Actas Dermosifiliogr. 2007;98:539-44

ORIGINAL ARTICLES

Analysis of Lymphocyte Populations in Psoriatic Plaques Following Inhibition of Tumor Necrosis Factor-α With Etanercept

L Mahiques,[a] G Pitarch,[a] JL Sánchez-Carazo,[a] A Pérez-Ferriols,[a] CJ Soriano,[b] and V Alegre[a]

[a]Servicio de Dermatología and [b]Servicio de Cardiología, Hospital General Universitario de Valencia, Valencia, Spain

Abstract. *Introduction.* Psoriasis is a disease with a strong immunological component in which there is a predominant T helper 1 cell-mediated immune response. Etanercept, a receptor for tumor necrosis factor α that blocks its action, is a new drug with proven efficacy in the treatment of psoriasis.

Objectives: The primary objective of this study was to assess the histological response to etanercept by analyzing the lymphocyte populations in psoriatic plaques. The secondary objectives were to assess the clinical response to the drug using the Psoriasis Area and Severity Index (PASI) and to analyze the effect of etanercept on peripheral blood lymphocyte populations.

Methods. Ten patients with plaque psoriasis and a PASI score greater than 10 were included in the study. A clinical assessment was performed in all patients along with a 4-mm skin punch biopsy of a plaque and analysis of peripheral blood lymphocyte populations at baseline and after 12 weeks of etanercept therapy at a dose of 50 mg per week.

Results. There was a significant reduction in different lymphocyte populations in the plaques following treatment with etanercept. The mean (SD) number of $CD4^+$ T lymphocytes per microscopic field decreased from 16.93 (8.13) at baseline to 6.51 (3.46) after treatment with etanercept ($P < 007$). $CD8^+$ T lymphocytes also decreased from 17.73 (9.77) before treatment to 10.50 (9.4) after treatment ($P < 005$). An overall improvement in PASI score was also observed: 33.30 (10.71) at baseline versus 15.20 (13.28) following treatment ($P < 008$). Nine out of 10 patients showed improvement. No significant differences were observed in peripheral blood lymphocyte populations before and after treatment.

Conclusions. Etanercept leads to clinical improvement of psoriasis and reduces inflammatory infiltration of the lesions without affecting peripheral blood lymphocyte populations.

Key words: psoriasis, lymphocytes, treatment.

ESTUDIO DE LAS POBLACIONES LINFOCITARIAS EN LAS LESIONES DE PSORIASIS TRAS EL BLOQUEO DEL FACTOR DE NECROSIS TUMORAL ALFA CON ETANERCEPT

Resumen. *Introducción.* La psoriasis es una enfermedad de fuerte base inmune, con un predominio de respuesta inmune celular o Th1. Entre los nuevos fármacos que han demostrado eficacia está el etanercept, un receptor para el factor de necrosis tumoral alfa que bloquea su acción.

Objetivos. Como objetivos nos planteamos en primer lugar determinar la respuesta histológica a etanercept mediante la determinación de las poblaciones linfocitarias en las lesiones psoriásicas; y en segundo lugar, determinar la respuesta clínica al fármaco mediante el *Psoriasis Area and Severity Index* (PASI) y valorar el efecto de etanercept sobre las poblaciones linfocitarias en sangre.

Métodos. Tratamos 10 pacientes con psoriasis en placas con PASI > 10. Se les realizó una evaluación clínica, un punch de 4 mm en la placa y una determinación de poblaciones linfocitarias en sangre antes del comienzo y tras 12 semanas de tratamiento con etanercept 50 mg/semana.

Correspondence:
Laura Mahiques Santos.
Servicio de Dermatología.
Hospital General Universitario de Valencia.
Avda. Tres Cruces, s/n. 46014 Valencia. Spain
E-mail: laura_mahiques@aedv.es

Manuscript accepted for publication May 9, 2007

Resultados. Hubo un descenso significativo en las distintas poblaciones linfocitarias de las lesiones tras el tratamiento con etanercept. Así, los linfocitos T CD4+ antes y después del tratamiento con etanercept registraron los siguientes valores: 16,93 ± 8,13 y 6,51 ± 3,46 (p < 0,007), respectivamente. Y los linfocitos T CD8+ antes y después del tratamiento con etanercept registraron: 17,73 ± 9,77 y 10,50 ± 9,4 (p < 0,005), respectivamente. El PASI mejoró globalmente tras 12 semanas de tratamiento. En un principio el PASI basal mostraba 33,30 ± 10,71, y tras el tratamiento se registró 15,20 ± 13,28, p < 0,008. Nueve de los diez pacientes mejoraron y una paciente se mantuvo sin mejoría. No hubo diferencias significativas en las poblaciones linfocitarias sanguíneas antes y después del tratamiento.
Conclusiones. Etanercept es un fármaco que mejora clínicamente la psoriasis y disminuye la infiltración inflamatoria de las lesiones sin afectar a las poblaciones linfocitarias sanguíneas.

Palabras clave: psoriasis, linfocitos, tratamiento.

Introduction

Psoriasis is a chronic disease that affects 2% to 3% of the world's population.[1] Until the 1980s, it was considered a primary disorder of keratinocytes in which abnormal differentiation led to hyperproliferation of those cells.[2,3] Nowadays it is classified as one of the most common T-cell-mediated autoimmune diseases.[4] The characterization of T-cell infiltration in psoriatic plaques has revealed a predominance of $CD4^+$ cells in the dermis and $CD8^+$ cells in the epidermis.[5,6] Several studies have demonstrated the importance of tumor necrosis factor-α (TNF-α) in the development and persistence of psoriatic lesions.[7,8]

The disease-modifying drug etanercept (Enbrel) is a fully human fusion protein that is made up of the extracellular 75 kd portion (p75) of the natural TNF-α receptor linked to the Fc portion of immunoglobulin G1.[9,10] It acts as a competitive inhibitor of the cytokine TNF-α by binding to it and preventing it from interacting with its receptors on the surface of different cell populations. Unlike other TNF inhibitors, etanercept does not induce complement-mediated cell death in vitro.

We designed a study to evaluate the effect of TNF-α inhibition on T cells in psoriasis by analyzing the response of T-cell populations to the administration of etanercept in a group of patients with psoriasis.

Objective

The primary objective of the present study was to evaluate histologic response to treatment with etanercept by quantifying T-cell populations ($CD4^+$ and $CD8^+$) in psoriatic lesions. As secondary objectives, we analyzed the clinical response to etanercept, measured with the Psoriasis Area and Severity Index (PASI), and the effect of etanercept on $CD4^+$ and $CD8^+$ cell populations in peripheral blood.

Materials and Methods

We enrolled 10 patients with moderate to severe plaque psoriasis over a period of 6 months. None of the patients had received systemic therapy in the preceding 8 weeks and they had all stopped using topical treatment 2 weeks beforehand. Biological therapies had not been used in any of the patients. Signed informed consent was obtained from all the patients prior to both treatment and biopsies.

Body mass index (BMI) was measured before treatment was started in all cases.

The study was approved by the hospital's ethics committee.

Etanercept (Enbrel) was administered as monotherapy at a dose of 25 mg twice a week for 12 weeks.

Disease involvement was determined using the PASI, with scores being measured before treatment and at 12 weeks.

In all cases, 4-mm punch biopsies were obtained from representative plaques before treatment and after 12 weeks of treatment.

Anesthesia was achieved using mepivacaine without vasoconstrictor, and the biopsy site was closed using nonresorbable monofilament suture material.

The plaque biopsy specimens were fixed in 10% buffered formalin. Once the diagnosis of psoriasis had been confirmed using hematoxylin–eosin staining, we performed immunohistochemical stains using the Bond Polymer Detection System (BioSystems Vision, Melbourne, Australia) with antibodies to identify $CD4^+$ cells and $CD8^+$ cells (Novocastra Laboratories, Newcastle, United Kingdom).

The different populations of $CD4^+$ and $CD8^+$ cells were quantified at 400× magnification. In the epidermis, all the labeled cells in the microscopic field were counted from the basement membrane to the stratum corneum, and in the dermis, the cells were counted to a depth of 600 #mm

throughout the specimen (4 mm). The mean number of cells per field was then calculated. The total number of cells in each biopsy specimen was expressed as the number of positive cells per microscopic field at 400× magnification.

T-cell populations were counted automatically and expressed as the number of cells per microliter.

Statistical Analysis

Continuous variables were expressed as means (SD) and categorical variables as frequencies. The Wilcoxon test for paired data was used to compare continuous variables before and after treatment with etanercept. The Spearman correlation coefficient was used to calculate the correlation between PASI scores after treatment and both T cells and BMI. Statistical significance was set at a 2-tailed value of $P<.05$. All the statistical analyses were performed using the SPSS statistical package, version 11 for Windows (SPSS Inc, Chicago, Illinois, USA).

Results

Patients

Ten patients aged between 30 and 56 years, with a mean (SD) age of 48.44 (12.02) years, participated in the study; the group included 6 (60%) women and 4 (40%) men. The BMI ranged from 20.96 to 36.52 kg/m^2, with a mean (SD) value of 20.87 (5.39) kg/m^2.

Dermatopathology

Twenty biopsies from 10 patients were analyzed, and total T-cell populations were quantified in both the dermis and epidermis (Table 1). We observed a considerable reduction in the different populations following treatment with etanercept. The total number of CD4$^+$ cells before and after treatment with etanercept was 16.93 (8.13) and 6.51 (3.46), respectively ($P<.007$); the corresponding figures for CD8$^+$ cells were 17.73 (9.77) and 10.50 (9.46) ($P<.005$). The number of CD4$^+$ cells in the dermis decreased from 14.46 (6.42) at baseline to 6.11 (3.41) after treatment with etanercept ($P<.007$), and the number of CD8$^+$ cells decreased from 17.29 (7.17) to 11.57 (6.50) ($P<.005$). The corresponding reductions in cell populations in the epidermis were 2.47 (2.18) to 2.18 (0.29) for CD4$^+$ cells ($P<.008$) and 17.73 (9.77) to 10.50 (9.46) for CD8$^+$ cells ($P<.005$).

We also found a significant difference in the CD4$^+$/CD8$^+$ ratio in the dermis: 0.94 (0.27) before treatment versus 0.62 (0.36) after treatment ($P<.047$).

Clinical Effects

There was an overall improvement in PASI scores after the 12 weeks of treatment. The score before treatment was 33.30 (10.71) compared to 15.20 (13.28) after treatment ($P<.008$). Nine of the 10 patients showed improvement and 1 continued without change.

We found no significant correlation between changes in the numbers of either CD4$^+$ or CD8$^+$ cell populations and PASI improvement: $\rho=-0.49$ ($P=.15$) for CD4$^+$ and $\rho=-0.21$ ($P=.55$) for CD8$^+$. We also found no correlation between BMI and PASI improvement ($\rho=-.30$, $P=.93$).

Blood

We analyzed 20 blood samples from 10 patients. There were no significant differences in T-cell populations in the blood before and after treatment with etanercept. The mean number of CD4$^+$ cells was 1204.40 (869.59) cells per microliter before treatment and 1021.67 (409.63) cells per microliter after treatment ($P<.441$). The corresponding figures for CD8$^+$ cells were 642.50 (351.52) and 583.11 (182.69) cells per microliter ($P<.55$).

Table. T-Cell Populations in the Skin, Dermis, and Epidermis[a]

	T-Cells Before Treatment	*After 12 Weeks of Treatment With Etanercept*	*P*
Total CD4± cells	16.93 (8.13)	6.51 (3.46)	<.007
Total CD8± cells	17.73 (9.77)	10.50 (9.46)	<.005
Epidermal CD4± cells	2.47 (2.18)	0.40 (0.29)	<.008
Epidermal CD8± cells	17.73 (9.77)	10.50 (9.46)	<.005
Dermal CD4± cells	14.46 (6.42)	6.11 (3.41)	<.007
Dermal CD8± cells	17.29 (7.17)	11.57 (6.50)	<.005

[a]Data are shown as means (SD).

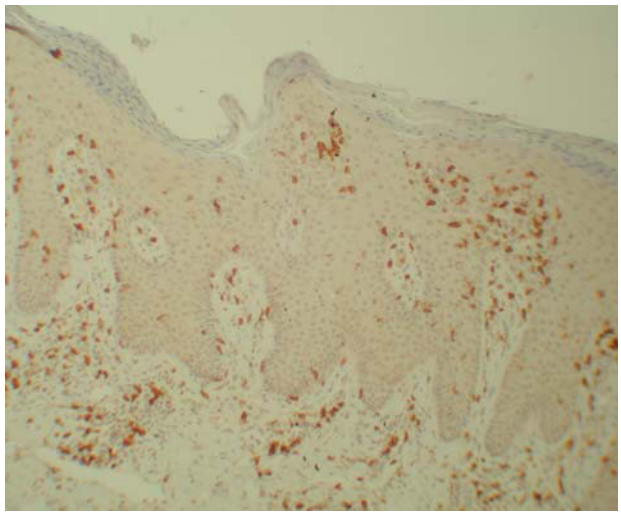

Figure 1. CD8+ cells before treatment (Bond Polymer Detection System, ×200)

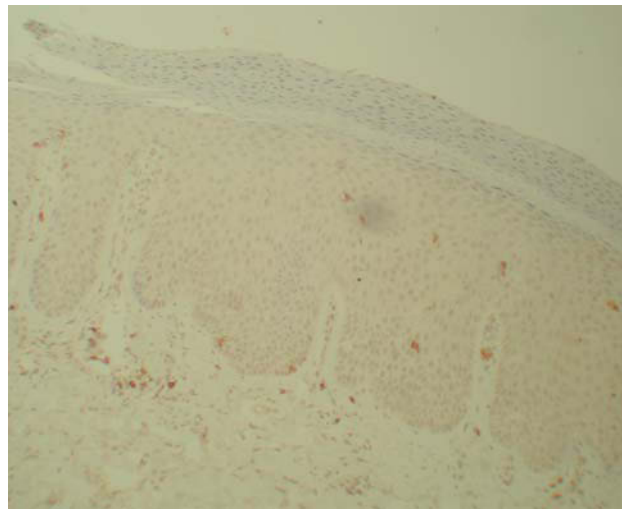

Figure 2. CD8+ cells after treatment (Bond Polymer Detection System, ×200)

Nine patients (90%) werexnegative for antinuclear antibodies (ANAs) and extractable nuclear antigens (ENAs) both before and after treatment. One patient (10%) was positive for ANAs with a titer of 1:640 and negative for ENAs before treatment. After treatment, the ANA titer increased to 1:1280 and anti-ribonucleoprotein antibodies were detected; the patient also experienced an outbreak of polyarthritis in the hands and wrists. The symptoms improved and the autoantibody levels decreased by prolonging treatment with etanercept and combining it with methotrexate at a dose of 25 mg per week.

Adverse Events

One patient developed impetigo of the nostrils due to staphylococcus aureus; the condition resolved without complications following the administration of systemic therapy. Another patient presented a radiation recall reaction at the injection site; this was successfully controlled with local steroids.

Discussion

Psoriasis is a chronic inflammatory skin disease that can develop in association with arthritis. The etiology of the disease has been suggested to include various genes that interact with each other and with the environment to produce the phenotype of the disease.[11,12] Psoriatic lesions are characterized by the excessive proliferation and abnormal maturation of keratinocytes and an inflammatory infiltrate composed primarily of T cells, with a predominance of CD4+ cells in the dermis and CD8+ cells in the epidermis.[13] The local activation of these cells has been shown to play a vital role in the development and persistence of psoriatic lesions.[4,14]

Etanercept is a fusion protein made up of 2 subunits of the p75 receptor for TNF-α; it acts by competitively binding to TNF-α and preventing it from interacting with its cell-surface receptors. It has proven to be an efficient means of treating both psoriasis and psoriatic arthritis, improving symptoms[8,15] and reducing the inflammatory infiltrate in the lesions. We believe that etanercept is an ideal drug with which to study the immune effect of TNF inhibition on psoriasis plaques because it acts by neutralizing TNF and not by depleting the cells that express this molecule on their surface.

We found that the overall number of T cells in psoriatic lesions decreased after just 12 weeks of monotherapy with etanercept. Our findings coincide with those of Gottlieb et al,[16] who also found a reduction after 24 weeks. In our study, CD4+ and CD8+ cell populations decreased in both the dermis and the epidermis, but not in peripheral blood. TNF inhibitors have also been seen to reduce T-cell populations in lesions produced in animal models, suggesting that the local proliferation of T cells in psoriasis is linked to the local production of TNF–a.[4] The reduction in the dermal CD4+/CD8+ ratio following treatment is consistent with findings that indicate that CD4+ cells but not CD8+ cells are capable of triggering psoriatic lesions,[17] and in our case would indicate that the lesions had resolved.

We analyzed autoantibody levels in search of possible increases following treatment with etanercept and found that the levels had increased in just 1 patient; that patient had tested positive for ANAs before treatment was started,

suggesting that autoantibody levels increase only in predisposed individuals.

The clinical improvement observed in 9 of our 10 patients coincides with reports in the literature.[18] The 1 patient who did not experience clinical improvement had ANAs prior to treatment with etanercept, and even higher levels following treatment. There were no clinical or histologic features of lupus, as proven by the negative results of the direct immunofluorescence study. The patient did, however, develop an acute episode of polyarthritis involving the hands and wrists, as has been described in several patients with rheumatoid arthritis who developed lupus following treatment with TNF inhibitors.[19] After the initial treatment period of 12 weeks, the patient was administered 25 mg of methotrexate a week in association with etanercept; as a result the lesions improved and the antibody levels decreased. Given that the lesions resolved without withdrawal of etanercept, we believe that the patient experienced an outbreak of psoriatic arthritis secondary to treatment rather than an episode of lupus.

We did not find a significant correlation between the decrease in T-cell numbers and improvement of PASI scores, perhaps because of the relatively short interval between the biopsies and our small sample size. It has also been suggested that the weak correlation between these 2 variables may be due to the fact that T cells act locally in the production of psoriatic lesions and are actually activated by dendritic cells, which would play a greater role in the development of the lesions.[4] Several studies have shown that the administration of etanercept for longer than an initial period of 12 weeks leads to the clinical improvement of psoriasis, even in patients who do not respond initially.[18,20] Thus, a long-term study might, perhaps, uncover a significant correlation between improvement in PASI scores and a decrease in T-cell numbers in the lesions.

The fact that the T-cell populations in the blood did not decrease has various explanations: blood levels of TNF do not vary in the early stages of treatment with TNF inhibitors,[21] there is less TNF in peripheral blood than in the lesions,[22] and etanercept does not cause lysis of cells that express TNF on their surface.[9]

The adverse effects during treatment were mild and well tolerated.

Conclusion

Etanercept leads to clinical improvement and a significant reduction in the number of $CD8^+$ cells in the epidermis and $CD4^+$ cells in the dermis of psoriasis plaques after just 12 weeks of treatment but has no impact on T-cell populations in peripheral blood. The study shows that the drug performs well in terms of safety and efficacy in the treatment of psoriasis but long-term studies are still required.

Conflicts of Interest
The authors declare no conflicts of interest. This study was not supported by external funding.

References

1. Nickoloff BJ, Nestle FO. Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. J Clin Invest. 2004;113:1664-75.
2. Lowes MA, Lew W, Krueger JG. Current concepts in the immunopathogenesis of psoriasis. Dermatol Clin. 2004;22:349-69.
3. Gottlieb AB. Psoriasis. Immunopathology and immunomodulation. Dermatol Clin. 2001;19:649-57.
4. Boyman O, Hefti HP, Conrad C, Nickoloff BJ, Suter M, Nestle FO. Spontaneous development of psoriasis in a new animal model shows an essential role for resident T cells and Tumor necrosis Factor-a. J Exp Med. 2004;199:731-6.
5. Austin LM, Coven TR, Bhardwaj N, Steinmann R, Krueger JG. Intraepidermal lymphocytes in psoriatic lesions are activated GMP-17(TIA-1) + CD8 + CD3 + CTLs as determined by phenotypic analysis. J Cutan Pathol. 1998;25:79-88.
6. Ferenczi K, Burack L, Pope M, Krueger JG, Austin LM. 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.
7. Chaudhari U, Romano P, Mulcahy LD, Dooley LT, Baker DG, Gottlieb AB. Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. Lancet. 2001;357:1842-7.
8. Mease P, Goffe B, Metz J, Van der Stoep A, Finck B, Burge DJ. Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. Lancet 2000;356:385-90.
9. Magliocco MA, Gottlieb AB. Etanercept therapy for patients with psoriatic arthritis and concurrent hepatitis C virus infection: Report of 3 cases. J Am Acad Dermatol. 2004;51:580-4.
10. Estebaranz JL. Etanercept. Estructura química, farmacocinética y mecanismo de acción. Actas Dermosifiliogr. 2005;96 Supl 3:2-9.
11. Toby AM, Kirby B. TNF-a inhibitors in the treatment of psoriasis and psoriatic arthritis. Biodrugs. 2005;19:47-57.
12. Capon F, Trembath RC, Barker JN. An update on the genetics of psoriasis. Dermatol Clin. 2004;22:339-47.
13. Mehlis S, Gordon KB. From laboratory to clinic: rationale for biologic therapy. Dermatol Clin. 2004;22:371-7.
14. Nickoloff BJ, Kunkel SL, Burdick M, Strieter RM. Severe combined immunodeficiency mouse and human psoriatic skin chimeras: validation of a new animal model. Am J Pathol. 1995;146:580-8.
15. Gottlieb AB, Matheson RT, Lowe N, Krueger GG, Kang S, Goffe BS, et al. A randomized trial of etanercept as monotherapy for psoriasis. Arch Dermatol. 2003;139:1627-32.
16. Gottlieb AB, Chamian F, Masud S, Cardinale I, Abello MV, Lowes MA, et al. TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. J Immunol. 2005;175:2721-9.

17. Nickoloff BJ, Wrone-Smith T. Injection of pre-psoriatic skin with CD4+ T cells induces psoriasis. Am J Pathol. 1999;155:145-58.
18. Leonardi CL, Powers JL, Matheson RT, Goffe BS, Zitnik R, Wang A, et al. Etanercept as monotherapy in patients with psoriasis. N Engl J Med. 2003;349:2014-22.
19. Debandt M, Vittecoq O, Descamps V, Le Loet X, Meyer O. Anti-TNF-alpha-induced systemic lupus syndrome. Clin Rheumatol. 2003;22:56-61.
20. Krueger GG, Elewski B, Papp K, Wang A, Zitnik R, Jahreis A. Patients with psoriasis respond to continuous open-label etanercept treatment after initial incomplete response in a randomized, placebo-controlled trial. J Am Acad Dermatol 2006;543 Suppl 2:S112-9.
21. Mastroianni A, Minutilli E, Mussi A, Bordignon V, Trento E, D'Agosto G, et al. Cytokine profiles during infliximab monotherapy in psoriatic arthritis. Br J Dermatol. 2005; 153:531-6.
22. Tigalonova M, Bjerke JR, Gallati H, Degré M, Jablonska S, Majewski S, et al. Serum levels of interferons and TNF-alpha are not correlated to psoriasis activity and therapy. Acta Derm Venereol Suppl (Stochk). 1994;186:25-7.