. For quantification of the epidermal K10, K16, elafin and human β-defensin (hBD)-2 stainings, the percentage of the epidermis with a positive signal was measured.

mRNA isolation and real-time quantitative polymerase chain reaction

Total RNA was isolated from the epidermis as previously described.[21] A DNase 1 treatment was performed according to the manufacturer's protocol (Invitrogen). First-strand cDNA was synthesized using an input of 1 μg of DNase 1-treated RNA with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, U.S.A.) according to the manufacturer's recommendation. Real-time qPCR was performed with the CFX96 Real-Time Detection System for quantification with SYBR Green and melting curve analysis (Bio-Rad) as previously described.[22] Primer validation, qPCR reactions and determination of relative mRNA expression were performed as previously described.[21] Expression of the genes of interest was normalized to that of human ribosomal phosphoprotein P0 (*RPLP0*). The relative quantity of gene expression was measured using the delta-delta cycle threshold ($\Delta\Delta C_t$) method.[23] Primers for qPCR (Biolegio, Nijmegen, the Netherlands) were only accepted if their efficiency was 100 ± 10%. Corrections were made for primer efficiency. Primer sequences and efficiency are shown in Table 2.

Statistical analysis

The results of the immunohistochemical analysis are expressed as means ± SEM, and time-related changes were assessed

Table 1 Primary antibodies

Target	Function and/or cell source	Designation and source	Dilution used
CD1a	Langerhans cells	M3571, 010; Dako, Glostrup, Denmark	1 : 100
CD3	T cells	17143, F7.2.38; Abcam, Cambridge, U.K.	1 : 500
Elafin	Host defence protein	HM2063/TRAB2F	1 : 500 000
Elastase	Neutrophils	M0752, NP57; Dako	1 : 10 000
hBD-2	Host defence protein	Abcam	1 : 50
IL-17	Interleukin 17	AF-317-NA, rhil-17; R&D Systems, Abingdon, U.K.	1 : 500
K16	Abnormal epidermal differentiation	LL025; Sanbio BV, Uden, the Netherlands	1 : 50
Ki67	Epidermal proliferation	M7240, MIB-1; Dako	1 : 100
T-Bet	T helper 1 cells	sc-21003, H-210; R&D Systems	1 : 1500

hBD, human β-defensin.

Table 2 Primer sequences

HUGO gene name	Synonym	Forward primer 5′-3′	Reverse primer 5′-3′	Efficiency[a]
CCL5	Rantes	TCTGCGCTCCTGCATCTG	GGGCAATGTAGGCAAAGCA	1·91
CXCL8	IL8	CTTGGCAGCCTTCCTGATTT	TTCTTTAGCACTCCTTGGCAAAA	2·10
CXCL10	IP10	TTCCTGCAAGCCAATTTTGTC	TCTTCTCACCCTTCTTTTTCATTGT	2·01
DEFB4	hBD-2	GATGCCTCTTCCAGGTGTTTTT	GGATGACATATGGCTCCACTCTT	1·99
DPP4	CD26	TCATTCAGTAAAGAGGCGAAGTATTATC	CAGTTTTTTGGAGGGCATCTG	1·89
ELANE	Elastase	GCCGTCGCAGCAACGT	GGAGGCAATTCCGTGGATTA	1·86
IFNG	Interferon-γ	GGAACTCTTTTCTTAGGCATTTTGA	GATGGTCTCCACACTCTTTTGGA	1·99
IL17A	Interleukin 17A	TTGATTGGAAGAAACAACGATGA	CTCAGCAGCAGTAGCAGTGACA	2·02
IL23A	Interleukin 23A	GAAGAGGGAGATGAAGAGACTACAAATG	AGGGCTATCAGGGAGCAGAGA	2·16
KRT16	Keratin 16	GATCATTGCGGCCACCAT	TGCTCATACTTGGTCCTGAAGTCA	2·01
MKI67	Ki67	AAACCAACAAAGAGGAACACAAATT	GTCTGGAGCGCAGGGATATTC	2·21
PI3	Elafin	CATGAGGGCCAGCAGCTT	TTTAACAGGAACTCCCGTGACA	2·02
RPLP0	hARP	CACCATTGAAATCCTGAGTGATGT	TGACCAGCCCAAAGGAGAAG	2·02
S100A7	Psoriasin	CTTCCTTAGTGCCTGTGACAAAAA	AAGGACAGAAACTCAGAAAAATCAATCT	1·89
TBX21	T-Bet	CAACACGCATATCTTTACTTTCCAA	GGGATGCTGGTGTCAACAGA	2·17
TNF	TNF-α	TCTTCTCGAACCCCGAGTGA	CCTCTGATGGCACCACCAG	2·00

hBD, human β-defensin; TNF, tumour necrosis factor. [a]Efficiency as fold increase in fluorescence per polymerase chain reaction cycle.

using a one-way ANOVA repeated measures test, followed by a Bonferroni *post hoc* test. For the qPCR experiments, statistical analysis by ANOVA was performed on ΔC_t values corrected for primer efficiency, followed by a Bonferroni *post hoc* test using SPSS v16.0 (SPSS, Benelux BV, Nieuwegein, the Netherlands). ΔC_t is the difference between the target gene and reference gene (*RPLP0*) C_t. The Spearman rank correlation analysis was used to explore the relationship between continuous variables. Throughout the analyses, $P < 0{\cdot}05$ was considered statistically significant.

Results

Clinical results

The baseline demographics and characteristics of the study population are specified in Table 3. The percentages of patients who previously received topical and systemic treatments are displayed in Figure 1. After 10 days of adalimumab therapy, a clinical improvement in PASI score was seen in half the patients. The mean PASI score decreased from 16·93 (SEM 2·64) at baseline to 14·94 (SEM 2·63) after 10 days, and to 1·07 (SEM 0·42) after 16 weeks of therapy. The mean SUM score from the target lesions declined from 6·90 (SEM 0·35) at baseline to 6·00 (SEM 0·69) after 10 days and to 0·40 (SEM 0·27) after 16 weeks. An overview of the improvement in clinical parameters during adalimumab treatment is depicted in Figure 2.

Table 3 Demographic characteristics of the study population

Characteristic	
Patients, *n*	10
Male	5
Female	5
Age (years)	55 (42–73)
Disease duration (years)	25·2 (6–47)
Age at onset (years)	29·1 (6–58)
PASI at baseline	16·93 (9·7–38·4)
BSA at baseline	24·96 (5·6–76·0)
SUM score at baseline	6·9 (5–9)

PASI, Psoriasis Area and Severity Index; BSA, body surface area; SUM, score of erythema, infiltration and desquamation. Values are mean (range) unless otherwise indicated.

Immunohistochemical analysis

An overview of the results of the immunohistochemical analysis is shown in Figure 3. At baseline, all tissue samples revealed the classical psoriatic features: acanthosis, parakeratosis, elongated rete ridges and a mixed cellular infiltrate.

Within the dermal compartment, high numbers of CD3+ cells, were present at baseline. After 10 days of treatment a slight decrease in CD3+ cells was found; however, differences were not statistically significant. The number of CD3+ cells decreased further until the end of the study. The course of the cells positive for the transcription factor for Th1-specific cytokines (T-Bet+ T cells) coincided with the CD3+ cells, with only a slight decrease after 10 days and a significant decrease after 16 weeks of treatment ($P < 0{\cdot}0001$).

Within the chronic psoriatic lesions, only small numbers of dermal elastase-positive PMNs were found. However, after 10 days of treatment with adalimumab, there was a significant decrease in elastase-positive cells ($P = 0{\cdot}0371$), and after 16 weeks, there were only a few sporadic cells positive for elastase present within the tissue samples.

The cells staining positive for IL-17 showed a moderate, gradual decrease, with only a minimal, nonsignificant, decrease at the end of the study.

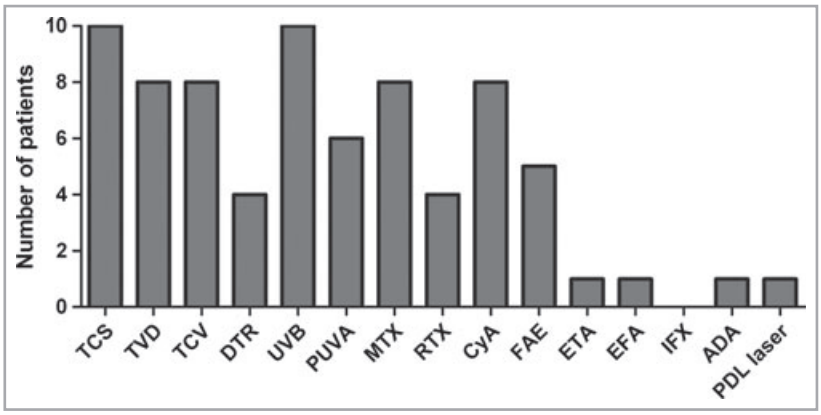

Fig 1. The number of patients who previously received topical and systemic treatments. ADA, adalimumab; CyA, cyclosporin; DTR, dithranol; EFA, efalizumab; ETA, etanercept; FAE, fumaric acid esters; IFX, infliximab; MTX, methotrexate; PDL, pulsed-dye laser; PUVA, psoralen plus ultraviolet A; RTX, retinoids; TCS, topical corticosteroids; TCV, combination therapy with TCS and topical vitamin D analogues (TVD); UVB, ultraviolet B.

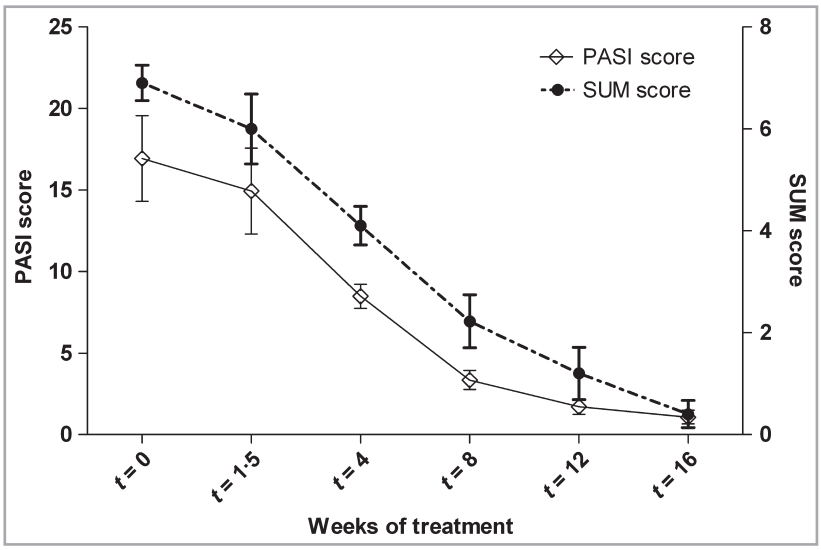

Fig 2. Clinical parameters during treatment with adalimumab as measured in the responding patients (n = 10). Dots indicate mean ± SEM. PASI, Psoriasis Area and Severity Index; SUM, score of erythema, infiltration and desquamation.

With respect to the epidermal compartment, along with the rapidly disappearing elastase+ cells, other markers of the innate immune system showed a significant decrease after only 10 days. This is depicted by the decrease of the elastase-specific protease inhibitor elafin (percentage elafin+ epidermal surface) and the skin antimicrobial peptide hBD-2 (percentage hBD-2+ epidermal surface). For both markers the same pattern is seen, with after 16 weeks hardly any epidermal surface area staining positive for elafin or hBD-2.

The proportion of abnormally differentiating keratinocytes diminished, depicted by the declining percentage of epidermal surface staining positive for K16 after 10 days and 16 weeks ($P < 0{\cdot}0001$). At the same time, there was a decrease in the number of cycling epidermal cells, reflecting keratinocyte proliferation, depicted by a decreasing number of Ki67+ nuclei within 10 days ($P = 0{\cdot}0098$). Regarding the density of the CD1a+ Langerhans cells within the epidermal compartment, a significant increase was seen after only 10 days of treatment, further increasing until the end of treatment ($P < 0{\cdot}0001$).

Correlation of protein levels and clinical scores

The correlations between the PASI score and all examined protein markers were calculated and are shown in Table 4. In addition, the correlation between the local SUM score and the overall PASI score was calculated. Results show a significant but weak-to-moderate correlation between the SUM score and the PASI score (Spearman $R = 0{\cdot}49$, $P = 0{\cdot}0043$), and a strong correlation between the change in SUM score (ΔSUM) and change in PASI score (ΔPASI) (Spearman $R = 0{\cdot}78$, $P < 0{\cdot}0001$). Regarding the correlations between the protein markers and the clinical scores, there were two markers showing a significant strong correlation between both the static PASI score, measured at a certain time point and the corresponding number of positive cells, and between the dynamic changes in PASI score (ΔPASI) and the expression of these proteins over time. These were found for the static and dynamic correlation of CD1a+ Langerhans cells (Spearman $R = -0{\cdot}75$ and $-0{\cdot}76$, respectively; $P < 0{\cdot}0001$ for both correlations) and for the course of the nuclei staining positive for Ki67 (Spearman $R = 0{\cdot}73$ and $0{\cdot}75$, respectively; $P < 0{\cdot}0001$ for both correlations) (Fig. 4).

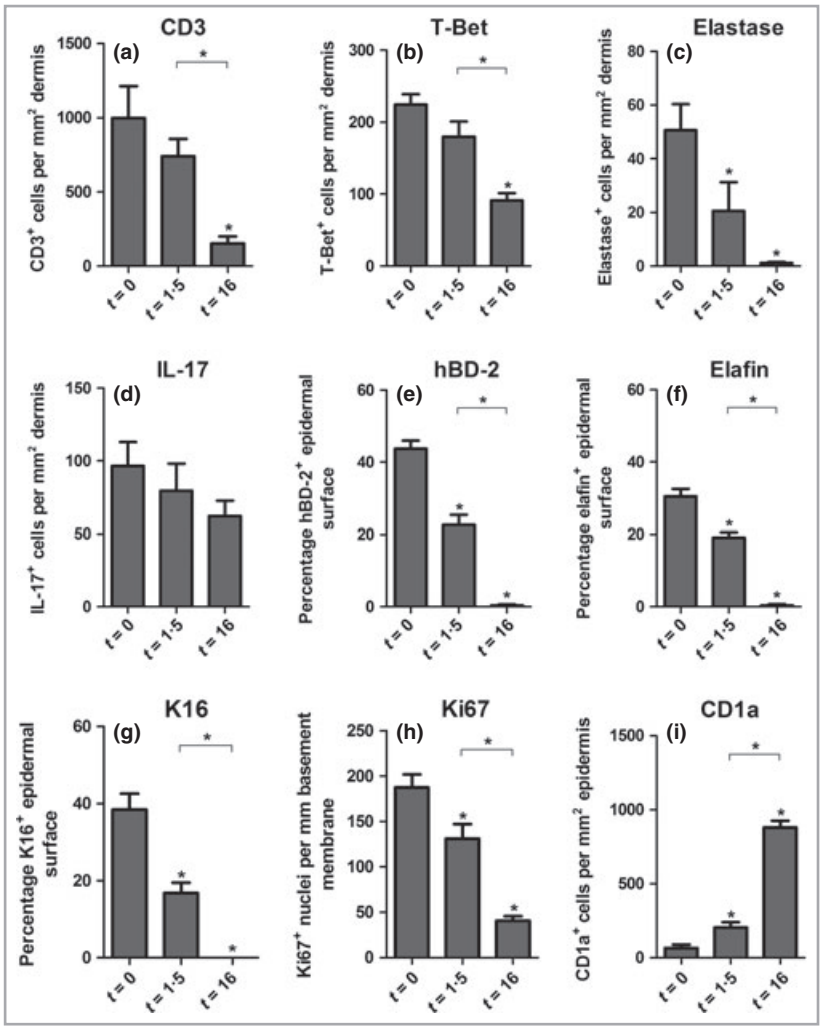

Fig 3. Overview of immunohistological results. The number of (a) CD3+, (b) T-Bet+, (c) elastase+ and (d) interleukin (IL)-17+ cells mm^{-2} dermis; percentage of (e) human β-defensin (hBD)-2+, (f) elafin+ and (g) keratin (K)16+ epidermal surface; (h) the number of Ki67+ nuclei per mm length of basement membrane; and (i) the number of CD1a+ cells mm^{-2} epidermis at baseline, and at 10 days and 16 weeks after adalimumab treatment. Note that protein levels are presented in different scales ($n = 10$); bars indicate mean ± SEM. *Statistically significant compared with baseline ($P < 0{\cdot}05$).

Gene expression levels during treatment

An overview of the mRNA expression levels of selected genes, at baseline and during treatment, is summarized in Figure 5. Most genes showed a significant decrease after 16 weeks of treatment. No significant decrease was detected in the mRNA levels of TNF-α, in contrast there was even a slight, but non-significant increase. In addition, the mRNA levels of CD26 and elastase showed a nonsignificant increase after 10 days of treatment, declining subsequently. Only the levels of IL-8 mRNA and Ki67 mRNA showed a significant reduction after just 10 days of treatment.

Discussion

This is the first study providing an overview of the dynamics of a broad range of important cell biological changes during adalimumab treatment at both the mRNA and protein levels in one single study population. As all results have been obtained from the same subjects, all results regarding the various processes can be compared with each other without the bias of interindividual variation.

As the biopsies taken from the target lesions were used for analyses as a reflection of overall disease severity, we calculated the correlation between the local SUM score and the PASI score concerning the total skin. We showed that there was a significant correlation between both scoring systems and it was concluded that the target lesions were essentially representative for the overall disease severity.

Within the first 10 days we found a notable decrease in the PASI score in half of the patients. In parallel with the fast clinical response, a rapid normalization of epidermal differentiation and proliferation was observed, depicted by the decline in the expression of K16 and number of cycling nuclei, respectively (Fig. 3g,h).

Along with the restoration of homeostasis within the epidermal compartment, a rapid decrease in the activity of the innate immune system was observed, whereas decrease of the adaptive immune system markers seemed to occur more slowly. At the mRNA level, a similar trend of a rapidly normalizing innate immune system was seen. However, only the decrease in IL-8 and Ki67 expression was significant after 10 days, probably because of large interindividual variations. At the protein level, we found a significant drop in the

number of dermal elastase+ cells, but the number of dermal CD3+ and T-Bet+ cells showed no significant decrease, whereas previous studies have shown that other antipsoriatic treatments such as potent topical corticosteroids[24] are capable of reducing the number of T cells after only 7 days of treatment. The elastase+ PMNs are important cellular representatives of the innate immune system in psoriasis. PMNs accumulating in the stratum corneum forming microabscesses of Munro and spongiform pustules in the stratum Malpighi (micropustules of Kogoj) are indicative of active psoriasis. Moreover, studies have shown that PMNs and mast cells are capable of producing IL-17, and that the release of IL-17 from these innate immune cells may be central in the pathogenesis of psoriasis.[25]

Besides the well-accepted importance of IL-17 in the pathogenesis of psoriasis, there is an established role for Th1 cells and IFN-γ as well.[26] It has been suggested that the IL-23/IL-17-directed pathway leads to an active eruptive psoriasis, whereas the IL-12/IFN-γ-directed pathway is predominant within chronic plaque psoriasis.[26] Moreover, the results from two previous studies,[20,27] investigating the involvement of elastase+, IL-17+ and T-Bet+ cells within normal skin in the acute phase after leukotriene B4 application and removal of the stratum corneum, respectively, showed that the acute phase of inflammation in challenged skin is dominated by elastase+ and IL-17+ cells, suggesting a more important role in the acute phase of inflammation. In contrast, T-Bet+ cells showed a more gradual course and may be more prominent

Table 4 Correlations between clinical score and protein levels (n = 11)

Protein marker	Correlation between PASI score and the protein expression		Correlation between ΔPASI and the change in protein expression	
	Spearman R[a]	P-value	Spearman R[b]	P-value
CD1a	**−0·75**	**< 0·0001**	**−0·76**	**< 0·0001**
CD3	0·05	0·0045	0·33	0·1394
Elafin	0·64	< 0·0001	0·67	0·0007
Elastase	0·53	0·0015	0·25	0·2546
hBD-2	**0·75**	**< 0·0001**	0·69	0·0003
IL-17	0·15	0·4174	0·17	0·4523
K16	**0·75**	**< 0·0001**	0·54	0·0091
Ki67	**0·73**	**< 0·0001**	**0·75**	**< 0·0001**
T-Bet	0·61	0·0002	0·64	0·0015

hBD, human β-defensin; IL, interleukin; K, keratin; PASI, Psoriasis Area and Severity Index. Bold indicates a strong correlation (Spearman R ≥ 0·7 and ≤ −0·7). [a]Correlation between the PASI score and the different protein levels. [b]Correlation between the change in PASI score (ΔPASI) and the change in the different protein levels.

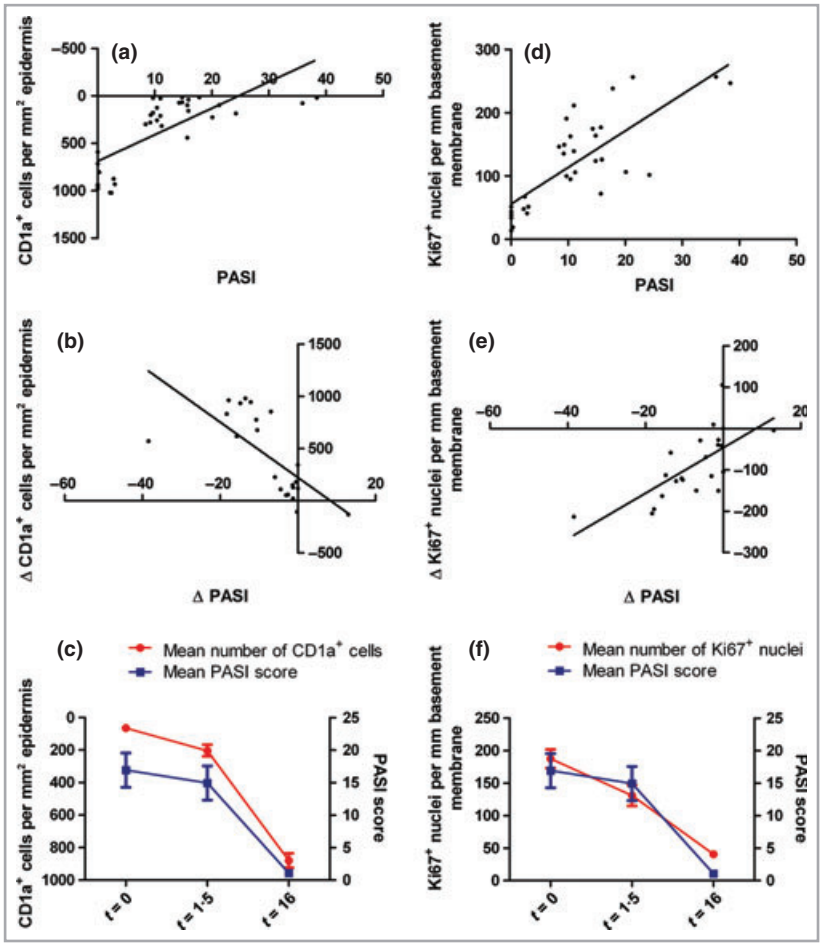

Fig 4. Overview of strong correlations. (a) Levels of CD1a+ Langerhans cells and (d) the number of Ki67+ nuclei, measured at three different times, with varying disease severity were plotted against the Psoriasis Area and Severity Index (PASI) score. A significant correlation was found; Spearman R = −0·75 (a) and 0·73 (d); P < 0·0001 for both correlations. (b, e) Correlation between the change in clinical score (ΔPASI) and (b) the change in the number of CD1a+ cells (Δ(ΔKi67+ nuclei)CD1a+ cells) or (e) the change in the number of nuclei expressing Ki67 (Ki67+ nuclei). Results show a strong correlation; Spearman R = −0·76 (b) and 0·75 (e); P < 0·0001, for both correlations (n = 11). Note that 10 patients showed clinical improvement (negative ΔPASI) and that there was only one patient who showed an exacerbation (positive ΔPASI). (c, f) An overview of the course of the PASI score in relation to (c) the number of CD1a+ cells and (f) the relation to the number of Ki67+ nuclei. Dots indicate mean ± SEM.

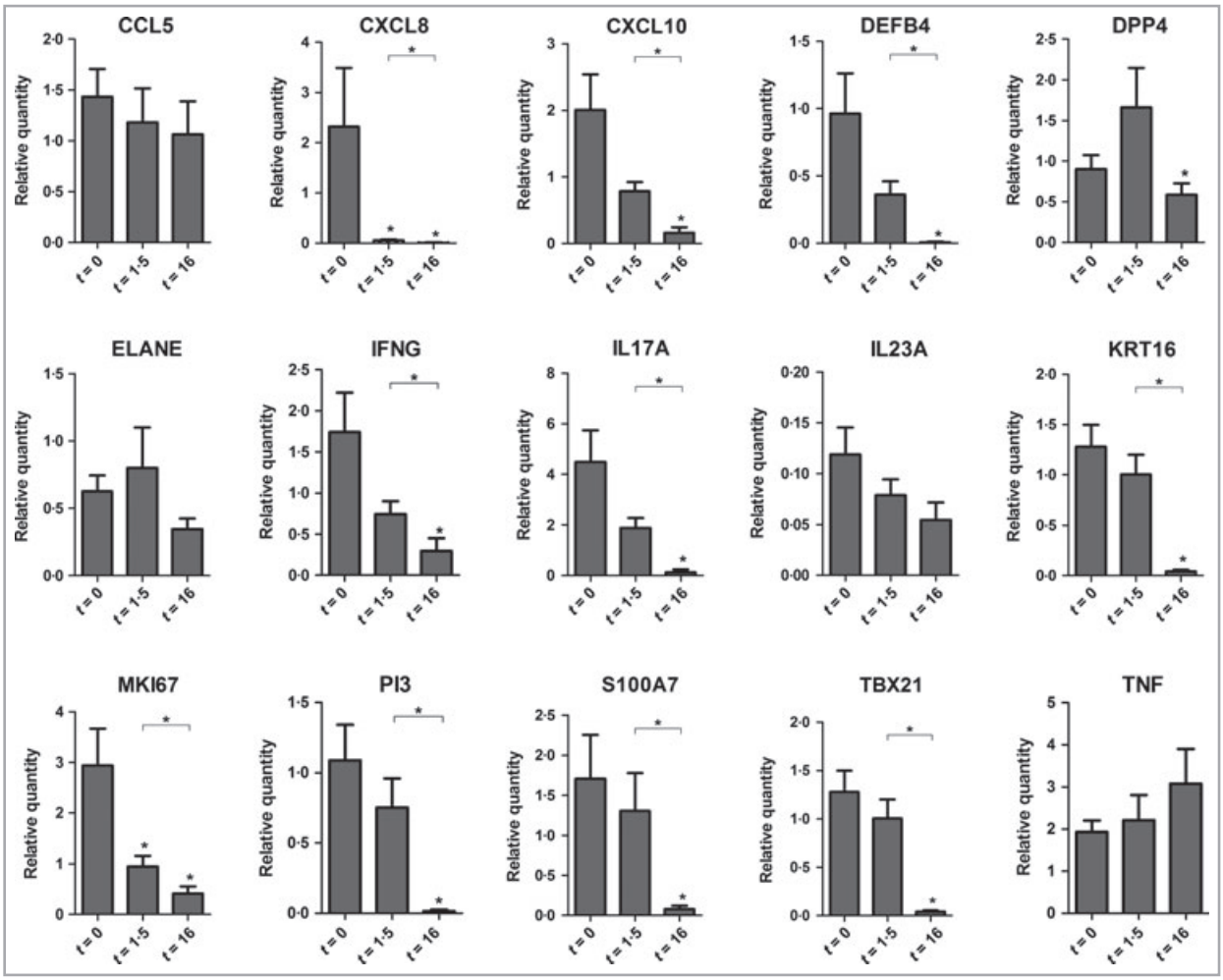

Fig 5. Relative epidermal mRNA expression levels of target genes; the intraindividual mRNA expression of the household gene (*RPLP0*) was used as a reference value in calculating the relative quantities of mRNA expression levels. Note the relative quantity is presented in different scales ($n = 9$); bars indicate mean ± SEM. *Statistically significant compared with baseline ($P < 0{\cdot}05$).

in the more chronic and stable situation. In the present study, all patients were suffering from longstanding chronic plaque psoriasis and were not in a phase of acute exacerbation. In these patients we observed relatively high numbers of T-Bet+ cells and relatively low numbers of elastase+ and IL-17+ cells. Moreover, only a nonsignificant reduction in IL-23A mRNA was found. Regarding IL-17A mRNA, a reduction was found, which was only significant after 16 weeks of treatment, whereas no significant reduction on the protein level was found during the study. These findings fit the hypothesis that differences in cytokine composition may be indicative of a more acute disease or a more chronic disease.

During the study, one patient had to be withdrawn from the study at week 12 because the psoriasis worsened despite adalimumab treatment. Therefore, the samples collected from this patient were not taken into account during analysis. However, there are some notable differences between the amount of cells stained positive for T-Bet within the baseline sample of this patient and the mean cell numbers found within the baseline samples of the 10 patients who completed the study, while PASI scores did not differ significantly. At baseline the mean number of T-Bet+ Th1 cells found in the responders was 224 cells mm^{-2} (SD 45), while only 106 cells mm^{-2} were found in the baseline sample of the nonresponder. Regarding the number of cells positive for IL-17 and elastase, no remarkable differences were found between the nonresponder and the responders. Results should be interpreted carefully, because they were derived from one patient only. However, it would be interesting to investigate the cytokine composition in the lesional skin of more patients with an insufficient response to adalimumab treatment.

As the disease severity of the target lesions was shown to be representative of the disease activity, as measured by the PASI score, we investigated the correlation between the PASI score and protein levels of the inflammation markers. We chose to investigate the correlation with protein levels because mRNA expression has high standard deviations. Only the presence and course of CD1a+ Langerhans cells and Ki67+ nuclei showed a strong significant correlation with the PASI score at a single time point as well as for the changes during follow-up. As

CD1a+ cells and Ki67+ nuclei are not only indicative of the disease activity of the target lesion but also proved to correlate with the overall skin involvement, it would be interesting to study whether reduction of these parameters precedes clinical improvement and whether these markers could serve as biomarkers predicting treatment response.

As has been described before, the expression of TNF-α mRNA did not change significantly during adalimumab treatment, as TNF-α expression in lesional psoriatic skin is regulated post-transcriptionally.[17,18,28] Other studies have demonstrated that the early effects of adalimumab therapy are mediated by the reduction in p38 MAPK phosphorylation and a subsequent decrease in the mRNA expression of IL-1β, IL-8 and IL-20, which are all regulated through the p38 MAPK signalling pathway.[18] Our findings confirm the rapid decrease in IL-8 expression, as only the expression of IL-8 mRNA (and Ki67 mRNA) showed a significant decrease after only 10 days of treatment.

In conclusion, this study provides a dynamic overview of the course of important players in the pathogenesis of psoriasis. During adalimumab treatment, representatives of both epidermal differentiation and proliferation, and those of the innate immune system revert rapidly to normal, while representatives of the adaptive immune system lag behind. As the principle of combining two drugs can be promising, it is attractive to speculate that a combination of drugs with different and synergistic mechanisms of action, for example targeting the innate and adaptive immune systems or targeting immunity and epidermal differentiation may provide a highly effective combination therapy. A synergistic approach may lead to superior effectiveness and may help to prevent excessive use of the individual components thereby preventing adverse events.

Acknowledgments

The authors would like to thank all patients who participated in this study, and I.M.J.J. van Vlijmen-Willems, D. Rodijk-Olthuis, K.M.G. Klaassen and R.T. de Boer-van Huizen for their valuable contribution to practical procedures during this project.

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Appendix

Conflicts of interest

A.G.M.H. has participated in trials funded by GalaxoSmithKline (GSK), Pfizer, Eli Lilly, Amgen, Allmirall and Celgene. H.M.J.v.d.V. has participated in trials funded by Schering Plough, Abbott, LEO Pharma, Centocor and Janssen-Cilag. E.A.W.W. has participated in a trial funded by GSK and Abbott. M.M.B.S. has served as a consultant for Wyeth, Pfizer and Abbott and received grants from Pfizer and LEO Pharma. M.C.P. has received grants from Pfizer and Janssen-Cilag. E.M.G.J.d.J. has acted as a consultant and/or paid speaker for and/or participated in research sponsored by companies that manufacture drugs used for the treatment of psoriasis including Abbott, Janssen-Cilag, MSD and Pfizer. P.C.M.v.d.K. has served as a consultant for Schering Plough, Celgene, Centocor, Allmirall, UCB, Wyeth, Pfizer, Soffinova, LEO Pharma, Actelion, Galderma, Novartis, Janssen-Cilag and Abbott; he has received research grants from Centocor, Wyeth, Schering Plough, Merck Serono, LEO Pharma, Philips Lighting, Pfizer, Janssen-Cilag and Abbott; and has carried out clinical trials for Allmirall, Celgene, GSK, Eli Lily, Amgen, Centocor, Wyeth, Schering Plough, Merck Serono, Abbott and Philips lighting.