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Rapid communication

Expression of antimicrobial peptides and proteins in etanercept-treated psoriasis patients

T. Gambichler *, S. Kobus, A. Kobus, C. Tigges, N. Scola, P. Altmeyer, A. Kreuter, F.G. Bechara, M. Skrygan

Department of Dermatology, Ruhr-University Bochum, Germany

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ABSTRACT

Recent papers highlight the role of dysregulated expression of antimicrobial peptides and proteins (AMPs) in the pathogenesis of psoriasis. Etanercept, a blocker of the pro-inflammatory cytokine tumour necrosis factor-α (TNF-α), is effective in the treatment of psoriasis. We aimed to evaluate the expression profiles of AMPs in psoriatic skin before and after a 6-week course of etanercept therapy. We included 12 psoriasis patients who underwent medium-dose etanercept treatment for 6 weeks. At baseline and at the end of therapy immunohistochemistry from lesional skin was performed for psoriasin, LL-37, and human ß-defensin 2 (hBD-2). After 6-week treatment, the modified psoriasis area and severity index significantly decreased from 37.5 ± 5.9 to 14 ± 13.4. Lesional immunoreactivity scores of psoriasin, LL-37, and hBD-2 also significantly decreased after a 6-week course of etanercept. We have demonstrated that etanercept-induced improvement of psoriasic lesions is associated with a significant decline of AMP protein expression.

1. Introduction

Psoriasis is one of the most common chronic dermatoses affecting approximately 2% of the general population in Western countries. There is a strong evidence for a genetic basis of psoriasis. Moreover, a large body of evidence has suggested a dysregulated interplay between keratinocytes and infiltrating immune cells underlying cutaneous inflammation in psoriasis. Cytokines and other soluble factors such as antimicrobial peptides and proteins (AMPs) secreted by resident or infiltrating cells are essential elements in this process of cell–cell communication in psoriasis [1]. Recent papers highlight the role of dysregulated expression of AMPs such as human β-defensins (HBD), cathelicidins (e.g., LL-37), and psoriasin (S100A7) in the pathogenesis of psoriasis and other inflammatory skin disorders [2–6].

The central role of the pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) in psoriasis has definitely come to light through the observations of the efficacy of anti-TNF-α biological therapies. Etanercept is a competitive inhibitor of TNF-α that prevents interaction between this cytokine and its cell surface receptors. In agreement with the guidelines of the British Association of Dermatologists, etanercept is recommended as the anti-TNF-α agent of choice for patients with moderate-to-severe stable psoriasis [7–10]. In the present study, we aimed to evaluate the protein expression profiles of AMPs in psoriatic skin before and after a 6-week course of etanercept therapy.

2. Material and methods

2.1. Study design and patients

This was a prospective study which was performed in the dermatology hospital of the Ruhr-University Bochum. The study was conducted in the light of the declaration of Helsinki and followed a protocol approved by our institutional review board. Patients were recruited in the study after being given informed consent. Inclusion criteria for enrolment into the study included moderate to severe plaque-type psoriasis for more than 6 months and PASI above 10. Exclusion criteria were as follows: Age younger than 18 years; pregnancy or lactation; autoimmune disease, or severe renal or hepatic disease; a history of previous treatments within the last 4 weeks before enrolment into the study including phototherapy, immunosuppressive and/or immunomodulating drugs (e.g., corticosteroids, methotrexate, cyclosporine) and biological agents such as etanercept, infliximab, adalimumab, and ustekinumab.

2.2. Etanercept therapy and clinical assessments

Medium-dose etanercept (Enbrel®, Wyeth) at a dose of 25 mg was subcutaneously administered twice weekly [9]. Before the beginning of the study, a marker lesion at the trunk was selected for the determination of the modified psoriasis area and severity index (M-PASI) [11]. In brief, the determination of the M-PASI was conducted as follows: The M-PASI is a modified area score in which the maximum of involved skin is considered to be 100%. Erythema, infiltration, and desquamation are each assigned a value from 0 to 4;

* Corresponding author at: Department of Dermatology, Ruhr-University Bochum, Gudrunstr. 56, D-44791 Bochum, Germany. Tel.: +49 234 5093458; fax: +49 234 5093445.
E-mail address: t.gambichler@klinikum-bochum.de (T. Gambichler).

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the sum of these values is multiplied with a score for the involved area [11]. Four millimeter punch biopsies were performed in lesional skin at baseline and after 6-week treatment. During the study, patients were only allowed to use topical emollients in addition to etanercept therapy.

2.3. Immunohistochemical analysis of AMP expression

Four μm paraffin-embedded sections were mounted on silanized slides and stored for 1 h in a humid chamber at 60 °C. Sections were deparaffinized in xylene and washed with 100%, 96%, 70% and 50% ethanol for 5 min each and rinsed with demineralized water. After washing with Target Retrieval Solution (DAKO), sections were stored for 20 min in a water bath (96 °C) with a pH of 6.0 for psoriasin and LL-37 or 9.0 for hBD-2. Protease blocking was performed in 3% hydrogene peroxide for 10 min. Staining for hBD-2 was performed using a goat anti-hBD-2 (dilution of 1:250) antibody (PeproTech Cell Concepts, NJ, USA). Staining for psoriasin was performed using a mouse anti-psoriasin (dilution of 1:50) antibody (Novocastra laboratories, Newcastle upon Tyne, United Kingdom). Staining for LL-37 was performed using a goat anti-LL-37 (dilution of 1:250) antibody (Abcam Inc., Cambridge, MA, USA). The following secondary antibodies were used: for hBD-2, anti goat IgG (dilution of 1:500; Vector Laboratories Inc., Burlingame, CA, USA) and for the other AMPs, anti-mouse and -goat IgG (DAKO), respectively. After washing with Wash Buffer 10 times for 2 min, streptavidin-AP (DAKO) was used as enzyme for 30 min. Chromogen red (Red permanent, DAKO) was used for visualization before counterstaining with haematoxylin and mounting in Mowiol (Roche Molecular Biochemicals, Mannheim, Germany).

2.4. Microscopic evaluation of immunohistochemistry

Immunopositivity of all AMPs under investigation was evaluated in each compartment of the epidermis within 5 fields of view. The semi-quantitative evaluation included the following score: 0 = no immunoreactivity at all; 1 = immunoreactivity in the stratum corneum; 2 = immunoreactivity in the stratum corneum and stratum granulosum; 3 = immunoreactivity in the stratum corneum, stratum granulosum, and stratum spinosum; 4 = immunoreactivity throughout the epidermis.

2.5. Statistics

Data analysis was performed using the statistical package MedCalc Software (Mariakerke, Belgium). Distribution of data was assessed by the D`Agostino-Pearson test. Means, standard deviation (SD), and 95% confidence intervals (CI) were calculated. M-PASI data and immunohistology scores were analyzed using paired t-tests. Exploratory assessment of correlation was performed using the Pearson procedures. Statistical significance was set at the 5% level (2-sided).

3. Results

We included 12 patients (7 males, 5 females) with moderate to severe psoriasis into the study. Patients mean ± SD age was 42 ± 12.7 years. After 6-week treatment using medium-dose etanercept, the M-PASI significantly decreased from 37.5 ± 5.9 to 14 ± 13.4 ($P = 0.0008$, CI −34.3 to −12.7). As shown in Fig. 1 immunoreactivity score of psoriasin was significantly decreased after a 6-week course of etanercept (3.5 ± 0.6 vs. 2.3 ± 0.6; $P = 0.0004$). After etanercept treatment, there was also significant decline of LL-37 immunopositivity (3.2 ± 0.6 vs. 2.1 ± 1.1; $P = 0.0018$; Fig. 2). Moreover, hBD-2 protein expression was significantly decreased after therapy when compared to baseline (1.4 ± 0.9 vs. 0.5 ± 0.6; $P = 0.0023$; Fig. 3). Correlation studies did not reveal a significant relationship between the expression levels of AMPs assessed ($r<0.1$; $P>0.05$). However, relative M-PASI reduction (62.5% ± 38.8%) moderately correlated ($r = 0.34$; $P = 0.045$) with the relative reduction of hBD-2 immunoreactivity score (63.4% ± 40.1). There was no significant correlation ($r<0.3$; $P>0.05$) between the relative M-PASI reduction and the relative reduction of the immunoreactivity scores of psoriasin (31.8 ± 20.4%) and LL-37 (36.1% ± 28.3%) protein expression.

4. Discussion

In several studies, it has been shown that AMPs such hBD-2, psoriasin, and LL-37 are strongly overexpressed in psoriatic plaques [2,3,12–14]. It was recently shown that the cathelicidin peptide LL-37 is able to suppress apoptosis induction in keratinocytes. Deregulation of apoptosis control is characteristic for keratinocytes in psoriasis and

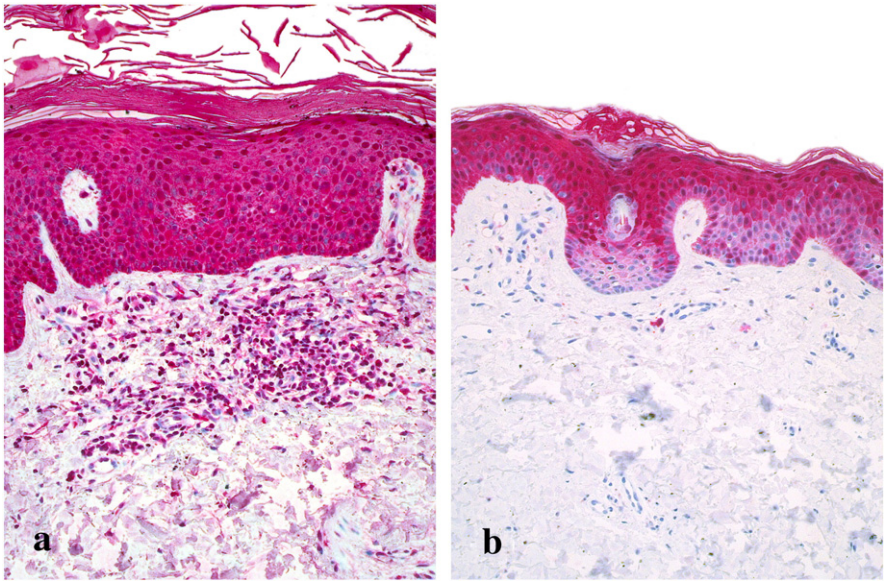

Fig. 1. Showing immunohistology of psoriasin expression before (a) and after (b) 6-week etanercept treatment. After therapy, reduced psoriatic skin morphology was accompanied by a decrease of psoriasin protein expression.