Effects of therapies on cytokine patterns in psoriasis
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Cyclosporine A and methotrexate are equally effective in reducing T cell numbers in psoriatic skin lesions but have no consistent effect on IFN-γ and IL-4 expression in psoriatic skin in situ.

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Introduction

T cells are involved in the pathogenesis of psoriasis. A classical systemic treatment of psoriasis i.e. cyclosporine A, is effective through inhibition of T cell activation. Cyclosporine A inhibits T cell activation via inhibition of calcineurin mediated Nuclear Factor of Activated T cells dephosphorylation. It also decreases the number of T cells in lesional psoriatic skin. Methotrexate is another effective systemic treatment of psoriasis. It inhibits folate-dependent enzymes which are important in cell proliferation. This could explain decreased keratinocyte proliferation in psoriasis after methotrexate treatment. In high doses, it also exerts anti-inflammatory effects through increased adenosine release. On the other hand, injection of methotrexate in low doses induces apoptosis of in vitro activated T cells from peripheral blood of patients with rheumatoid arthritis without causing adenosine release. The apoptotic action of methotrexate can be reversed by addition of purines. It is not known whether methotrexate affects T cells in psoriasis patients.

Psoriasis is characterized by a predominance of type 1 cytokine [interferon-γ (IFN-γ)], expression in peripheral blood and lesional skin of patients. High IFN-γ expression contributes to the inflammation in psoriatic skin via its proinflammatory effects. Systemic treatment with anti-inflammatory (interleukin-10 and interleukin-11) and type 2 [interleukin 4 (IL-4)] cytokines which counteract the inflammatory effects of IFN-γ, was found to cause clinical improvement in lesions of patients with psoriasis.

With respect to the crucial role of T cells and cytokines in psoriasis, we evaluated the in situ effects of therapy with either cyclosporine A or methotrexate on number of cutaneous T cells and type 1/ type 2 (IFN-γ/ IL-4) cytokine balance of psoriatic skin.

Materials and methods

Patients This study was part of a prospective randomized trial, comparing efficacy of oral cyclosporine A versus methotrexate in the treatment of severe psoriasis. The main clinical results of this study were reported elsewhere. Patients, of at least 18 years of age with chronic plaque type psoriasis were included in this study. Patients were recruited from the Academic Medical Center dermatological outpatient clinic in Amsterdam and other dermatological outpatient clinics throughout The Netherlands. Patients were included if the Psoriasis Area and Severity Index (PASI) at the time of randomization was 8 or higher and if topical therapy had failed. All patients gave their written informed consent and the study was approved by the local ethics committee. No active topical treatment for psoriasis was permitted during treatment. All anti-psoriatic medication was stopped. Patients were randomized for either cyclosporine A or methotrexate therapy. Cyclosporine A was given
orally (Neoral® capsules of 25 or 100 mg) as 3 mg/kg body weight/day for 13 weeks followed by weekly tapering to 2 mg/kg body weight/day, 1 mg/kg body weight/day and 0.5 mg/kg body weight/day. Thereafter, medication was withdrawn. Methotrexate was given orally (tablets of 2.5 mg) according to the Weinstein scheme as 15 mg/week in three equal doses of 5 mg each 12 hours apart for 13 weeks. The methotrexate dose was tapered weekly to 12.5 mg/week, 10 mg/week, 5 mg/week and 2.5 mg/week. Thereafter, medication was withdrawn. After the first four weeks of oral therapy, clinical efficacy was evaluated and in case of unsatisfactory results doses were adjusted to alternative, higher dosage schemes. Laboratory results were obtained in a blinded fashion before randomization (week 0) and at week 12 of therapy. The code was broken only after all definitive results were obtained from all participating patients.

**Biopsies and immunohistochemical staining** Four millimeters punch biopsies from lesional skin were taken under local anesthesia before and after 12 weeks of cyclosporine A or methotrexate therapy. Biopsies were snap frozen in liquid nitrogen and stored at -80°C. Sections (6 mm) from the frozen skin biopsies were fixed in acetone at 4°C. To block the endogenous peroxidase activity, sections were treated with 0.1% sodiumazide and 0.3% H₂O₂ in tris-HCl buffered saline for 20 minutes at room temperature. After brief washing in tris-HCl buffered saline, the sections were incubated with 10% goat serum (Dako, Glostrup, Denmark) for 20 minutes at room temperature followed by incubation with IL-4 antibody (Genzyme, Cambridge, MA) or IFN-γ antibody (R&D Systems, Minneapolis, MN) or CD3 antibody (Dako). For cytokine staining, overnight incubation at 4°C and for T cell staining, 1 hour incubation at room temperature were performed. After that, sections were sequentially incubated with biotinylated goat anti-mouse antibody (Dako) and horseradish peroxidase-conjugated streptavidin (Dako). Peroxidase activity was detected as a red colour by use of chromogen 3-amino-9-ethylcarbazole substrate (Sigma Aldrich Chemie, Munich, Germany). Counterstaining was performed with hematoxilin. Negative controls were obtained by using nonspecific isotype controls as primary antibodies. All preparations were evaluated by 3 investigators without clinical information. CD3+ lymphocytes were counted in 4 sequential high power fields (x400) of each section. Each high power field included epidermis and dermis. Cell counts from 2 sections of each biopsy were averaged. IL-4 and IFN-γ stainings were scored as follows: 0, no staining; 1+, weak staining associated with few cells; 2+, strong staining associated with several scattered or small groups of cells and; 3+, strong staining associated with many cells forming large groups.
Results

The skin biopsies were obtained from 5 patients receiving methotrexate and 5 patients receiving cyclosporine A therapy. The characteristics of patients can be seen in Table 1. The mean PASI of these patients markedly decreased after 12 weeks of both treatments from 13.8 to 4.4 in cyclosporine A group and from 13.5 to 4.0 in methotrexate group.

Table 1. Characteristics of patients from the cyclosporine A and methotrexate treatment groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cyclosporine A (n=5)</th>
<th>Methotrexate (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean age (range)</td>
<td>41.3 (22-55) years</td>
<td>45.2 (32-66) years</td>
</tr>
<tr>
<td>male/female ratio</td>
<td>4-1</td>
<td>3-2</td>
</tr>
<tr>
<td>mean PASI at entry (range)</td>
<td>13.8 (9.6-18.2)</td>
<td>13.5 (11.5-19.6)</td>
</tr>
<tr>
<td>mean PASI at week 12 (range)</td>
<td>4.4 (1-14.3)</td>
<td>4.0 (0.6-11.2)</td>
</tr>
</tbody>
</table>

Evaluation of psoriatic skin biopsies of all patients before treatment revealed a heavy infiltration of T cells, identified as CD3+ cells, especially in papillary dermis forming large clusters around capillaries before cyclosporine A treatment (Figure 1A) and methotrexate treatment (Figure 1B). In addition to decreased epidermal thickness in different degrees (data not shown), reduction of T cell numbers was observed after 12 weeks of systemic treatment with cyclosporine A (Figure 1C) or methotrexate (Figure 1D). A summary of the T cell countings of all five patients in both groups is given in Figure 2. T cells were diminished 47.8% (range 6.7%-85.8%) in the cyclosporine A group and 47.5% (range 10.84%-87.4%) in the methotrexate group. The reduction of T cell numbers was most prominent in areas which showed the highest reduction of the epidermal thickness upon treatment in both groups.

As concerns the presence of IFN-γ and IL-4 in the psoriasis lesions, both were variably expressed before treatment (Table 2). IFN-γ was located abundantly in papillary dermis (Figure 1E), whereas IL-4 expression was mainly characterized by scattered cells in epidermis and dermis (Figure 1F). Reduction of IFN-γ expression after 12 weeks of treatment was observed in 3 of 5 biopsies in both treatment groups (Figure 1G and Table 2). In 2 biopsies no major change of IFN-γ expression was found, in spite of prominent reduction in inflammatory cell infiltration. Further, one patient in the methotrexate group and two patients in the cyclosporine A group (having the highest IL-4 expression before therapy) showed decreased expression of IL-4 after therapy. In the biopsies of other patients, IL-4 was absent or vaguely expressed before treatment and remained so thereafter (Figure 1H and Table 2).
Figure 1. Immunohistochemical stainings of the skin biopsies. Representative stainings are shown from the patients treated with either cyclosporine A or methotrexate (200x magnification). Section-pairs before and after treatment belong to the same patient for each staining (a-c, b-d, e-g, and f-h). (a) Before cyclosporine A treatment, (b) before methotrexate treatment, (c) after cyclosporine A treatment, (d) after methotrexate treatment. Red stained CD3 + cells are abundantly expressed before treatment in upper dermis and also in epidermis (a, b). Number of positively stained cells is dramatically decreased after both treatments (c, d). (e) IFN-γ expression before methotrexate treatment, (f) IL-4 expression before methotrexate treatment, (g) IFN-γ expression after methotrexate treatment, (h) IL-4 expression after methotrexate treatment. Both cyclosporine A and methotrexate treatments caused decreased IFN-γ and IL-4 expression in skin sections of some patients. Sections of the patient from methotrexate group show that the IFN-γ expression mainly confined to papillary dermis (e) and IL-4 expression in dermis (f) are suppressed after the treatment (g, h) (page 195).
Discussion

The present study shows that the diminished T cell numbers in lesional skin are not only observed after cyclosporine A therapy of psoriasis, but also after methotrexate therapy. In last decade, many in situ studies widely documented the T cell number reducing effects of cyclosporine A in psoriatic skin\textsuperscript{12,13}, but surprisingly though, in situ effects of methotrexate therapy on psoriatic T cells has not been studied until now. The main cause of the lack of these studies is probably the strong proliferation inhibitory effects of methotrexate which has been considered as the major therapeutic mechanism in psoriasis by the suppression of keratinocyte growth. It has also been shown that methotrexate suppresses neutrophil functions in psoriatic skin. Several studies postulated that the beneficial effects of methotrexate on T cells in other diseases like rheumatoid arthritis and graft versus host disease maybe mediated by T cell death and subtype changes\textsuperscript{14}. Obviously, inhibitory effects of methotrexate on T cells also operate in the treatment of psoriasis. In the present study we show that this
change comprises mainly reduction of T cell numbers in skin. This decrease could be the result of decreased cutaneous lymphocyte associated antigen expressing T cells in the peripheral blood after methotrexate treatment of the patients in this study, which could result eventually in decreased recruitment of T cells into the skin (unpublished peripheral blood data from this study). On the other hand one cannot exclude the possibility of T cell apoptosis in the skin, which has been shown to occur in in vitro studies. Both methotrexate and cyclosporine A are known to influence cytokine expression regulation. It has been shown that IFN-γ related molecules i.e. IP-10 and HLA-DR are downregulated in psoriatic skin by cyclosporine A therapy. Nevertheless, no significant change in IFN-γ expression in psoriatic skin before and after cyclosporine A therapy has been found. Although cyclosporine A has been shown to restore the abnormal cytokine balance in atopic dermatitis, it seems to suppress expression of both IFN-γ and IL-4 in 3 of 5 cases of psoriatic skin lesions. Similarly, methotrexate causes decrease of type 1 and increase of type 2 cytokines in rheumatoid arthritis patients. However, in this study it was not found to have a major effect on IFN-γ/IL-4 balance, but reduction of IFN-γ in 3 and IL-4 in 1 of 5 cases.

In conclusion, we have demonstrated that cyclosporine A and methotrexate were equally effective in decreasing T cell numbers in psoriatic skin. However, none of the systemic treatments had effect on lesional IFN-γ and IL-4 expression, although a reduction of cytokine expression was observed in some patients.

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References


