

# Delayed-type Hypersensitivity in Palmoplantar Pustulosis: Effect of Cyclosporin A Treatment on Skin Testing with Recall Antigens

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Delayed-type hypersensitivity reactions to skin antigens are an indirect measure of cellular immune response. We studied in a double-blind manner whether clinically effective doses of cyclosporin A in palmoplantar pustulosis would diminish delayed-type hypersensitivity reactions *in vivo*. For testing delayed-type hypersensitivity, we applied intradermally a standardized panel of seven recall antigens and a vehicle control in 30 patients with palmoplantar pustulosis, and 28 were tested both at baseline and after 4 weeks. For 4 weeks 14 patients were treated with 2.5 mg/kg/day cyclosporin A and 14 patients with placebo. Cyclosporin A but not placebo caused a significant decrease in clinical disease parameters. In contrast, no significant differences in delayed-type hypersensitivity reactions between treatment groups were observed. The results do not support the view that the efficacy of low-dose cyclosporin A in dermatological disorders can be entirely explained by cyclosporin A's inhibitory actions on effector T-cells. **Key words:** intradermal test; double-blind study; mechanism of drug action.

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Müller & Herrmann first showed that cyclosporin A (CsA) is effective in the treatment of psoriasis (1). In further controlled studies the efficacy of CsA has been shown in psoriasis (2), atopic dermatitis (3) and palmoplantar pustulosis (PPP) (4). Additionally, a wide variety of other skin diseases have been shown to respond to CsA therapy (5, 6).

CsA exerts effects on various cell types *in vitro*. However, when CsA concentrations similar to the *in vivo* situation are used, the most pronounced effect is seen on T-lymphocytes with a blocking effect on the transcription of several lymphokine genes including interleukin-2 (7). Effects on other cell types of the skin, such as keratinocytes and Langerhans' cells, are observed with higher CsA concentrations not present in the *in vivo* situation (8, 9). Many dermatoses studied display inflammatory infiltrates rich in T-lymphocytes, especially of the CD4 phenotype, which suggests that cellular immunity plays a role in the pathogenesis of these diseases. This further supports the concept that the *in vivo* action of CsA in skin diseases is mediated through the inhibition of lymphokines in these cells. However, several of the CsA-responsive dermatoses also have infiltrates of other inflammatory cells, e.g. neutrophilic granulocytes (psoriasis, PPP, pyoderma gangrenosum) and eosinophils (atopic dermatitis). Delayed-type hypersensitivity (DTH) reactions to skin antigens are an indirect measure of cellular immune response. This study was planned to determine whether clinically effective doses of CsA in palmoplantar pustulosis (PPP) would actually result in measurable impairment of DTH *in vivo*. PPP was chosen for this study

because it shows a restricted location, so that it can possibly be assumed that the skin test sites represent normal healthy skin.

## MATERIAL AND METHODS

### Patient and control groups

The study was approved by the Ethics Committee of the Department of Dermatology, Helsinki University Central Hospital. Informed consent was obtained from all patients. Forty patients with PPP were consecutively randomized to two preassigned treatment groups, placebo or CsA at 2.5 mg/kg/day (4). Both treatment groups were comparable in terms of age, sex, disease duration, disease activity, and several other parameters. All 40 patients were asked to volunteer for serial intradermal testing; 30 patients volunteered and 28 were studied both at baseline and 4 weeks. Additionally, 13 healthy control subjects volunteered for testing. The baseline characteristics of the patient groups studied both at baseline and after 4 weeks and healthy control subjects are shown in Table I.

### Treatment schedule

As previously published, the first 4 weeks of treatment were performed as a double-blind study with placebo or CsA at a dosage of 2.5 mg/kg/day (4). After 4 weeks the study was continued as an open trial. Clinical evaluation of the lesions was determined primarily by counting the number of fresh pustules in the palmar and plantar lesions. Successful treatment was defined as a more than 50% reduction in the number of pustules compared to baseline. If the treatment was a success the same treatment was continued, if not, the treatment was continued in the placebo group with 1.25 mg/kg/day CsA and in the CsA group with 3.75 mg/kg/day CsA, which was the highest CsA dose used in the study. The patients were evaluated every 4 weeks for the success or failure of treatment. CsA treatment was terminated after 16 weeks and the patients were followed up until week 24.

### Skin testing

DTH tests were performed in all patients at the beginning of treatment and in some of the patients after 4, 16 and 24 weeks. The control subjects were tested for reactions in DTH simultaneously on both right and left arm. Skin testing for DTH was performed using Multitest CMI (Institut Mérieux, Lyon, France) (10). The eight-pronged Multitest applicator contained 7 preloaded antigens and 1 vehicle

Table I. Baseline characteristics of the study groups: the healthy controls and the PPP patients studied at baseline and after 4 weeks

Study groups	Total (n)	Sex (n) F/M (ratio)	Age Mean (range)
1. Healthy controls	13	9/4 (2.25)	35.2 (24–60)
2. PPP patients	28	19/9 (2.1)	40.4 (24–62)
receiving CsA	14	10/4 (2.5)	39.7 (24–58)
2.5 mg/kg/day for 4 weeks			
receiving placebo for 4 weeks	14	9/5 (1.8)	41.0 (29–62)

Table II. The antigens included in Multitest CMI

Antigen*	Concentration
Tetanus toxoid	550,000 U/ml
Diphtheria toxoid	1,100,000 U/ml
Streptococcus group C	2,000 U/ml
Tuberculin	300,000 TU/ml
Candida albicans	2,000 U/ml
Trichophyton mentagrophytes	150 U/ml
Proteus mirabilis	150 U/ml

\* All antigens were applied in 70% glycerol; a vehicle control was included in the test.

control (Table II). All applicators were purchased at the same time and were from the same batch. The test site was the forearm and the tests were read after 48 h. Consecutive tests on the same patient were performed on the forearm not previously tested. All tests were evaluated by the same person. A test reaction of at least 2 mm in diameter was regarded as positive. For each positive test reaction the average diameter of the induration (mm) was calculated; results were given as sums of the average diameters (the Merieux score), and also as a number of positive antigens.

#### Statistical methods

The data are presented as the mean  $\pm$  SEM. The levels of statistical significance between the groups were calculated using Students unpaired *t*-test. For differences within one group at various time-points, a paired *t*-test was used. Levels of  $p < 0.05$  were regarded as significant. For assessment of reliability of two methods for scoring intradermal tests (Merieux score and number of positive antigens), coefficient of repeatability (11) was used.

## RESULTS

#### Reproducibility of the intradermal test method

In a left-to-right comparison of intradermal DTH reactions in healthy controls the reproducibility was better with Merieux score than with the number of positive antigens. The coefficient of repeatability (11) was 0.22 of the mean for Merieux score and 0.28 of the mean for number of positive antigens. Consequently, Merieux score was chosen as the parameter for this study.

#### DTH characteristics of study populations

None of the tested subjects were anergic at the beginning of the study. The Merieux score seemed higher in PPP patients than in healthy controls ( $14.4 \pm 1.8$  vs.  $10.1 \pm 1.5$ ; not significant (n.s.)). The age of the test subjects did not seem to influence DTH (not shown). The Merieux score seemed higher in healthy males when compared to healthy females ( $12.6 \pm 2.0$  vs.  $9.0 \pm 2.0$ ; n.s.); this sex difference was significant in PPP patients ( $22.3 \pm 3.2$  vs.  $10.7 \pm 1.4$ ;  $p < 0.005$ ). The Merieux score baseline values were highly similar in PPP patients starting double-blind CsA or placebo treatment ( $14.7 \pm 3.1$  vs.  $14.2 \pm 1.9$ ).

#### Effect of 4-week placebo or CsA treatment on DTH

As shown in Fig. 1, the patient group receiving CsA did not show any notable suppression of DTH when compared to the placebo group. No statistical differences between any groups at weeks 0 or 4 were detected. At the same time the disease activity was significantly reduced in the CsA group when compared to the placebo group (4).

#### DTH after 16 weeks of treatment

In patients studied for at least 16 weeks, the group initially receiving placebo and receiving a maximal dose of 1.25 mg/kg/day of CsA ( $n=4$ ) showed modest suppression in the Merieux score ( $13.8 \pm 2.2$  at 0 wk,  $12.4 \pm 2.5$  at 16 wk; n.s.). The group receiving a maximal dose of 2.5 mg/kg/day of CsA ( $n=10$ ) also showed a slight suppression in the Merieux score ( $13.1 \pm 3.8$  at 0 wk,  $11.7 \pm 2.2$  at 16 wk; n.s.). When the patients receiving a maximal dose of 3.75 mg/kg/day of CsA ( $n=4$ ) were studied, they also showed a similar suppression in the Merieux score ( $11.8 \pm 3.9$  at 0 wk,  $10.8 \pm 2.1$  at 16 wk; n.s.). The differences between these groups at 16 weeks were not significant.

#### DTH after 16 weeks of treatment and an 8-week follow-up period

When compared to baseline, in those patients who completed the whole study on DTH skin reactions there seemed to be

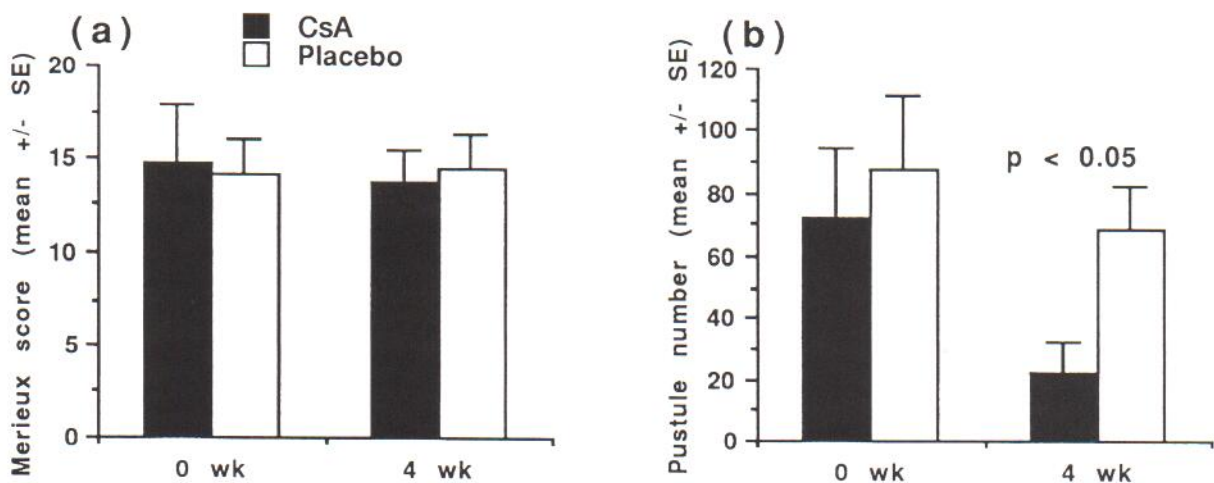


Fig. 1. The effects of CsA on DTH reactions (a) and on clinical response (b), calculated as the number of fresh pustules (4) during the first 4 weeks of treatment with either CsA or placebo.

Table III. Effects of CsA on DTH or patch test reactions in various dermatological diseases

Disease (Ref.)	CsA dose (mg/kg/d)	Treatment time (weeks)	Effect on recall antigens* or patch test reactions
Atopic dermatitis (18)	5	4	None
Psoriasis (2)	0, 3, 5, 7.5 Mean 5 (3-7.5)	8 16	None* Decrease*
Contact dermatitis (16)	5	2	Decrease
(17)	5	4	Slight decrease

suppression of DTH. There was a decline in the mean Merieux score in the group receiving a maximal dose of 1.25 mg/kg/day of CsA ( $n=8$ ;  $14.2 \pm 2.0$  at 0 wk to  $11.7 \pm 2.9$  at 24 wk; n.s.), in the group receiving a maximal dose of 2.5 mg/kg/day of CsA ( $n=14$ ;  $13.8 \pm 3.1$  at 0 wk to  $10.9 \pm 2.0$  at 24 wk; n.s.) and in the group receiving a maximal dose of 3.75 mg/kg/day of CsA ( $n=7$ ;  $17.2 \pm 3.5$  at 0 wk to  $12.2 \pm 1.8$ ;  $p=0.076$ ). One patient in the initial placebo group was anergic at the end of the study.

## DISCUSSION

In the present double-blind study we found that treatment with CsA at 2.5 mg/kg/day for 4 weeks did not have a significant effect on in vivo DTH reactions. This was in contrast with the rapid effect of CsA in clinically measurable parameters, e.g. formation of new pustules in patients with PPP (4). The stronger DTH reactions in men as compared to women in this study could at least partly be explained by the immunization program that men receive in the army.

Earlier the effects of CsA on DTH and contact hypersensitivity have been studied in other dermatological diseases, including psoriasis, chronic dermatitis, allergic contact dermatitis and atopic dermatitis (Table III).

Van der Heyden et al. (12) reported a lack of suppression of DTH reactions to secondary antigens in patients undergoing kidney transplantation. However, the study was an open trial using patients with systemic disease, a high dose of CsA (14 to 6 mg/kg/day), and the controls consisted of healthy individuals instead of patients not receiving CsA.

Ellis et al. (2) showed that DTH reactions in patients with plaque-type psoriasis did not differ after 8 weeks among different treatment groups including placebo. However, after 16 weeks the DTH reactions were reduced in patients with a mean CsA dose of 5.0 mg/kg/day, a higher mean dose than that used in this study. Interestingly, 9 of 42 patients (21.6%) were anergic even before therapy. Several studies on DTH in psoriasis support the finding of an increased occurrence of anergy and a decreased reactivity in DTH skin tests in psoriasis patients when compared to healthy controls (13-15). In contrast, none of the PPP patients in this study were anergic at the beginning.

Higgins et al. (16) reported that when patients with chronic contact dermatitis were tested on the back skin during CsA treatment, the patch test reactions were reduced. However, when Flori et al. (17) treated 15 patients with allergic contact dermatitis with CsA, the patch test reactions were not clearly modified. Munro et al. (18) treated 14 patients with atopic dermatitis with CsA and found that immediate and late phase reactions to intradermal house dust mite antigen were increased, but patch test responses were not influenced.

In conclusion, the results of this study suggest that the efficacy of low-dose CsA in vivo in PPP does not have to be related to inhibition of DTH. One interpretation could be that T-cell responses are not involved in PPP. An alternative argument might be that the cytokines responsible for PPP are suppressed by 2.5 mg/kg of CsA, whereas interferon-gamma involved in delayed hypersensitivity is not. Finally, it seems that in dermatoses responding to low-dose CsA, pathomechanisms other than inhibition of DTH to putative antigens may be involved.

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