Etanercept induces apoptosis of dermal dendritic cells in psoriatic plaques of responding patients

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Background: Etanercept is a tumor necrosis factor-α binding fusion protein that demonstrates efficacy in the treatment of psoriasis, which is accompanied by decreased plaque infiltration of T cells and myeloid dendritic cells. We hypothesized that etanercept decreases inflammatory cell infiltration by inducing apoptosis.

Objective: We sought to investigate the effect of etanercept on circulating and plaque leukocyte apoptosis in psoriasis.

Methods: Blood and plaque specimens were obtained from 10 patients with psoriasis treated with etanercept (25 mg subcutaneously twice weekly) for 24 weeks. Apoptosis was determined in tissue samples using immunohistochemistry and flow cytometry.

Results: Etanercept selectively induced apoptosis in dermal dendritic cells in plaques of responding patients at 1 month, before most of the clinical and histologic response was achieved. No apoptosis was detected in plaque or circulating T cells or in circulating monocytes.

Limitations: Addition of multiple time points earlier than 1 month would be valuable to establish the time point of maximum induction in cell apoptosis.

Conclusion: Etanercept selectively induces apoptosis of pathogenic dermal dendritic cells in responding patients early in the course of treatment. (J Am Acad Dermatol 2006;55:590-7.)

Etanercept is a tumor necrosis factor (TNF)-α blocking biologic that shows efficacy in the treatment of moderate to severe psoriasis.[1,2] Its capacity to bind and neutralize soluble TNF-α is responsible for producing therapeutic effects in patients with psoriasis. However, the mechanism of action of etanercept in psoriasis is not fully understood. We recently demonstrated that after 1 month

Abbreviations used:

DC:	dendritic cell
FITC:	fluorescein isothiocyanate
IL:	interleukin
NF:	nuclear factor
PASI:	Psoriasis Area and Severity Index
PBMC:	peripheral blood mononuclear cell
TNF:	tumor necrosis factor

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Supported in part by an investigator-initiated study (Dr Gottlieb) funded by Amgen, by general support to the Psoriasis Center of Excellence by Beiersdorf (to Dr Gottlieb), and by a grant from the David Ju Foundation (to Dr Gottlieb).

Disclosure: Dr Gottlieb is an investigator, consultant, and speaker for Amgen and Wyeth, and a consultant and investigator for Centocor and Abbott. Dr Krueger receives research funding from Amgen. Dr Magliocco had received a fellowship from Amgen in the past.

Accepted for publication May 1, 2006.

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Published online July 5, 2006.

0190-9622/$32.00

doi:10.1016/j.jaad.2006.05.004

of treatment, which was before clinical and histologic clearance, etanercept decreased T-cell, dendritic-cell (DC), and neutrophil infiltration into psoriatic plaques.[3] Decreased chemokine and adhesion molecule messenger RNA expression, demonstrated in this study, may account for the decrease in the inflammatory infiltrate but other mechanisms were not investigated.

Cell depletion by induction of cellular apoptosis is a possible mechanism. In psoriasis, impaired apoptosis of keratinocytes and infiltrating inflammatory lymphocytes and DCs have been described.[4-6] Excess TNF-α in psoriasis may contribute to the inhibition of cell apoptosis through the activation of transcription factor nuclear factor (NF)-κB.[7] We recently reported that both nonlesional and lesional skin from patients with psoriasis have increased NF-κB protein expression and that treatment with etanercept down-regulates activation of NF-κB in plaques.[8] These data suggest that etanercept may produce a therapeutic effect by inducing apoptosis of infiltrating inflammatory cells. Infliximab (a monoclonal anti-TNF-α antibody) but not etanercept has been reported to induce apoptosis of lamina propria T cells and circulating monocytes/macrophages in patients with Crohn's disease.[9-11] However, similar studies in rheumatoid arthritis synovium demonstrate macrophage apoptosis with both infliximab and etanercept.[12]

To investigate whether apoptosis induction could account for the observed decrease in inflammatory infiltration in psoriatic plaques,[3] we used tissue specimens from patients with psoriasis treated with etanercept (25 mg subcutaneously twice weekly) as in our previous studies,[8] and determined cell apoptosis in blood and plaques. Our data demonstrate that etanercept induces dermal DC apoptosis early in the course of clinical and histologic response only in the psoriatic plaques of responding patients. Circulatory cell apoptosis was not observed either in responding or in nonresponding patients.

METHODS

Study design and patient entry criteria

The study protocol was approved by our institutional review board. Patient recruitment, treatment dose, treatment schedule, Psoriasis Area and Severity Index (PASI) score assessment, and skin punch biopsy specimen collection have been previously described.[3,8] Heparinized blood samples were obtained from patients at baseline, at 24 hours, weekly for the first month, then monthly for a total of 6 months and were processed immediately after the collection.

Narrowband UVB exposure of human volunteers

Under an institutional review board-approved protocol, healthy volunteers (18-65 years) were recruited and their minimum erythema dose was determined. The volunteers were irradiated with 2 minimum erythema doses of UVB on either left or right hip and 6-mm skin punch biopsy specimens were taken from each patient at 48 hours and snap frozen. Baseline biopsy specimens were obtained before UVB light exposure and were used as control.

Isolation and treatment of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood obtained from healthy volunteers using Ficoll-Paque density gradients (Amersham Biosciences, Piscataway, NJ) following manufacturer's instructions. A total of 5 mL of PBMC suspension (2×10^6 cells/mL in phosphate-buffered saline) was either irradiated with 30 mJ/cm^2 UVB or sham-irradiated in 100 mm Petri dishes as described.[13] After irradiation the cells were resuspended in Roswell Park Memorial Institute media containing 5% heat-inactivated human serum, penicillin/streptomycin, gentamycin, and 1 mmol/L 4-(2-hydroxyethyl)-1 piperazine ethane sulphonic acid buffer and incubated in a 37°C incubator with 5% carbon dioxide for 24 hours.

Western blot analysis

The proteolytic activation of caspase-3 was detected by immunoblotting analyses following the procedure described (Oral communication: Tan JK, Salahuddin K, Malaviya R, Sun Y, Lipets I, and Gottlieb AB. Alefacept induces late-onset apoptosis in infiltrating dermal cells in psoriatic plaques from clinically responsive patients. Psoriasis: From gene to clinic. December 3, 2005.). The antibodies used for the analyses included polyclonal antihuman anticaspase-3 active (R and D Systems, St Paul, Minn), and anticleaved caspase-3 antibodies (Cell Signaling Technology Inc, Beverly, Mass). β-Actin was used to normalize protein in different samples.

Blood phenotyping and caspase staining

Monoclonal phycoerythrin-anti-CD3, phycoerythrin-anti-CD14, peridinin-chlorophyll-protein-anti-CD45 and control antibodies of appropriate isotype (BD Biosciences, Palo Alto, Calif) were used for phenotyping of circulating T cells and monocytes. Apoptosis was determined using CaspACE-FITC-VAD-FMK (Promega, Madison, Wis), a fluorescein isothiocyanate (FITC)-conjugated, cell-permeable in situ caspase marker that binds to all the active