younger satellite lesion on the elbow, make it more likely that the elimination of the infection is due to some direct chemical action rather than an effect of increased turnover rate or destruction of stratum corneum as a substrate, discussed as possible reasons for the spontaneous healing (1, 2, 7).

The investigation concerns only one person. The maximum size of an experimental infection, including the perilesional invasion of the horny layer observed in this study, will certainly differ from person to person, depending on the rapidity with which delayed sensitivity develops.

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Cyclosporin A Does Not Modify Langerhans' Cell Number and Distribution in Normal Human Skin

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We used the model of human skin graft on nude mouse to investigate a possible influence of systemically administered cyclosporin A (CsA) on epidermal Langerhans' cells (LC). This experimental model has the advantage of being independent of the systemic humoral and cellular influences of a human host. No change in the human LC distribution or number could be observed after 3 weeks of CsA therapy as revealed by anti-CD1 and anti-HLA-DR immunohistochemical stainings and the cell counting, despite the evidence of the drug effect on the keratinocyte proliferation. However, our findings do not rule out the possibility that CsA influences the LC functional capacities responsible for the local cell-mediated immunity responses. ***Key words: Langerhans' cell counting; 'Nude' mouse; Skin graft.***

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Cyclosporin A (CsA) is an immunosuppressive agent which has been demonstrated to inhibit T-lymphocyte-mediated immune reactions (1). A recent report indicated that topically applied CsA inhibited a local contact hypersensitivity to nickel (2). Since this type of immune response is known to be mediated by Langerhans' cells (LC), the question of CsA influence on the LC population has been raised (2–4). The results of LC quantitation after topical application of CsA were rather contradictory. No influence of the drug on the number of LC could be observed in the human patch-test experiments (2), although a significant reduction of ATPase-positive dendritic cells in CsA-treated mice has been reported (3).

Systemically administered CsA did not modify LC number in mice (3, 4) nor in CsA/prednisone immunosuppressed renal transplant patients (5). The turnover of epidermal LC (and also their number) is maintained in normal conditions by recruitment of circulating LC precursors from the monocyte–macrophage lineage and, to a lesser degree, by an *in situ* slow-rate LC division (6, 7). Since the systemic phenomena may influence the epidermal LC number, we used a model of human skin graft to nude mice, which is free of the human systemic control mechanisms, for studies on the direct effect of CsA on human epidermal LC distribution and density.

MATERIALS AND METHODS

Normal human skin was obtained from mammoplasty and whole thickness grafts (approximately 1 cm^2) were performed

Table I. *Quantitation of CD1 and HLA-DR-positive epidermal cells in the grafted normal human skin after 3 weeks of treatment with Cyclosporin A*

Monoclonal antibody	Number of positive cells: Per 1 mm² of epidermal section area (mean ± SD) Control ($n=8$)	CsA[b] ($n=7$)	Per 1 mm of epidermal section length (mean ± SD) Control ($n=8$)	CsA[b] ($n=7$)
BL6	89.1±25.6	87.0±23.7	7.2±2.7	5.9±2.2
BL2	79.5±24.5	87.5±24.2	5.9±2.2	5.6±2.3
LN3[a]	75.1±23.5	85.6±22.6	6.4±2.7	6.7±2.7

[a] Results obtained with deparaffinized sections.
[b] No statistically significant differences between the three groups or when compared with the controls.
n=number of grafts; SD = standard deviation.

on Swiss nu/nu athymic mice (Iffa-Credo, Les Onçins, France) according to the technique described in detail elsewhere (8). The wound dressings were removed after 2 weeks and one group of animals was injected subcutaneously with 50 mg/kg of CsA (Sandoz Inc, Basle, Switzerland) once a day for 3 weeks, whereas control mice received subcutaneous injections of the solvent alone (olive oil). During the third week, the mice from both groups were injected intraperitoneally three times a day the physiological saline solution of 5-bromo-2′-deoxyuridine (BrdU, 100 mg/kg, Sigma Chemicals, St. Louis, USA). At the end of the experiment, the animals were sacrificed and the grafted human skin was excised with a margin of the surrounding mouse tissue. One part of each graft was immediately frozen in liquid nitrogen, another fixed in Baker's solution. Blood levels of CsA were measured by radio-immunologic assay.

Human Langerhans' cells were visualized on frozen and deparaffinized tissue sections using immunohistochemical stainings with: 1) BL6, anti-CD1a antigen monoclonal antibody (Immunotech, Marseille, France), diluted 1:50; 2) BL2, anti-HLA-DR antigen monoclonal antibody (Immunotech, Marseille, France), diluted 1:100; 3) LN3, anti-HLA DR monoclonal antibody reactive on deparaffinized sections (Biotest diagnostics, Dreieich, West Germany), diluted 1:10. The sections were incubated with the primary antibodies at 4°C overnight, washed in phosphate-buffered saline, and the immunoreactivity was visualized with an avidin–biotin–alkaline phosphatase technique (ABC kit, Vector Lab., Burlingame, U.S.A.). For detection of BrdU-positive (S-phase) cells, the sections were preincubated in 4 N HCl (15 min, 37°C) and washed in 0.1 M borax buffer (pH 8.5, 10 min) before use of an anti-BrdU primary antibody (Becton-Dickinson, Mountain View, California) diluted 1:20. The counting of positive cells was performed according to the methods described previously (9, 10) using a semiautomatic image analyser (Videoplan, Kontron, Munich, West Germany). The rate of keratinocyte proliferation was evaluated as the mean number of the BrdU-positive cells counted in the epidermal sections per 100 basal layer keratinocytes. Student's t-test was used for the statistical analysis of results.

RESULTS

All the 15 human skin grafts performed were successful. Human dendritic cells were preserved in the grafted skin, as revealed by the anti-CD1 and anti-HLA-DR labelling. No positive cells were observed with these human-specific antibodies in the adjacent mouse epidermis. The Langerhans' cells (LC) in the grafts were distributed regularly in the mid-epidermis and no anti-CD1 reactivity could be detected in the dermis. This normal distribution of LC was not affected by cyclosporin A (CsA) treatment.

The results of counting of epidermal LC are summarized in Table I. In the controls, the number of cells positive with BL6 monoclonal antibody was not significantly different from the results of quantitation with the two anti-HLA-DR antibodies; nor could any significant modification of the LC population density be observed in the grafts after treatment with CsA. Irrespective of the mode of presentation of the data, per unit section length (independent of the mean epidermal thickness) or per unit section area (relative to the epidermal volume), the relations between the control and CsA groups remained unchanged. This indicates that there was no CsA-induced variability of the epidermal thickness (acanthosis) or irregularity of the dermal–epidermal junction. An increased hair growth could be observed in the CsA-treated mice as from the second week of the drug administration. The keratinocyte proliferation rate was significantly ($p<0.001$) decreased after CsA treatment: 27.3±6.9 BrdU+ cells per 100 basal layer keratinocytes versus 61.1±13.5 in the controls. The mean plasma level of CsA in the treated animals was 679 ng/ml.

DISCUSSION

Previous studies demonstrated that about 85–90% of LC observed in the initial biopsies were still present in the human skin grafts on 'nude' mice at 4 weeks after grafting (7). Human inflammatory cells disappear from the grafts which are cut off from the systemic (cellular and humoral) influences of human origin (8, 11). Therefore, human skin graft onto 'nude' mice represents a useful and 'pure' model for investigation of human LC outside of the human body.

In the present study, we demonstrate the absence of the direct effect of systemically administered CsA on the density and distribution of human epidermal LC. In our experimental model, we avoided the possibility of recruitment of the LC precursors from the circulating blood pool to the CsA-treated epidermis. Therefore, our data unequivocally confirm previous results obtained in the mouse LC studies (3, 4) and the immunosuppressed renal transplant patients (5). Contrary to other authors, we verified the plasma levels of CsA in the treated animals and were thus able to confirm that these were similar to the CsA blood concentration usually observed in human patients (12). Additionally, an increased rate of hair growth in the CsA-treated mice, similar to the common side effect in humans, was indicative of the biological effectiveness of the drug. Using the same human skin graft model, we observed, as others did in the in vitro studies (13), a suppressive influence of CsA on proliferation of interfollicular keratinocytes, and this result also indicated that the intracellular levels of the molecule are sufficient to exert a direct action. Therefore, the epidermal keratinocyte reactivity to CsA seems to be independent of the drug influence on the LC population in normal human skin.

Even if the numerical fluctuations of epidermal LC are not induced by CsA, the possibility that it has some influence on the LC function has to be considered. Several authors have demonstrated the in vitro inhibition of the accessory function of macrophages and dendritic cells from spleen and lymph nodes by CsA at concentrations of 5 to 500 ng/ml (14–16). Recently, CsA has been reported to inhibit the antigen-presenting capacity of mouse LC in vitro (4). This latter result, however, could only be observed with the high drug concentration (about 5 µg/ml) and could not be reproduced in vivo. Also, the mixed leukocyte reaction studies with pre-treated cell subsets have indicated that CsA blocks T-cell but not dendritic cell function in vitro (17). Further studies are required to clarify the effect of CsA on the functional capacity of LC in Man.

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Topical Cyclosporin A in Alopecia Areata

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We conducted a trial of topical application of 10% cyclosporin A in an oil preparation in 10 patients with alopecia areata and alopecia universalis. After 12 months of therapy, no beneficial response was observed in any of the 10 patients.

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Because of the immunosuppressive effect of CS, combined with its hair growth promotion (1), several studies have been performed in order to investigate a possible beneficial effect of the drug in alopecia areata (AA) and alopecia universalis (AU) (2, 3). Parodi & Rehora (2) and others (3) described a significant hair growth in patients with AA and AU following topical CS application, whereas Manduit et al. (4) deny any benefit from the drug in AA. Because of these conflicting reports we conducted a study focused on patients with severe AA and AU.

MATERIALS AND METHODS

Ten patients with severe AA and AU were enrolled into this study. They were 6 male and 4 female patients, ranging in age from 20 to 54 years. The duration of their disease ranged from 2 to 18 years. Six patients demonstrated AU and 4, AA. One patient had associated autoimmune thyroiditis. All subjects were in good health, as documented by history, physical examination and laboratory evaluation.

Each patient had the following laboratory examinations performed prior to treatment, and again once a week in the first month of treatment; thereafter, twice a month: complete blood cell count, urinalysis, serum urea nitrogen, uric acid, alkaline phosphatase, SGOT, bilirubin, creatine kinase, calcium, phosphorus, lactic dehydrogenase. The CS blood levels of each patient were monitored by both the radio-immunoassay (RIA) and high pressure liquid chromatography (HPLC) method (5) once a week in the first month and once a fortnight thereafter. Scalp biopsy specimens were obtained from each patient prior to treatment and at the completion of the study. Photographs were taken before and monthly during the treatment phase. Response to treatment was defined as growth of terminal hair. All evaluations of hair growth were performed by the same observer (A. G.). The solution contained 10% CS in an oily preparation. The solution (0.5cc) was applied twice a day. Informed consent was obtained from each patient prior to treatment.

RESULTS

After 12 months of treatment, as well as during the follow-up, no significant response was observed in any of the treated patients. Growth of vellus hair was noted in 3 patients with AU. Scalp hair loss was observed in one patient with AA. The histological finding confirmed the clinical observations of unresponsiveness. All patients failed to show any signs of systemic absorption of CS by the HPLC technique, which is highly specific for unchanged drug. The less specific RIA technique detected traces of CS in samples from 2 patients. Laboratory studies did not show any change in the various tests reported before.

DISCUSSION

In animal studies, we were able to show that both systemic and topical CS caused hair growth in nude mice engrafted with normal human scalp and then with skin obtained from patients with AA and AU (6, 7). Moreover, we have conducted a study of topical CS in patients with male pattern alopecia and found a significant hair growth in 2 out of 8 patients (8). In contrast to the animal study, we were unable to show any effect of CS in any of the 10 patients treated.

The lack of effect of topical CS in our severe AA patients, contrary to hair growth observed following