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Serum Levels of IL-17 and IL-22 Are Reduced by Etanercept, but not by Acitretin, in Patients with Psoriasis: a Randomized-Controlled Trial

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Abstract
Introduction There are no controlled trials comparing etanercept and acitretin efficacy and therapeutic mechanisms in psoriasis.
Materials and methods In the present study, 30 patients were given etanercept 50 mg twice weekly and 30 patients acitretin 0.4 mg/kg per day, both for 12 weeks. Before and after treatment, psoriasis area and severity index was calculated, and serum levels of interleukin (IL)-17, IL-22, and IL-23 were investigated.
Results After treatment, psoriasis area and severity index was significantly lower for both groups. However, etanercept-treated patients showed lower psoriasis area and severity index than acitretin-treated ones. Psoriasis patients showed higher IL-17 and IL-22 levels than controls, while no IL-23 was found in any serum. Furthermore, a correlation between IL-17 levels and psoriasis severity was found. Only etanercept was able to reduce IL-17 and IL-22 levels.
Conclusions Our findings suggest that etanercept is more effective than acitretin in the treatment of psoriasis and that it is able to affect Th17 system.

Keywords Psoriasis · IL-17 · IL-22 · IL-23 · etanercept

Introduction

Psoriasis is a common cutaneous inflammatory disease affecting 2–5% of the world's population. Although for a long time it have been under debate whether psoriasis might be considered a primary disease of keratinocytes, it is now clear that its pathogenesis is mainly T-cell-mediated [1]. Until recently, T helper (h) 1 cells were implicated as the main pathogenic cells, and large amounts of Th1 cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-2 have been found in psoriatic skin [2], while a reduced expression of IL-4 and IL-10 have been reported [3]. However, there are growing evidences that a recently recognized subset of T cells, Th17 cells, may play an important role in the pathogenesis of psoriasis [4–12]. The Th17 lineage has emerged from discovery of a new family of cytokines, the IL-17 family, that comprises IL-17 and IL-22 [13]. Th17 cells differentiate from naïve $CD4^+$ T cells under the stimulation of IL-1, IL-6, and transforming growth factor-β [4], and their proliferation is driven by IL-23 [14]. Th17 cells appear to have evolved as an arm of the adaptive immune system specialized for enhanced host protection against extracellular bacteria and some fungi, microbes probably not well covered by Th1 or Th2 immunity [13]. They can produce IL-17, IL-22, IL-6, and TNF-α [15] and are now considered responsible for many of the inflammatory autoimmune responses once attributed to Th1 cells [16–18].

TNF inhibitors are emerging drugs for the treatment of several inflammatory diseases. Although their efficacy for the treatment of chronic plaque psoriasis has been well established in clinical trials and through real-world experience, there are little data on their therapeutic mechanisms in such a disease [19, 20]. In the present study, we have investigated the effects of etanercept on the serum levels of Th17-related cytokines, in comparison with acitretin. Acitretin is a second-generation systemic retinoid that has been demonstrated to be effective in the treatment of psoriasis by several studies [21]. Although both drugs were successful in clearing the

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disease, only etanercept, and not acitretin, modulates the expression of IL-17 and IL-22.

Materials and Methods

Patients

The present trial was approved by the medical ethical committee of our hospital and was conducted according to the Declaration of Helsinki; all patients provided written informed consent. We enrolled consecutively 60 patients with moderate-to-severe plaque-type psoriasis without joint involvement, who were required to have a baseline Psoriasis Area and Severity Index (PASI) of ≥10 and at least 10% of total body surface area affected by psoriasis. All the patients were comparable in terms of previous treatments, duration of psoriasis, and phase of psoriasis activity. Patients who were treated in the previous month with any topical or systemic psoriasis therapy, as well as those who had a history or risk of serious infection, lymphoproliferative disease, or active or latent tuberculosis, were excluded.

Half the patients were given etanercept (Enbrel®) 50 mg twice weekly (group 1) and the other half acitretin (Neotigason®) 0.4 mg/kg per day (group 2), both for 12 weeks. No topical treatment was administered during the trial. Patients were randomly assigned to one of the two groups. At the baseline (T0) and at the end of the treatment (T1), a blind clinical assessment by calculating PASI was made, and blood samples were taken to evaluate IL-17, IL-22, and IL-23 levels. All the patients (30 etanercept-treated patients, 13 males and 17 females, age range 28–67, and 30 acitretin-treated patients, 11 male and 19 female, age range 31–65) concluded the trial successfully. As controls, ten healthy donors (five male and five female, age range 28–59) were included in the study.

Cytokine Analysis

Blood samples were collected from patients and controls and were centrifuged for 20 min at 10,000×*g* (5417R microcentrifuge; Eppendorf, Hamburg, Germany). Then, serum samples were subdivided into small aliquots to be stored at −80°C until tested for cytokine levels. Solid-phase enzyme-linked immunosorbent assay kits were used to determine IL-17, IL-22 (R&D Systems, Minneapolis, MN, USA), and IL-23 p19–p40 heterodimer (BioSource Europe s.a., Nivelles, Belgium) serum levels, according to the manufacturer's instructions. Results are expressed in picogram per milliliter as mean±standard deviation. Samples and standards were analyzed in duplicates and only variation coefficients <15% were accepted.

Statistical Analysis

Statistical analysis was performed with a Wilcoxon signed rank test between the pretreatment and posttreatment groups, and a Wilcoxon rank sum test was used for comparing group 1 with group 2. Correlation was calculated by Spearman's test. Differences were considered as statistically significant when $p<0.05$.

In order to verify the homogeneity of the two groups, we preliminarily compared clinical and serological parameters between the two pretreated series.

Results

At T0, both groups 1 and 2 showed a similar PASI (21.54±9.09 and 22.25±5.73, respectively). At T1, 17 of 30 patients (56.7%) of group 1 and eight of 30 patients (26.7%) of group 2 attained PASI-75 responses, while 26 of 30 patients (86.7%) of group 1 and 20 of 30 patients (66.7%) of group 2 achieved PASI-50 results. Mean PASI at T1 was significantly lower than at T0 both for group 1 and for group 2 (4.61±2.75, $p<0.001$ and 9.62±4.64, $p<0.001$, respectively). However, group 1 showed lower PASI values than group 2 after treatment, with statistical significance ($p=0.005$; Fig. 1).

IL-17 and IL-22 were detected in all the patients and controls, while no detectable IL-23 was found in any serum. Psoriasis patients showed significantly higher IL-17 levels than controls, without differences between the two treatment groups (52.86±27.63 in group 1, 52.01±21.38 in group 2, and 19.68±9.78 in controls; group 1 vs controls: $p=0.001$; group 2 vs controls: $p=0.003$; Fig. 1). A statistically significant positive correlation between PASI and IL-17 concentrations was found in all the psoriasis patients ($p=0.003$; Fig. 2).

Etanercept achieved a significative reduction in IL-17 levels (21.48±14.01, $p<0.001$), while acitretin did not (48.60±19.86). Furthermore, at T1, IL-17 levels of group 1 patients were similar to those of healthy controls (Fig. 1).

As for IL-17, IL-22 levels were similar in the two groups of patients and were higher in all psoriasis patients than in controls (50.58±46.15 in group 1, 52.81±42.49 in group 2, and 6.52±2.31 in controls; group 1 vs controls: $p<0.001$; group 2 vs controls: $p<0.001$; Fig. 1). No statistical significative correlation was found between IL-22 levels and PASI scores (Fig. 2).

Etanercept significantly reduced IL-22 levels in a 12-week-lasting therapy (22.02±25.36, $p=0.01$), while acitretin did not affect IL-22 expression (48.69±38.5; Fig. 1).

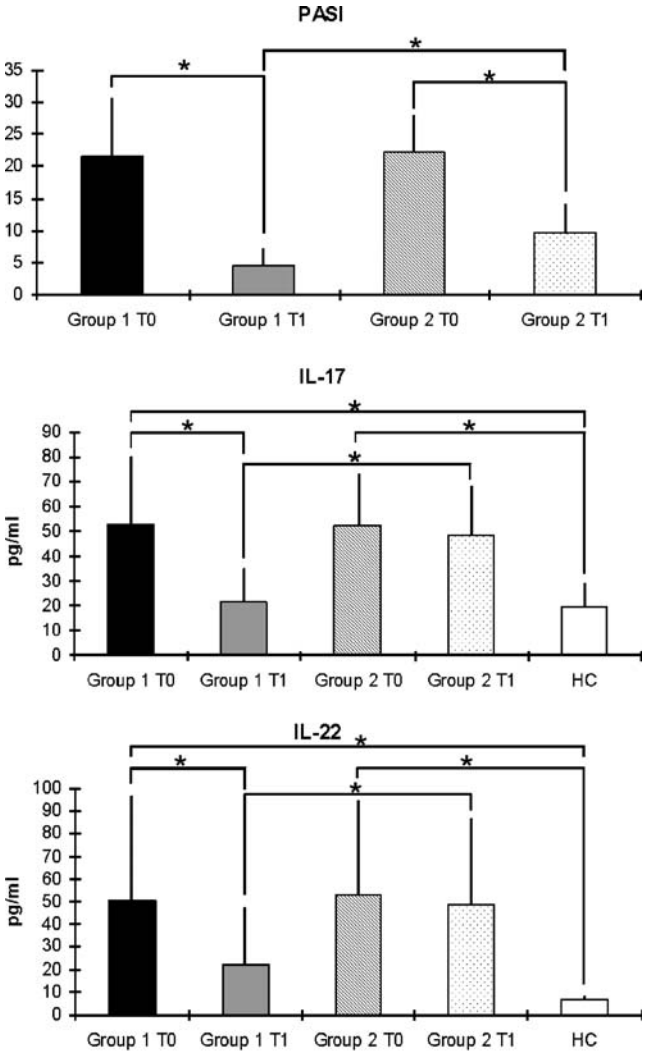

Fig. 1 PASI, IL-17, and IL-22 mean values±standard deviation at baseline (*T0*) and after 12 weeks (*T1*) in etanercept-treated patients (*group 1*), acitretin-treated patients (*group 2*), and healthy controls (*HC*). * = $p<0.05$

Discussion

In the present trial, several topics have been investigated in psoriasis patients: the first one has been the comparison between clinical efficacy of etanercept and acitretin, the second one has been the role of serological markers of a novel T cell lineage, namely Th17 cells, and the last one has been the effects of etanercept (and acitretin) on such markers.

To our knowledge, there are no controlled trials in the literature comparing the effects of etanercept and acitretin in the treatment of moderate-to-severe chronic plaque psoriasis. Although our data confirmed that both drugs were able to induce a significative reduction in the PASI index after a 12-week-lasting course [22], etanercept was more effective than acitretin, with a posttreatment PASI lower than acitretin.

The second topic investigated in the present study has been the presence of the Th17 inducer cytokine IL-23 and of the Th17 effector molecules IL-17 and IL-22 in the sera of patients with psoriasis. Although recent works have shown higher levels of IL-17, IL-22, and IL-23 in psoriasis lesions [4–6, 8–12], there are only few studies regarding these molecules in the serum. In particular, while higher circulating IL-22 expression in psoriasis patients has been demonstrated [5, 7], IL-17 serum levels have not revealed any increase [23] and, to date, no studies are present in the literature about serum levels of IL-23 in psoriasis.

According to recent studies suggesting an important role of Th17 cells in the pathogenesis of psoriasis [4–12], in our series, psoriasis patients showed higher IL-17 and IL-22 levels than controls, and IL-17 values correlates with psoriasis severity. Both IL-17 and IL-22 appear to serve as inducers of keratinocytes to produce antimicrobial peptides, S100 acute-phase proteins, and chemokines (such as IL-8) and chemokine receptors, thus leading to neutrophilic chemotaxis within psoriasis lesions [24, 25]. Furthermore, IL-22 strongly induces hyperplasia of in vitro reconstituted human epidermis [25, 26] and may be responsible for the acanthosis typically found in psoriasis.

On the contrary, no IL-23 was found in any patient or control serum in our study. Such finding is probably related

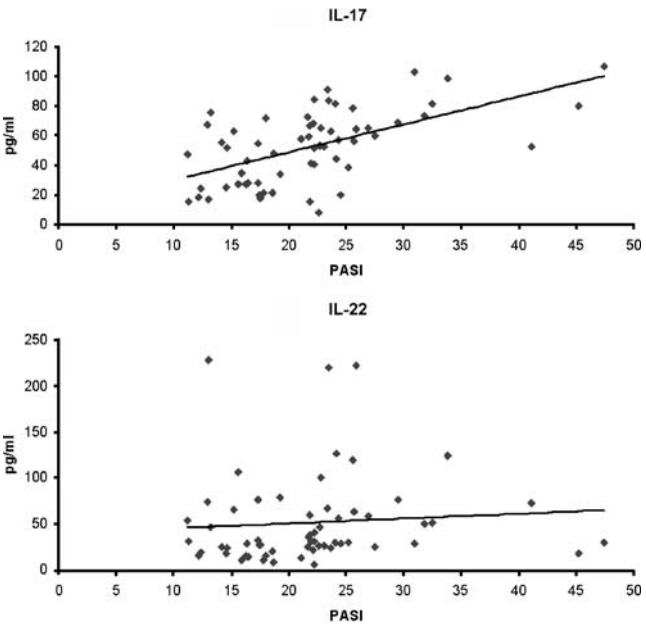

Fig. 2 IL-17 and IL-22 serum levels and PASI in psoriasis patients before treatment. A positive correlation between IL-17 concentrations and PASI values was found ($p=0.003$), while no statistical significative correlation was present between IL-22 levels and PASI scores

to the role of IL-23 in Th17 biology: in fact, IL-23 is an inducer of Th17 cells and of Th17-related cytokines IL-17 and IL-22 [13], so it may be involved in the early phases of psoriasis pathogenesis and may be absent in chronic and stable forms of the disease. However, according to the findings of an augmented expression of IL-23 in psoriasis lesions [4, 8, 11], another possibility is that such a cytokine may be produced in lesional sites and then rapidly involved in autocrine or paracrine mechanisms of cell stimulation, without the capability to reach blood vessels in enough amounts to be detected.

The most important aim of our study has been the comparison between etanercept and acitretin effects on Th17-related cytokines. According to a recent study, which has shown that etanercept is able to downregulate Th17 products in cutaneous lesions of psoriasis [4], our data demonstrated a reduction of IL-17 and IL-22 serum levels after a 12-week-lasting therapy with etanercept in psoriasis patients. It has been proposed that etanercept effects on Th17-related molecules may be explained by the inhibition of inflammatory dendritic cell cytokine production and maturation, leading to a reduction in the activity of Th17 cells [4]. In addition, TNF-α directly induces IL-17 production [13], so its blockade by etanercept may further explain the reduction of IL-17 serum levels.

Finally, our findings suggest that while etanercept may modulate IL-17 and IL-22 production via TNF inhibition, acitretin is not able to affect their serum levels and to interfere with Th17 pathway; thus, acitretin efficacy in psoriasis clearing is probably only due to direct effects on the keratinocyte differentiation and proliferation via the interaction with its specific nuclear receptor [27].

To sum up, our study provides further evidences of the role of Th17 pathway in the pathogenesis of psoriasis. Moreover, for the first time, a comparison of the clinical efficacy therapeutic mechanisms between etanercept and acitretin has been performed. Our findings suggest that etanercept is more effective than acitretin in the treatment of psoriasis and that it is able to downregulate IL-17 and IL-22 serum levels, while acitretin is not. However, other studies are required to further investigate the differences in the therapeutic mechanisms of these two widely used drugs for psoriasis treatment.

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