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of less than 6.5 mg per kg) is currently the drug of first choice.<sup>2</sup> If these measures fail, a wide variety of other medications have been advocated. These include thalidomide,<sup>3</sup> dapsone,<sup>4</sup> oral gold,<sup>5</sup> alpha interferon,<sup>6</sup> and retinoids.<sup>7</sup> All have been shown, in at least one study, to produce a good clinical response in patients with DLE who have failed to respond to the combination of topical steroids, sunblocks and hydroxychloroquine. However, in our experience, we have encountered a number of patients with active DLE who have failed to respond to the above treatments. In addition, the toxicity of some treatments, such as thalidomide, oral gold or retinoids, may limit their use. We were therefore interested to assess the potential use of MTX in the management of DLE, which has not been reported previously, and which might be a useful addition to the therapeutic armamentarium for this condition. Low-dose weekly oral MTX is a relatively safe drug and hepatotoxicity is usually limited to psoriatics whose condition is complicated by a secondary factor such as alcohol excess, diabetes mellitus, arsenic ingestion, or obesity. The American Rheumatism Association does not recommend routine pre-treatment liver biopsy for patients with uncomplicated rheumatoid arthritis receiving MTX,<sup>8</sup> as very few problems have been reported with its use in this condition. Side-effects reported in patients with SLE have included neutropenia and oral ulceration, and have tended to be confined to those patients with active disease with renal impairment.<sup>1</sup> Thus, short-term treatment of DLE with MTX is most unlikely to be complicated by any significant side-effects, in the absence of an additional factor. Possible mechanisms of action of MTX in DLE might include inhibition of T-cell activation and the inhibition of migration of inflammatory cells into the affected area.

As only a small number of patients were included in this study, the conclusions drawn must be limited. However, the reasonable response seen in two of the four patients suggests that MTX may be of help in patients with therapy-resistant DLE which has failed to respond to hydroxychloroquine, and in whom other treatments, such as thalidomide, oral gold or retinoids, have either failed or are contraindicated.

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## Transglutaminase-positive cells in psoriatic epidermis during treatment with calcitriol ( $1\alpha,25$ dihydroxy vitamin $D_3$ ) and tacalcitol ( $1\alpha,24$ dihydroxy vitamin $D_3$ )

SIR, The keratinization process in human epidermis involves the formation of an insoluble cross-linked protein envelope. Involucrin, filaggrin and other major precursor proteins of the cornified cell envelope are expressed late during epidermal differentiation.<sup>1</sup> Involucrin expression starts in the upper spinous cell layers in normal human skin.<sup>2</sup> Filaggrin expression is restricted to the granular layer and the stratum corneum.<sup>3</sup> These and other precursor proteins become cross-linked by the activity of transglutaminase K, the rate limiting enzyme in the formation of the cornified envelope, via  $\epsilon$ -( $\gamma$ -glutamyl) lysine isopeptide bonds.<sup>4</sup>

It has been demonstrated that membrane-associated transglutaminase activity and the number of cross-linked envelopes are markedly increased in psoriatic skin.<sup>5</sup> It is also well established that in psoriatic epidermis involucrin expression is significantly increased, whereas filaggrin expression is decreased or even absent.<sup>6</sup>

In keratinocytes *in vitro*, it has been demonstrated that cornified envelope formation and transglutaminase activity are enhanced by calcitriol ( $1\alpha,25$  dihydroxy vitamin  $D_3$ ), tacalcitol ( $1\alpha,24$  dihydroxy vitamin  $D_3$ ), and calcipotriol.<sup>7-9</sup> Recently, we reported that  $1\alpha,25$  (OH) $_2$  $D_3$  and  $1\alpha,24$  (OH) $_2$  $D_3$  reduce the expression of involucrin and increase filaggrin expression in psoriatic skin.<sup>6,10</sup>

A mouse monoclonal antibody (IgG2a) against human keratinocyte transglutaminase (BTI) has become available for the immunoperoxidase staining technique.<sup>11-13</sup> The aim of the present investigation was to examine the effect of  $1\alpha,25$  (OH) $_2$  $D_3$  and of  $1\alpha,24$  (OH) $_2$  $D_3$  on the expression of human keratinocyte transglutaminase *in vivo* during treatment of psoriatic plaques with these derivatives.

Twenty patients with stable psoriasis vulgaris were included in this study. Ten patients were treated with calcitriol in petrolatum (3  $\mu$ g/g) twice daily. Before treatment, and after 1, 2 and 4 weeks of treatment, punch biopsies (3 mm) were taken from the psoriatic lesions. Calcitriol was obtained from Solvay Duphar, Amsterdam, the Netherlands. The other 10 patients were treated with tacalcitol ointment (4  $\mu$ g/g) once daily on one half of the body, and the ointment base alone on the other half, for 8 weeks. Before treatment, and after 8 weeks of treatment, punch biopsies (3 mm) were taken from both body halves.

To visualize transglutaminase type I, which is expressed during differentiation of the epidermis, we used immuno-

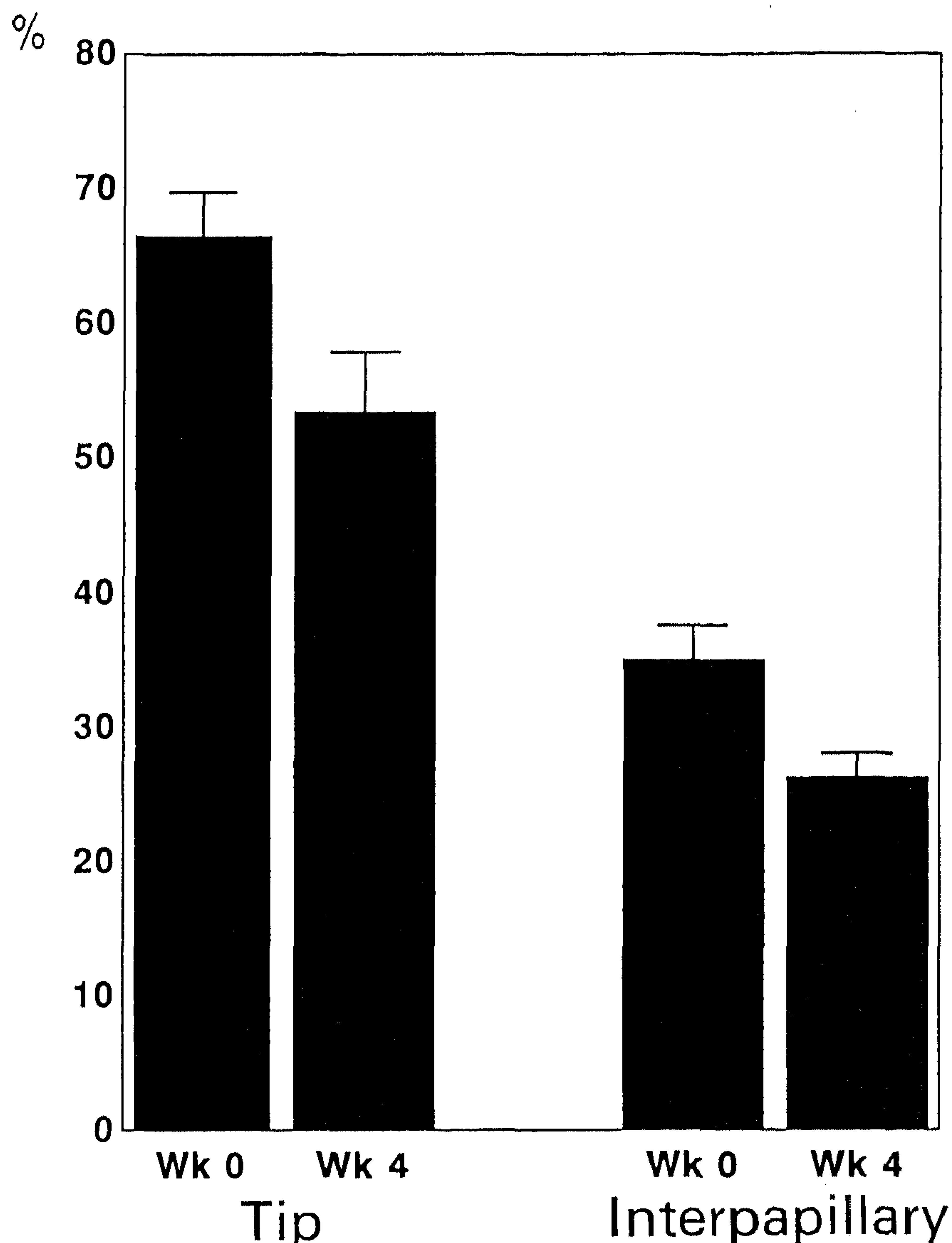


Figure 1. Plasma membrane-bound transglutaminase before treatment, and after 4 weeks of treatment with calcitriol 3 µg/g in petrolatum, twice daily ( $n = 10$ ). Mean  $\pm$  standard error of the mean.

peroxidase staining with an anti-human keratinocyte transglutaminase (mouse monoclonal antibody, IgG2a).<sup>11-13</sup> The histological examination was performed blinded. Trans-

glutaminase expression was assessed by calculating the ratio positive cell layers/total cell layers of the living epidermis. This procedure was performed at two sites: at the tip of a dermal papilla, and in the interpapillary region. On every slide, two representative areas were examined, and the mean of these observations was assessed. The Wilcoxon ranking test for matched pairs was used for statistical analysis.

The calcitriol-treated lesions showed a statistically significant reduction of transglutaminase expression at the tip of a dermal papilla after 4 weeks of treatment ( $P < 0.03$ ). In the interpapillary region, the transglutaminase staining showed a tendency to decrease after 4 weeks of treatment with calcitriol ( $P < 0.075$ ).

The tacalcitol-treated lesions also showed a statistically significant decrease of transglutaminase expression at the tip of a dermal papilla ( $P < 0.03$ ), whereas the expression in the interpapillary zone was not significantly affected by this treatment ( $P < 0.125$ ). The transglutaminase expression in the biopsies of the lesions treated with placebo did not change significantly ( $P = 0.2$  in both areas). Figures 1 and 2 demonstrate the mean values  $\pm$  the standard error of the mean. Figure 3 shows the distribution of transglutaminase before and after 4 weeks of treatment with calcitriol.

It has been demonstrated that membrane-associated transglutaminase activity and the number of cross-linked envelopes are markedly increased in psoriatic skin—fivefold and tenfold, respectively.<sup>5</sup> Recently, we demonstrated by immunohistochemistry that the number of transglutaminase-positive cells is markedly increased in lesional skin compared with the clinically uninvolved skin of psoriatic patients. The distribution pattern of transglutaminase comprised relatively more cell layers compared with the distribution pattern of involucrin. To date, the trigger for the premature expression of transglutaminase in psoriatic skin has still to be identified. It is remotely possible that involucrin expression stimulates the expression of its cross-linking enzyme directly. Alternatively,

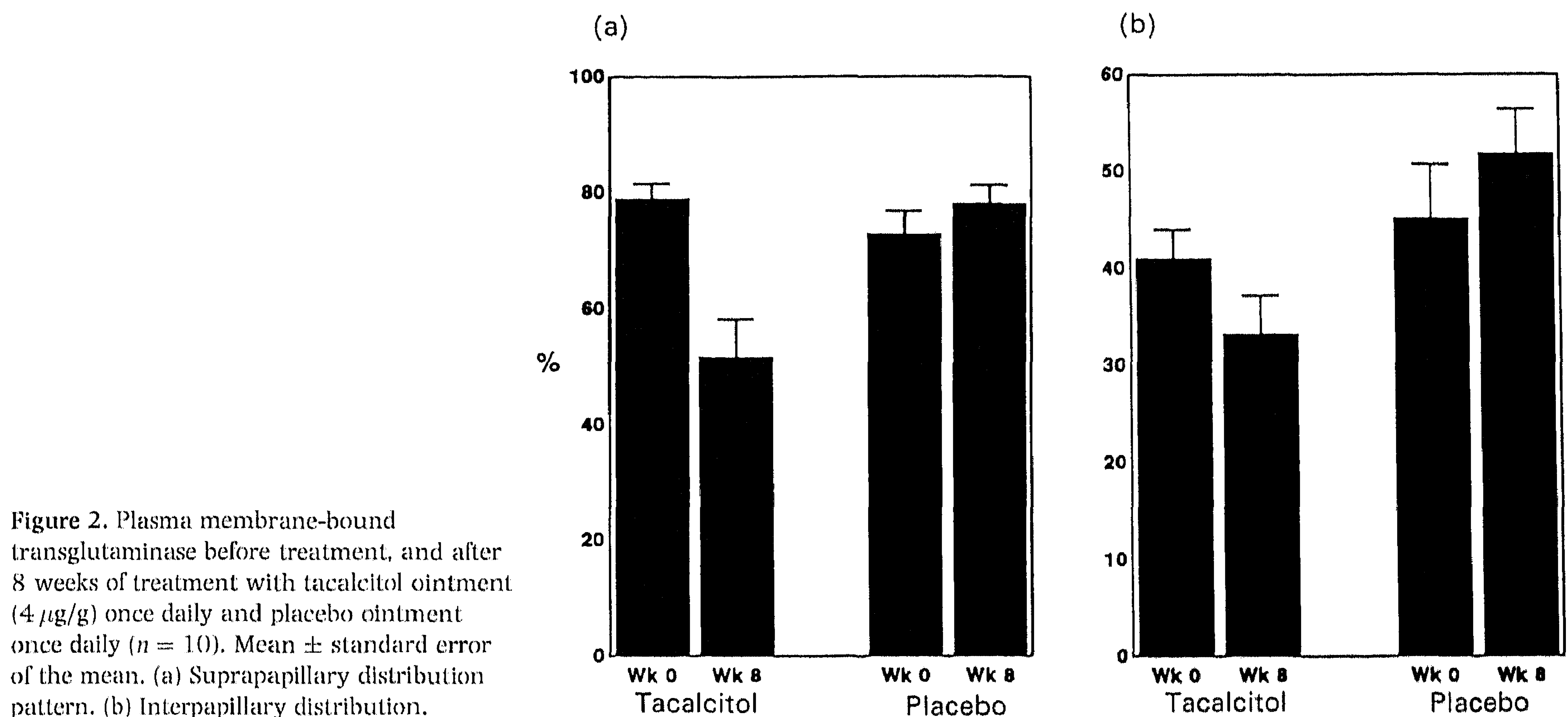


Figure 2. Plasma membrane-bound transglutaminase before treatment, and after 8 weeks of treatment with tacalcitol ointment (4 µg/g) once daily and placebo ointment once daily ( $n = 10$ ). Mean  $\pm$  standard error of the mean. (a) Suprapapillary distribution pattern. (b) Interpapillary distribution.

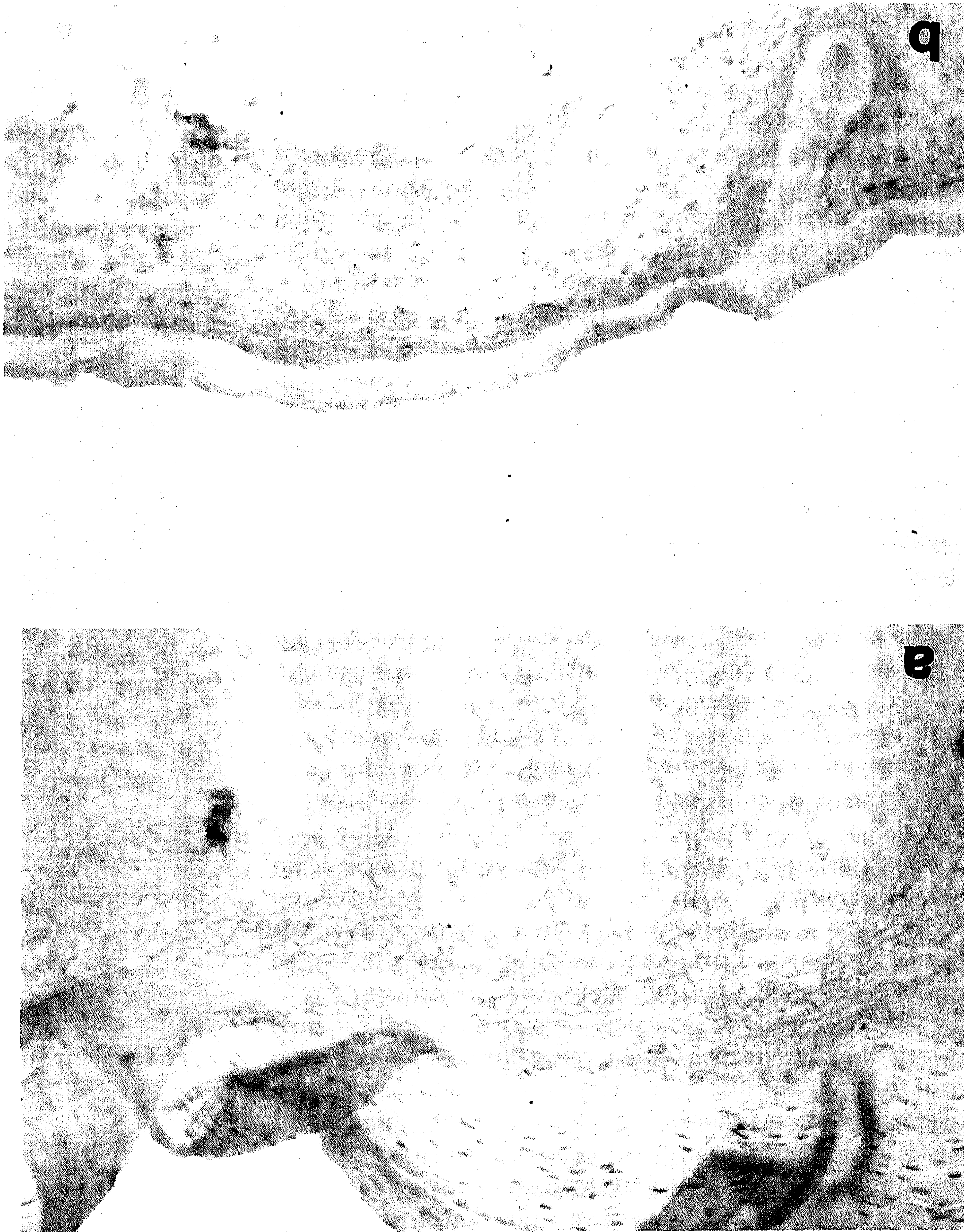


Figure 3. The distribution of plasma membrane-bound transglutaminase (a) before treatment, and (b) after 4 weeks of treatment with calcitriol (immunoperoxidase with mouse monoclonal IgG2a).

As transglutaminase activity is enhanced by active vitamin D<sub>3</sub> *in vitro*, the reduced number of transglutaminase-positive cells in vitamin D<sub>3</sub>-treated psoriatic skin is likely to be the result of a modification of compartmentalization of the epidermal cell population by this treatment. In untreated lesional skin of psoriatics, the number of cycling cells is increased 3(-fold) compared with normal skin. The increased recruitment of cycling epidermal cells in the germinal pool results in a 2(-fold) increase in cell production.<sup>16</sup> The number of differentiating cells is increased threefold compared with normal skin.<sup>17</sup> The increased transglutaminase capacity in untreated lesional skin could well be a compensatory phenomenon related to the increased production rate of epidermal cells. It has been shown that the increased recruitment of cycling epidermal cells is inhibited at an early stage of the treatment with calcitriol and tacalcitol.<sup>6,10</sup> A redistribution of the number of cycling and differentiating cells is accompanied by a decrease in the number of transglutaminase-positive cells.

the increased phosphatidylinositol turnover in lesional psoriatic skin enhances liberation of calcium from the intracellular calcium stores.<sup>14</sup> In an *in vitro* study, an increase of involucrin expression and transglutaminase activity was observed in association with a high level of extracellular calcium.<sup>12</sup> However, increased transcription of transglutaminase by an increase of the intracellular calcium concentration has not been proved. *In vitro*, active vitamin D<sub>3</sub> analogues enhance transglutaminase activity.<sup>2-9</sup> In this respect it is of interest that active vitamin D<sub>3</sub> enhances calcium entry from the extracellular compartment.<sup>15</sup> Hausler *et al.* found evidence that binding of calcitriol to keratinocyte receptors results in intracellular calcium mobilization.<sup>16</sup> *In vivo*, the effect of active vitamin D<sub>3</sub> on the activity of transglutaminase remains unsubstantiated. However, the present study demonstrates that the number of transglutaminase-positive cell layers is reduced markedly during treatment with active vitamin D<sub>3</sub>.

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### Treatment of recurrent aphthous stomatitis with pentoxifylline

SIR, Recurrent aphthous stomatitis (RAS) is one of the most common diseases affecting the oral mucosa. Many drugs, including analgesics, antibiotics, topical and systemic corticosteroids, dapsone, colchicine and thalidomide, have been used to relieve pain and to reduce the frequency of relapse. They are not always effective, and side-effects are a complicating factor.<sup>1</sup> Pentoxifylline (PTX) is a methylxanthine derivative with haemorrhological and antithrombotic properties.<sup>2</sup> Recent experimental and clinical observations have demonstrated that PTX also has immunomodulating and anti-inflammatory activities,<sup>3</sup> which seem to be related, at least in part, to the inhibitory effect of PTX on tumour necrosis factor (TNF)-alpha production.<sup>4</sup> Thalidomide, which is one of the treatments of choice for severe RAS,<sup>5</sup> also inhibits TNF-alpha production.<sup>6</sup> This observation led us to speculate that PTX and thalidomide could share certain therapeutic effects, such as the prevention of RAS.<sup>7</sup> Recently, we reported six patients with RAS who were successfully treated with PTX. Oral therapy with PTX (400 mg two–three times daily) suppressed the recurrence of aphthae in five patients, and led to a reduction in the number and duration of ulcers, with symptomatic improvement, in one patient.<sup>8</sup> We now report 22 additional cases.

Twenty-two patients (14 women and eight men) between 19 and 75 years of age (mean 34 years), were enrolled in this open study. All the patients were diagnosed as having minor RAS, and had a disease duration of 2–8 years (mean 3.5 years). They all had multiple oral aphthous ulcers which lasted for 7–15 days, and experienced recurrences at least every 2 months. Seventeen patients had neither clinical nor analytical evidence of any underlying systemic or cutaneous disease. The remaining five patients suffered from ulcerative colitis (which was treated with low-dose oral corticosteroids and mesalazine), rheumatoid arthritis (treated with non-steroidal anti-inflammatory drugs), subacute cutaneous lupus erythematosus (treated with topical corticosteroids and sun-screening cream), anorexia nervosa and Parkinson's disease. All the patients received oral therapy with PTX at a dose of 400 mg three times daily. Patients were studied monthly for a 6-month period. Two patients (9%) had gastrointestinal intolerance, and the drug was discontinued in the first month. The drug was well tolerated by the remaining 20 patients, including the patient with ulcerative colitis. No relapses of aphthous ulcers during the course of treatment were observed in 11 patients (50%), including those cases with anorexia nervosa, ulcerative colitis and subacute cutaneous lupus erythematosus. Six patients (27%) showed recurrence of the lesions during the period of the study, but there was a reduction in the number and duration of ulcers, as well as in the pain and difficulty with eating. Recurrence of aphthous ulcers without symptomatic improvement was observed in three cases (14%).

The present study, and our previous observations,<sup>8</sup> suggest that continuous PTX treatment can prevent minor RAS, or significantly reduce its severity, in most patients. In addition,

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