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CD1a expression in psoriatic skin following treatment with propylthiouracil, an antithyroid thioureydene

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Abstract

Background: The antithyroid thioureylenes, propylthiouracil (PTU) and methimazole (MMI), are effective in the treatment of patients with plaque psoriasis. The mechanism of action of the drugs in psoriasis is unknown. Since the drugs reduce circulating IL-12 levels in patients with Graves' hyperthyroidism, the effect of propylthiouracil on CD1a expression in psoriatic lesions was examined in biopsy samples of patients with plaque psoriasis. CD1a is a marker of differentiated skin antigen presenting cells (APC, Langerhans cells). Langerhans cells and skin monocyte/macrophages are the source of IL-12, a key cytokine involved in the events that lead to formation of the psoriatic plaque.

Methods: Biopsy specimens were obtained from six patients with plaque psoriasis who were treated with 300 mg propylthiouracil (PTU) daily for three months. Clinical response to PTU as assessed by PASI scores, histological changes after treatment, and CD1a expression in lesional skin before and after treatment were studied.

Results: Despite significant improvement in clinical and histological parameters the expression of CD1a staining cells in the epidermis did not decline with propylthiouracil treatment.

Conclusions: It appears that the beneficial effect of propylthiouracil in psoriasis is mediated by mechanisms other than by depletion of skin antigen-presenting cells.

Background

Psoriasis is a common skin disorder affecting 1 to 3 percent of the world's population.[1,2] Treatment of the disease results in costs that range between 0.7 to more than 4 billion dollars annually in the US alone [3]. One of the most cost-effective forms of potential treatment for the disease is the use of antithyroid thioureylenes such a propylthiouracil. We previously reported the effectiveness of thioureydene treatment in patients with plaque psoriasis [4–7], a finding since confirmed by others [8]. The mechanism of action of antithyroid thioureylenes in psoriasis

remains unclear. The drugs have been studied with relation to their effect in Graves' hyperthyroidism which, like psoriasis, is an autoimmune disorder [9–12] Antithyroid thioureylenes, of which propylthiouracil, methimazole and carbimazole are used in clinical practice, have known immunomodulatory properties [13,14] and, in the case of psoriasis, also have antiproliferative effects as demonstrated by their ability to produce a decline in markers of cellular proliferation such as proliferative cell nuclear antigen (PCNA)[15].

Skin antigen-presenting cells (Langerhans cells) appear to play an important role in the initiating events that lead to the cytokine cascade that results in keratinocyte proliferation and development of psoriatic lesions [16–18]. Effective therapeutic approaches to psoriasis treatment, such as psoralen UV-A therapy (PUVA), which result in depletion of APCs in the skin [19,20], and UV-B [21], lead to one of the most sustained improvement in psoriasis. IL-12, a product of Langerhans cells and skin monocytes plays a pivotal role in T-cell activation that leads to formation of the psoriatic plaque [22]. IL-12 levels are also significantly raised in patients with Graves' hyperthyroidism and are reduced following treatment with propylthiouracil [23].

CD1a is expressed in differentiated antigen-presenting cells and monocytes and is used as a marker for the presence of these cells in the skin [24–26]. The CD1 molecules are the products of five CD1 genes located on chromosome 1. The genes are closely related to the mixed histocompatibility complex (MHC) genes indicating origin from a common precursor [27]. CD1 molecules, however, have limited sequence homology to MHC class I and II molecules, and unlike the MHC molecules are also capable of binding non-protein antigens [28,29].

The present study was performed to examine the effect of propylthiouracil treatment of patients with plaque psoriasis on CD1a expression in their psoriatic lesions in order to determine whether this form of therapy resulted in depletion of APCs from affected skin, that could account for the therapeutic benefit of PTU in this disorder.

Methods

Patients

Six patients (4M, 2F) with stable plaque psoriasis were recruited for study. The patients ranged in age from 21 to 63 years, mean \pm SD, 44 ± 16 years. None of the patients was taking any form of topical or systemic treatment, other than emollients, for psoriasis for at least six weeks prior to entry into the study. Pregnant women and patients with thyroid dysfunction determined by pre-enrollment screening thyroid function tests were excluded from the study. The Institutional Review Board of the University of California, Irvine, approved the study.

Clinical Scoring

Clinical evaluation was performed by the same dermatologist (VSN) at the time of patient entry into the study and monthly intervals until completion of the study. Scoring was based on the PASI scoring system [30].

Biopsy Samples

Biopsy samples were taken from patients at the start and after 3 months of PTU treatment from the same psoriatic lesion. Skin samples were obtained using a 5 mm punch

biopsy under aseptic conditions with local anesthesia. The biopsies were frozen and stored at -70°C until processed. Indirect immunoperoxidase staining was used to identify CD1a.

Histological Scoring

Biopsy specimens were viewed by a dermatopathologist (RJB). Histological scoring was made with a 5-point scoring system graded from 0 to 4 (0 = normal, 1 = slight, 2 = moderate, 3 = severe, 4 = very severe) based on criteria noted below after examination of four consecutive high-power fields. Histological scoring took into account hypogranulosis, parakeratosis and the inflammatory infiltrate in the epidermis and superficial dermis. The total score reported was the sum of the individual parameters. Epidermal hyperplasia was measured with an oculomicroscope from top of the epidermis to the base of the ridges.

Laboratory Tests

Thyroid function tests were performed in all volunteers prior to entry into the study and again at monthly intervals until study completion at twelve weeks. The tests consisted of measurement of serum free T_4 (FT_4) and thyroid-stimulating hormone (TSH). Complete blood counts (CBC) were also obtained at the same time intervals.

Immunoperoxidase Staining

Tissue samples from biopsy specimens stored at -70°C were taken and embedded in paraffin. 4 μm sections were coated onto slides and allowed to dry overnight at 55°C . An RBC block (2.5 ml, 30% H_2O_2 in 100 ml methanol) was applied for 40 min after which the slides were washed in tap water until clean. Following a series of washing with PBS, the slides were washed with blocking serum (5% horse serum) for 20 min after which antibody for CD1a (1:100 dilution) was applied for 30 minutes. The slides were washed with PBS for 5 min after which anti-mouse antibody (1:200 dilution) was applied and the slides washed again in PBS for 5 minutes. Tertiary antibody (ABC kit) was added for 30 min and the slides were then washed for with PBS. DAB was applied in the dark (10 min), the slides were washed in distilled water (5 min), counterstained with hematoxylin, washed with tap water, dehydrated and mounted.

Quantification

Results were expressed as the absolute number of positive staining dendritic cells counted over a 1 mm length of the epidermis. Only cells with clearly defined nuclei present in the suprabasal layers of the epidermis were counted.

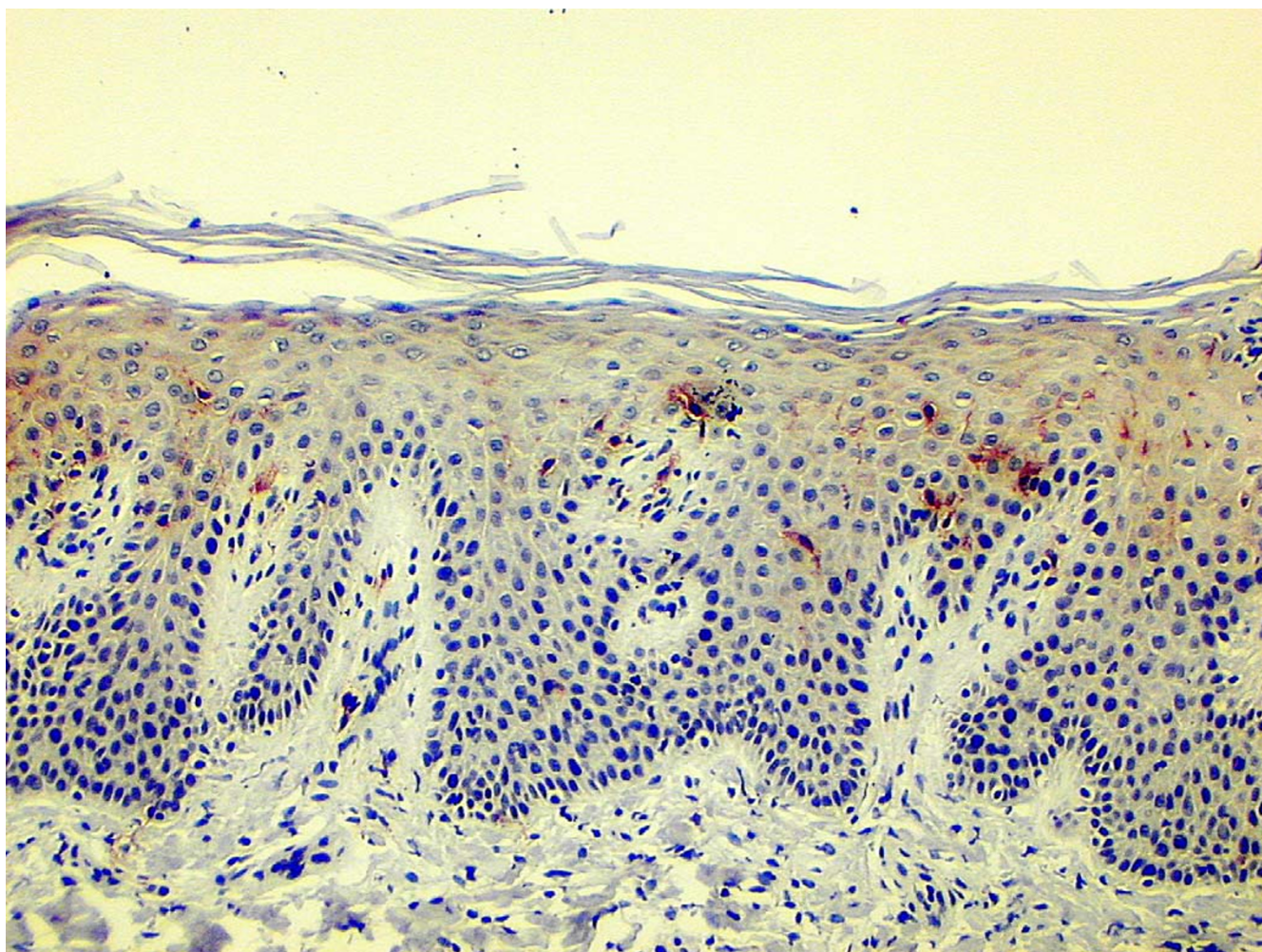


Figure 1
CD1a staining Langerhans cells before treatment with 300 mg propylthiouracil for 12 weeks

Statistical Analysis

The results are expressed as mean \pm SD. Statistical analysis was performed using the t-test for paired observations. Level of significance was $p < 0.05$.

Results

Clinical Response and PASI Scores

All patients showed some improvement in their psoriasis with near-complete resolution in 4 patients. PASI scores declined from 21.40 ± 6.81 at baseline to 7.85 ± 4.35 at completion of the study ($p < 0.004$). Clinical improvement became apparent about three weeks after therapy.

Histological Scores

Histological scores also declined significantly from 7.17 ± 2.78 at baseline to 3.00 ± 2.61 at the end of 12 weeks PTU

treatment ($p < 0.002$). Epidermal thickness decreased from 0.52 ± 0.17 mm to 0.26 ± 0.11 mm ($p < 0.007$).

Serum TSH, FT_4 and CBC

CBC remained normal (7.85 ± 0.53 vs. 8.05 ± 1.31 cells/ μ L, $p = ns$) during the entire study with no patient demonstrating any leucopenia. Serum TSH concentrations remained within the euthyroid range with no patient exhibiting clinical signs or offering complaints suggestive of hypothyroidism. Serum free thyroxine (FT_4) showed no significant change. Serum TSH showed a slight rise in some patients with the group as a whole showing no significant elevation of TSH (1.72 ± 0.98 μ U/ml vs. 2.73 ± 1.43 μ U/ml, $p = ns$). The rise did not exceed the normal range, and all patients remained clinically euthyroid.

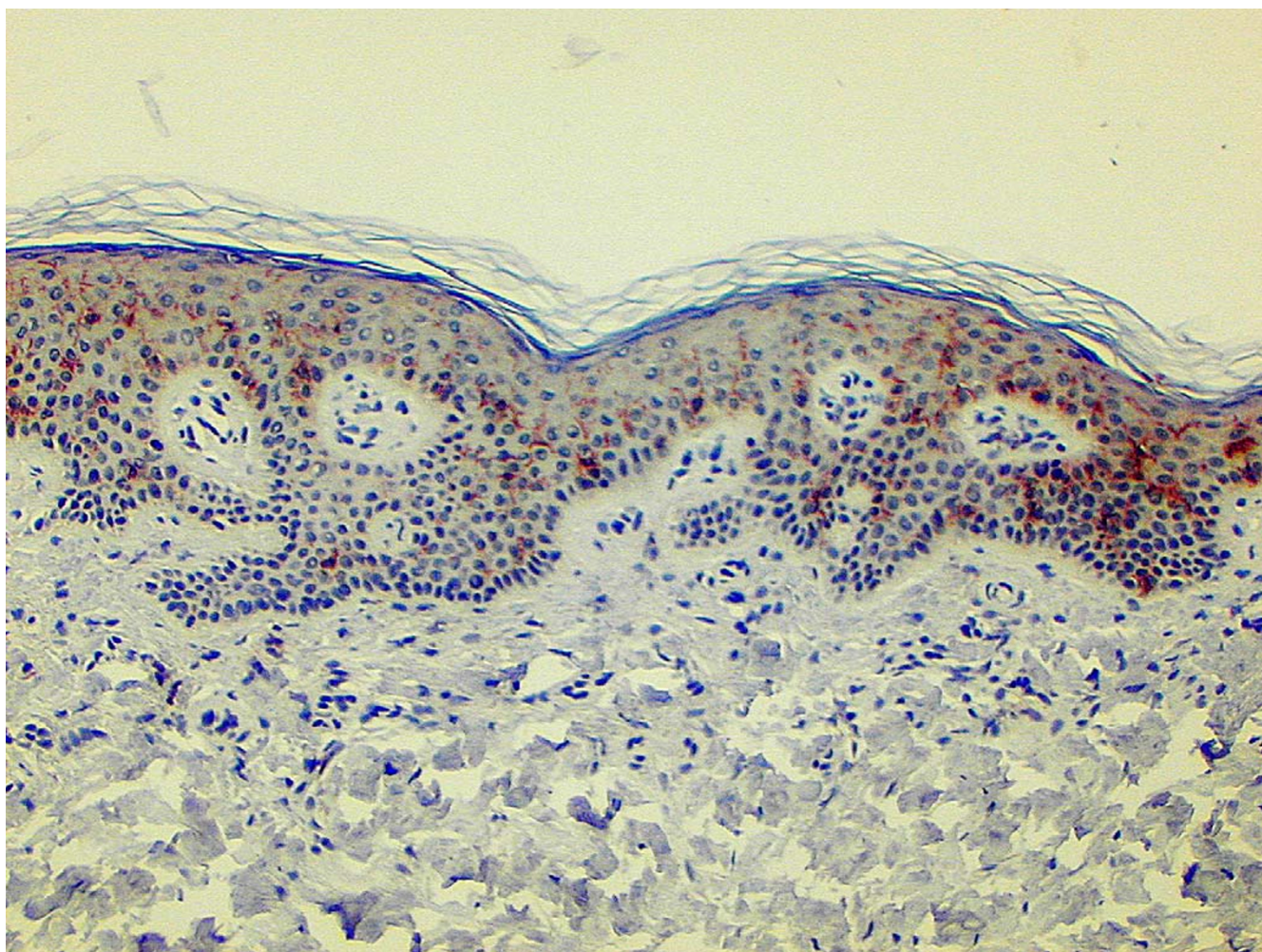


Figure 2
CD1a staining Langerhans cells after treatment with 300mg propylthiouracil for 12 weeks.

Expression of CD1a in Psoriatic Plaques

As expected, CD-1a with characteristic stellate profiles typical for Langerhans cells were readily seen in the epidermis in all pre- and post-treatment biopsy samples. The number of CD-1a staining cells showed an apparent increase after propylthiouracil therapy. The number of such cells rose from 5.67 ± 4.08 pretreatment to 10.00 ± 4.20 post-treatment ($P < 0.02$) (Fig 1, 2).

Discussion

The efficacy of antithyroid thioureylenes as agents in the treatment of plaque psoriasis is well established [4,5,7,8]. The mechanism of action of these agents in psoriasis remains unclear. The drugs can potentially act by reducing the cytokine signals that lead to keratinocyte proliferation or by reducing keratinocyte proliferation. A key element

in the generation of the cytokine signals responsible for formation of a psoriatic plaque is the skin antigen-presenting cell or Langerhans cell. Therapeutic modalities deplete such cells, that often lead to improvement in psoriasis. The best example of such an approach is treatment with UV-A and psoralens, as well as UV-B, which result in depletion of APCs and produce one of the most long-lasting remissions of the disease which might last in some instances for as long as 6 months [31,32]. A key antigenic marker of skin APCs is CD1a. Langerhans cell precursors arise from CD34 positive monocytes/macrophages in the marrow [33,34]. They circulate in the blood before localizing in the skin, primarily in dermal connective tissue. In the dermis, the Langerhans precursors are strongly HLA-DR (Ia) positive, and are relatively non-phagocytic, "indeterminate cells." CD1a expression is not pronounced in

the precursors and is not significantly expressed until the cells become differentiated.[35–38] Differentiated cells localize to the midepidermis, demonstrate characteristic Birbeck granules, and exhibit dendritic appendages. These differentiated cells, although usually stable, sometime exhibit proliferative activity and increase in many cutaneous immune response states [23,39,40]. The inability of PTU to reduce CD1a expression in biopsy samples of patients with psoriasis suggests that the mechanism of action of this drug is psoriasis is not due to any effect on skin antigen-presenting cells or production of IL-12, a motive cytokine that is responsible for initiation and maintenance of the cytokine stimulatory cascade that leads to formation of the psoriatic plaque [16]. In support of this argument is the fact that circulating IL-12 did not show any significant change with PTU treatment (submitted). Additional studies are being performed which address whether PTU treatment of patients with plaque psoriasis leads to reduced *in situ* IL-12 expression, alters production of the IL-35 or IL-40 components of the IL-70 heterodimer, as well as exerts an effect by enhancing apoptosis in the proliferated keratinocytes that make up the psoriatic plaque which would result in clinical improvement.

Competing Interests

None declared.

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