British Journal of Dermatology 1998; 139: 984–991.

Calcipotriol inhibits the proliferation of hyperproliferative CD29 positive keratinocytes in psoriatic epidermis in the absence of an effect on the function and number of antigen-presenting cells

A.M.JENSEN, M.BØRRESEN LLADÓ, L.SKOV, E.R.HANSEN, J.K.LARSEN* AND O.BAADSGAARD

Department of Dermatology, Gentofte Hospital, University of Copenhagen, Niels Andersens Vej 65, 2900 Hellerup, Denmark
**Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark*

Accepted for publication 25 June 1998

Summary

The aim of this study was to elucidate some of the possible mechanisms of action of the vitamin D analogue calcipotriol *in vivo*. Calcipotriol is finding increasing use in the treatment of psoriasis, but the primary target cell *in vivo* has not yet been identified. We treated psoriatic patients and healthy volunteers with calcipotriol and placebo ointment for 4 and 7 days, and obtained epidermal cell suspensions from treated areas. Epidermal cells were cocultured with autologous T cells, isolated from peripheral blood, in the absence or the presence of a classical antigen or a superantigen. In both psoriatic and normal skin, calcipotriol treatment did not alter the capacity of epidermal antigen-presenting cells to stimulate the proliferation of autologous T cells, either in the absence or in the presence of exogenous antigen. Epidermal cell suspensions were analysed further by staining for infiltrating leucocytes (CD45+) and Langerhans cells (CD1a+). Flow cytometric analyses showed that calcipotriol did not alter the number of CD45+ cells or Langerhans cells in psoriatic skin. These results indicate that calcipotriol does not alter either the number or the function of epidermal antigen-presenting cells in psoriatic epidermis. In contrast, we found that calcipotriol significantly inhibited the proliferation of epidermal cells isolated from psoriatic skin after *in vivo* treatment, as determined by propidium iodide staining and flow cytometry. More specifically, we stained for CD29+ keratinocytes and found an even more significant reduction in proliferative capacity. This cell type contains the population of hyperproliferative keratinocytes in psoriatic epidermis. In conclusion, calcipotriol seems to act via an inhibitory effect on hyperproliferative basal keratinocytes of psoriatic epidermis, rather than via an effect on infiltrating leucocytes, including antigen-presenting cells.

The hyperproliferative skin disease psoriasis is characterized by increased keratinocyte proliferation and infiltration of leucocytes, predominantly activated T cells.[1] Activated T cells are believed to initiate the psoriatic lesions,[2,3] although it is not known how the T cells achieve and maintain their activated state. One of the classical mechanisms of T-cell activation is through contact between the T-cell receptor and a major histocompatability complex class II-expressing antigen-presenting cell.

The vitamin D analogue calcipotriol has been found to be effective in the treatment of psoriasis, but the mechanisms of action of calcipotriol in the skin *in vivo* have not been determined. From *in vitro* studies, calcipotriol and calcitriol have been shown to inhibit the proliferation of both keratinocytes and T cells[4–6] but recently, the inhibition of keratinocyte proliferation was shown to be dependent on culture conditions. In cultures containing high extracellular calcium, calcitriol was found actually to stimulate keratinocyte proliferation.[7] The effect of calcipotriol on keratinocyte proliferation *in vivo* therefore needs further examination. So does the effect of calcipotriol on antigen-presenting cells and their ability to activate T cells. In an earlier study, calcipotriol caused a decreased T-cell proliferation; however, this decrease may have been caused by a direct effect on the T cells, instead of an effect on antigen presentation, as calcipotriol was added to

Correspondence: Ole Baadsgaard. E-mail: Baadsgaard@nethotel.dk.

in vitro cocultures of antigen-presenting cells and T cells.[8]

Our intention was to elucidate the possible primary mechanisms of action of calcipotriol in the treatment of psoriasis and to carry out studies as close to the situation *in vivo* as possible. This was accomplished by performing all analyses on epidermal cell suspensions obtained from the skin of patients with psoriasis and healthy volunteers treated with calcipotriol and placebo ointment *in vivo* for a maximum of 1 week. After 1 week of treatment, the lesions begin to clear, and changes observed hereafter may be secondary to the primary changes. The epidermal cells were analysed by immunostaining and flow cytometry for keratinocyte proliferation and for their ability to stimulate autologous T cells.

Materials and methods

Subjects

Psoriatic patients and healthy volunteers participated in the study, which was approved by the local ethics committee. All subjects gave informed consent.

Group I

Six healthy volunteers were treated for 4 days on four symmetrical areas of the buttocks or upper inner arms with calcipotriol ointment (Dovonex, 50 μg/g calcipotriol; Leo Pharmaceutical Products, Ballerup, Denmark) and calcipotriol placebo ointment (Leo Pharmaceutical Products). Two spots were treated open, twice daily, and two spots were treated under occlusion.

Group II

Three healthy volunteers were treated at three areas on the upper inner arms with calcipotriol ointment, placebo ointment and the steroid mometasone furoate (Elocon; Schering-Plough, Farum, Denmark), under occlusion for 7 days.

Group III

Seven healthy volunteers were treated with calcipotriol and placebo ointment for 7 days, twice daily, on two symmetrical areas of the buttocks.

Group IV

Thirteen psoriatic patients were treated for 7 days, twice daily, with calcipotriol and placebo ointment on two symmetrical plaques of equal severity.

Epidermal cell preparations

From the treated areas, suction blisters were raised (groups I and II) or keratome biopsies were taken (groups III and IV). Keratome biopsies were incubated overnight in dispase (2 U/mL; Gibco BRL, Paisley, U.K.) at 4 °C, and epidermis was separated from dermis. An epidermal cell suspension was obtained from epidermal sheets of keratome biopsies and suction blister roofs as described previously.[9] Epidermal cells used for T-cell proliferation studies were resuspended in RPMI-1640 (Gibco BRL) containing 1% glutamine and 10% autologous serum (subsequently referred to as medium), whereas epidermal cells used for flow cytometric studies were resuspended in phosphate-buffered saline (PBS) with 0·1% bovine serum albumin (BSA) and 0·02% NaN_3.

Isolation of autologous T cells

Heparinized blood samples were taken from volunteers (groups I and II) and psoriatic patients (group IV), and mononuclear cells (MNCs) were isolated by density gradient centrifugation on Ficoll–Hypaque (Pharmacia, Uppsala, Sweden). Monocytes were removed by plastic adherence for 1 h at 37 °C, and purified resting T cells were obtained from the monocyte-depleted MNCs using immunomagnetic beads as described previously.[10] Briefly, monocyte-depleted MNCs were incubated with murine IgG antibody to human HLA-DR, -DP (IVA12; ATCC, Rockville, MD, U.S.A.). Immunomagnetic beads coated with antibody to mouse IgG (Dynal, Oslo, Norway) were incubated with the cells for 30 min at 4 °C under rotation. HLA-DR, -DP-positive cells bound to the beads were removed using a magnet for 2×5 min. The cells left in suspension were purified resting T cells. The procedure was repeated from the addition of antibody. Using this method, less than 0·4% HLA-DR/-DP+ T cells were present after depletion. The T cells were finally resuspended in medium.

T-cell stimulation assay

In 96-well, round-bottomed microtitre plates, cultures of epidermal cells and autologous T cells were mixed in a total volume of 200 μL of medium. Approximately 50,000 T cells were cocultured with 1, 5, 10, 20 and 40×10^3 epidermal cells from calcipotriol-, placebo- or

steroid-treated skin, alone or in the presence of 2 μg/mL tetanus toxoid (TT; Statens Serum Institut, Copenhagen, Denmark) or 0·5 μg/mL *Staphylococcus* enterotoxin B (SEB; Sigma, St Louis, MO, U.S.A.). Culture duration before the addition of 0·5 μCi of [^{3}H]TdR (Amersham, U.K.) was 3 days for wells with SEB and 6 days for wells with TT and wells without exogenous antigen. Cells were harvested after 18 h using a cell harvester (Skatron, Tranby, Norway), and [^{3}H]TdR incorporation was measured by liquid scintillation. All cultures were performed in triplicate, and the results are given as mean c.p.m. ± SD). When data for the individual persons are pooled, results are given as mean c.p.m. ±SEM, owing to large interindividual differences.

Flow cytometric studies

In order to analyse the percentage of CD1a+ and CD45+ cells in epidermis, epidermal cells from skin treated with calcipotriol and placebo ointment were incubated with murine monoclonal antibodies against CD1 (Dako-CD1a; Dako, Denmark) or CD45 (anti-HLe-1; Becton Dickinson, San Jose, CA, U.S.A.) in a 1 : 10 dilution. The cells were incubated at 4 °C for 30 min. After washing, the cells were incubated with the secondary goat antimouse fluorescein isothiocyanate (FITC)-conjugated F(ab)$_2$ anti-IgG diluted 1 : 50. The cells were then kept in 70% ethanol at − 20 °C until the day before flow cytometric analysis, when they were washed and stained with 50 μg/mL propidium iodide (PI; Sigma). The nuclear staining was used to gate diploid cells with a normal DNA content, to avoid cell debris and cell aggregates and to analyse the proliferation of the CD45+ cells.

Analysis of the percentage of proliferating epidermal cells was performed via staining with murine monoclonal phycoerythrin (PE)-conjugated antibodies against CD29 (4B4-RD1L; Coulter, Hialeah, FL, U.S.A.). Before flow cytometric analysis, the cells were permeabilized in ethanol and stained with 25 μg/mL 7-amino-actinomycin D (7-AAD; Calbiochem, La Jolla, CA, U.S.A.). Initially, cells were incubated with 10% normal goat serum (Zymed, San Francisco, CA, U.S.A.) to block non-specific binding. Isotype control was antiglial fibrillary acidic protein (GFAP; IgG1, Dako). Both types of nuclear staining (PI and 7-AAD) were performed in the presence of 1 mg/mL ribonuclease A (RNase; Sigma) to avoid staining of cytoplasmic RNA. Cells were analysed on a FACS Vantage cell sorter (Becton Dickinson).

Statistics

The difference between the results for epidermal cells treated with calcipotriol and epidermal cells treated with placebo was compared using the Wilcoxon test for paired data. Statistical analysis was done with SYSTAT for Windows (SystatInc, Evanston, IL, U.S.A.).

Results

Epidermal cells from calcipotriol-treated skin do not inhibit antigen-induced T-cell proliferation

In order to examine whether calcipotriol had an inhibitory effect on the function of epidermal antigen-presenting cells, epidermal cells isolated from normal skin treated with calcipotriol or placebo ointment were cocultured with autologous T cells in the absence or the presence of antigen (TT) or superantigen (SEB). After 4 days treatment of normal skin, no difference was found between the capacity of calcipotriol-treated epidermal cells and placebo-treated epidermal cells to stimulate the proliferation of autologous T cells (Fig. 1), either in the absence of antigen (mean c.p.m. ± SEM: calcipotriol 1770 ± 1140; placebo 1196 ± 560; $n = 5$, $P = 0{\cdot}5$) or in the presence of TT (mean c.p.m. ± SEM: calcipotriol 24,603 ± 5751; placebo 23,950 ± 5684; $n = 6$, $P = 0{\cdot}9$) or SEB (mean c.p.m. ± SEM: calcipotriol 77,580 ± 2939; placebo 90,268 ± 11,598; $n = 4$). Similar results were found for normal skin treated with calcipotriol and placebo for 4 days under occlusion (data not shown). Nor did treatment with calcipotriol and placebo ointment for 7 days *under occlusion* have any effect on the epidermal antigen-presenting capacity (Fig. 1). However, in contrast to calcipotriol, treatment with the corticosteroid mometasone furoate totally abrogated the TT-induced T-cell proliferation (Fig. 2).

Similar experiments were performed with epidermal cells from psoriatic skin, which was treated for 7 days, twice daily, with calcipotriol and placebo ointment. As found for normal skin, calcipotriol did not alter the capacity of epidermal cells to activate autologous T cells (Fig. 1), either in the absence of antigen (mean c.p.m. ± SEM: calcipotriol 1085 ± 526; placebo 722 ± 495; $n = 6$, $P = 0{\cdot}1$) or in the presence of TT (mean c.p.m. ± SEM: calcipotriol 12,414 ± 5388; placebo 17,985 ± 9265; $n = 5$, $P = 0{\cdot}4$) or SEB (mean c.p.m. ± SEM: calcipotriol 52,452 ± 9493; placebo 53,643 ± 8016; $n = 6$, $P = 0{\cdot}8$). Others have found that psoriatic epidermal cells induced higher T-cell proliferation than normal epidermal cells in the absence

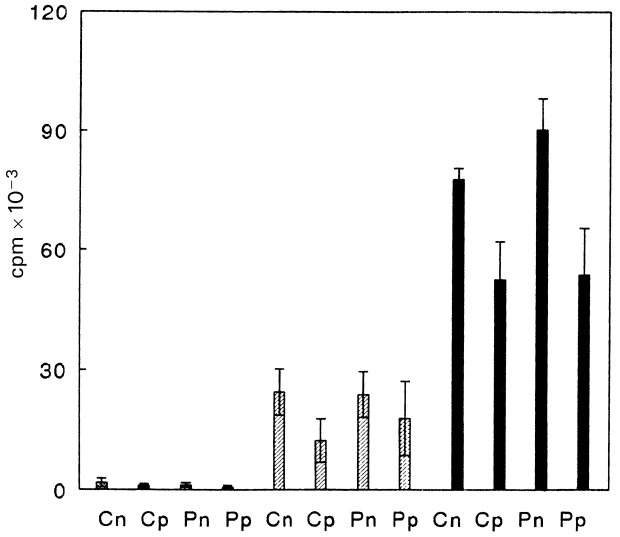

Figure 1. Six healthy volunteers were treated for 4 days with calcipotriol (Cn) and placebo (Pn) ointment on symmetrical areas of arms or buttock, and six psoriatic patients were treated for 7 days, twice daily, with calcipotriol (Cp) and placebo (Pp) ointment. Epidermal single-cell suspensions were obtained, and 20×10^3 epidermal cells were cocultured with 50×10^3 purified autologous T cells in the absence of antigen or in the presence of either 2 μg/mL tetanus toxoid (TT) or 0·5 μg/mL *Staphylococcus* enterotoxin B (SEB) in triplicate. After 3 (wells with SEB) and 6 days incubation (wells without antigen and with TT), cultures were pulsed with [methyl-^{3}H]thymidine for 18 h, cells were harvested and analysed by liquid scintillation. Solid bars represent mean c.p.m. values ± SEM for cultures incubated with SEB (normal skin: $n=4$; psoriasis: $n=6$), hatched bars represent mean c.p.m. ± SEM for cultures incubated with TT (normal skin: $n=6$; psoriasis: $n=5$) and chequered bars represent mean c.p.m. ± SEM for wells without antigen (normal skin: $n=5$; psoriasis: $n=6$).

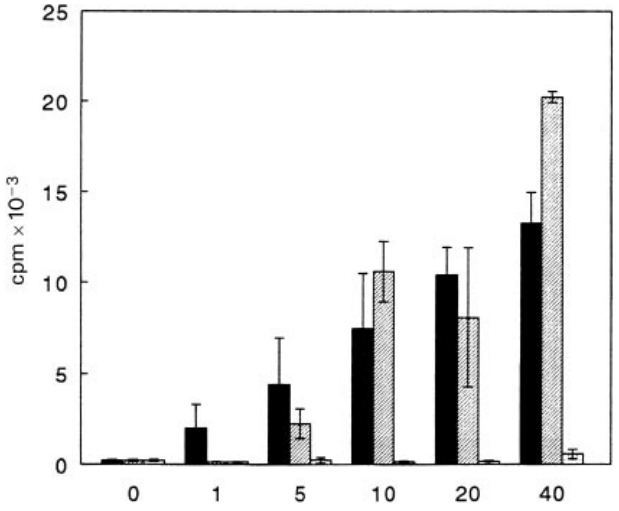

Figure 2. Representative dose–response study ($n=3$) for one healthy volunteer treated with calcipotriol, mometasone furoate and placebo ointment for 7 days under occlusion. Approximately $1–40\times10^3$ epidermal cells were incubated with 50×10^3 T cells in the presence of tetanus toxoid. The numbers of epidermal cells ×1000 are shown on the *x*-axis. Solid bars represent mean c.p.m. ± SD for autologous T cells incubated with epidermal cells treated with calcipotriol, hatched bars represent epidermal cells treated with placebo and dotted bars represent epidermal cells treated with mometasone furoate. In contrast to calcipotriol, steroid treatment of epidermal cells was able to block T-cell proliferation completely.

of antigen, which may result from a higher number of T cells used in these studies.[18,19] Our results demonstrate that alteration of the epidermal antigen-presenting capacity is not one of the primary mechanisms of action of calcipotriol in the treatment of psoriasis.

Calcipotriol does not alter the number of Langerhans cells and infiltrating leucocytes in psoriatic epidermis

We then wished to examine whether calcipotriol was able to influence the number of Langerhans cells and infiltrating leucocytes in psoriatic and normal epidermis. Epidermal cells from seven healthy volunteers and from seven psoriatic patients, treated with calcipotriol and placebo ointment twice daily for 7 days, were stained with either anti-CD1a/FITC or anti-CD45/FITC and PI and analysed by flow cytometry. CD45 identifies all bone marrow-derived cells, including Langerhans cells, whereas CD1a identifies Langerhans cells only. As shown in Table 1, calcipotriol did not alter the number of either CD45+ cells or CD1a+ cells in psoriatic skin. In normal skin, calcipotriol tended to decrease the number of Langerhans cells, although not at a significant level, whereas there was no change in the number of CD45+ cells.

Thus, we have demonstrated that, after 1 week of treatment, calcipotriol does not influence either the function or the number of the antigen-presenting cells in psoriatic epidermis, as Langerhans cells and infiltrating leucocytes are the only antigen-presenting cells in epidermis. The efficacy of calcipotriol in the treatment of psoriasis does not therefore appear to be caused by a modulation of the antigen-presenting capacity in the epidermis.

Calcipotriol inhibits the proliferation of basal keratinocytes in psoriatic skin

As the effect of calcipotriol on the proliferation of keratinocytes *in vitro* is dependent on culture conditions, it is important to carry out *in vivo* studies. Calcipotriol is known to reduce the hyperplasia of psoriatic lesions, but the mechanism has not been elucidated. Recently, the keratinocytes responsible for

Table 1. Epidermal cells from psoriatic and normal skin treated with calcipotriol and placebo ointments for 7 days were analysed by flow cytometry for expression of CD1a or CD45

		Number of $CD45^+$ cells as a percentage of total epidermal cells (mean ± SD)			Number of $CD1a^+$ cells as a percentage of total epidermal cells (mean ± SD)		
	n	Calcipotriol	Placebo	*P*	Calcipotriol	Placebo	*P*
Psoriatic patients	7	5·29 ± 2·85	5·08 ± 2·37	0·4	2·23 ± 0·89	2·10 ± 0·65	1·0
Healthy volunteers	7	1·81 ± 0·41	1·90 ± 0·46	0·6	1·98 ± 0·44	2·16 ± 0·49	0·06

the hyperproliferative state in psoriatic skin were shown to be present in the basal proliferative compartment of psoriatic epidermis.[11] These basal keratinocytes can be identified by anti-CD29. In order to examine the effect of calcipotriol on the proliferation of unfractionated epidermal cells and, more specifically, the proliferation of the basal CD29+ keratinocytes isolated from skin treated *in vivo*, epidermal cell suspensions were double stained with PE-conjugated anti-CD29 and the nuclear marker 7-AAD and analysed by flow cytometry.

As shown in Figure 3a, calcipotriol significantly inhibited the proliferation of psoriatic epidermal cells when compared with placebo-treated cells [mean percentage of cells in $(S + G_2 + M) \pm SD$: calcipotriol 11·49 ± 1·94; placebo 14·05 ± 2·33; $n = 10$, $P = 0·01$]. Gating of CD29+ cells gave an even more significant reduction in the percentage of proliferating cells after calcipotriol treatment [mean percentage of cells in $(S + G_2 + M) \pm SD$: calcipotriol 14·61 ± 2·42; placebo 18·05 ± 3·0; $n = 10$, $P = 0·009$] (Fig. 3b). In contrast, there was no

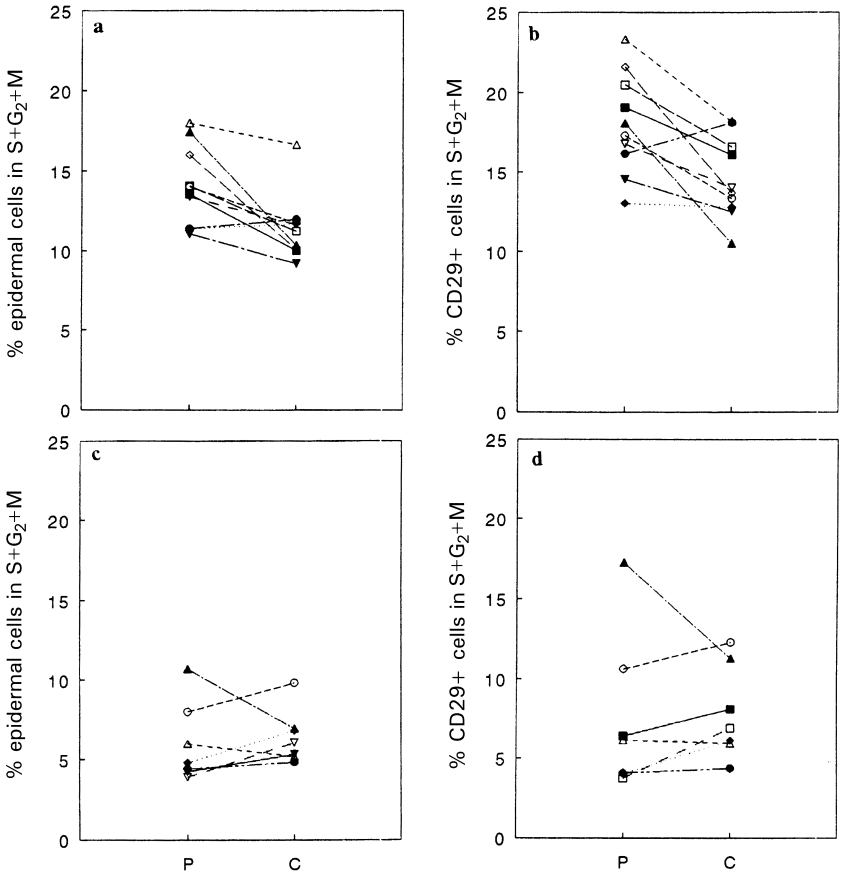

Figure 3. Epidermal single-cell suspensions from 10 psoriatic patients and seven healthy volunteers, treated with calcipotriol (C) and placebo (P) ointment, were stained with PE-conjugated anti-CD29. 7-AAD was used for nuclear staining. The percentage of total epidermal cells and CD29+ cells in the proliferative $(S + G_2 + M)$ phases of the cell cycle were analysed by flow cytometry. (a) Proliferation of total epidermal cells, psoriatic skin; (b) proliferation of CD29+ cells, psoriatic skin; (c) proliferation of total epidermal cells, normal skin; (d) proliferation of CD29+ cells, normal skin. Values for calcipotriol- and placebo-treated skin for the same person are identified by the same symbol connected with a line. In contrast to normal skin, calcipotriol significantly inhibited the proliferation of the total epidermal cell suspension and of the CD29+ cells from psoriatic skin.

statistically significant difference between calcipotriol- and placebo-treated skin from healthy volunteers. This was found for both unfractionated epidermal cells (Fig. 3c) [mean percentage of cells in $(S + G_2 + M) \pm SD$: calcipotriol $6{\cdot}43 \pm 1{\cdot}58$; placebo $6{\cdot}0 \pm 2{\cdot}31$; $n = 7$, $P = 0{\cdot}4$] and gated CD29+ cells [mean percentage of cells in $(S + G_2 + M) \pm SD$: calcipotriol $7{\cdot}85 \pm 2{\cdot}71$; placebo $7{\cdot}47 \pm 4{\cdot}58$; $n = 7$, $P = 0{\cdot}3$] (Fig. 3d).

As CD45+ cells also express CD29, we analysed the effect of calcipotriol on the proliferation of the CD45+ cells. We found no difference between the proliferation of CD45+ cells from calcipotriol- and placebo-treated psoriatic [mean percentage of cells in $(S + G_2 + M) \pm SD$: calcipotriol $9{\cdot}95 \pm 3{\cdot}33$; placebo $10{\cdot}96 \pm 5{\cdot}19$; $n = 7$, $P = 0{\cdot}7$] or normal skin [mean percentage of cells in $(S + G_2 + M) \pm SD$: calcipotriol $13{\cdot}59 \pm 4{\cdot}6$; placebo $11{\cdot}05 \pm 7{\cdot}04$; $n = 7$, $P = 0{\cdot}1$]. This allows us to conclude that calcipotriol specifically inhibits the proliferation of the CD29+ keratinocytes in psoriatic skin, as only keratinocytes and CD45+ cells express CD29 in skin. Thus, we have demonstrated that calcipotriol significantly inhibits the proliferation of the CD29+ keratinocytes in psoriatic skin. These cells contain the compartment of hyperproliferative keratinocytes in psoriatic epidermis. Therefore, our data indicate that one of the primary effects of calcipotriol in psoriatic skin is inhibition of the basal hyperproliferative keratinocytes.

Discussion

In this study, we wished to elucidate some of the primary effects of calcipotriol in the skin *in vivo*. This was obtained by treatment for a maximum of 1 week. We have demonstrated that calcipotriol does not influence the ability of epidermal antigen-presenting cells, isolated from *in vivo*-treated skin, to stimulate or inhibit the proliferation of autologous T cells, either in the absence of antigen or in the presence of classical antigen (TT) or superantigen (SEB). These results were found for antigen-presenting cells from both healthy skin treated for 4 and 7 days and for psoriatic skin treated for 7 days. Our data are in contrast to earlier studies, in which calcipotriol was shown to reduce the proliferation of T cells after incubation with epidermal cells.[8,12] However, in these studies, calcipotriol was either preincubated with epidermal cells *in vitro* or added directly to co-cultures of T cells and epidermal cells. The inhibition found was therefore likely to be caused by either a direct effect on the T cells or a carry-over effect, where calcipotriol was released to the supernatant from the epidermal cells.

In our study, epidermal cell suspensions were obtained from skin treated with calcipotriol *in vivo*, and the epidermal cells were then cocultured with autologous T cells. A possible bias in our study design was the risk of washing out calcipotriol from the epidermal cells during the culture period. We therefore included treatment with the corticosteroid mometasone furoate as a positive control, as topical steroid therapy is known to reduce the epidermal antigen-presenting capacity.[13] Corticosteroids and vitamin D are similar, because they both act through intracellular steroid receptors, with which they form complexes that interfere with gene regulation. As expected, mometasone furoate was able to abrogate the epidermal antigen-presenting capacity in our study.

The antigen-presenting cells of epidermis are resident Langerhans cells and infiltrating bone marrow-derived cells, such as macrophages. All bone marrow-derived cells can be identified with antibodies against CD45, whereas Langerhans cells are identified by anti-CD1. In order to analyse possible early changes in the number of antigen-presenting cells in the epidermis, we stained epidermal cell suspension with anti-CD1 and anti-CD45. Flow cytometric analysis showed that 7 days treatment with calcipotriol did not alter the number of either Langerhans cells or CD45+ cells in psoriatic skin. These results are in agreement with others who found that there was no significant change in the number of Langerhans cells, T cells and macrophages after 1 week of calcipotriol treatment of psoriatic skin as determined by immunohistochemical staining with anti-OKT6, anti-CD2 and anti-CD14, respectively.[14] In a later flow cytometric study, the same authors found that calcipotriol treatment twice daily did not alter the number of inflammatory cells in psoriatic epidermis, after staining for antivimentin, which identifies all non-keratinocytes in the epidermis.[15] Thus, the primary mechanism of action of calcipotriol *in vivo* is not an effect on either the number or the function of epidermal antigen-presenting cells.

Recently, Bata-Csorgo *et al*.[11] were able to identify the primary hyperproliferative population of keratinocytes in psoriatic epidermis. These were identified as CD29+K1/K10− cells. CD29+K1/K10− cells are part of the normal basal keratinocyte layer but, in normal skin, they do not hyperproliferate. A puzzle in the understanding of the mechanisms behind psoriasis and its treatment with vitamin D analogues is the fact that vitamin D analogues reduce the hyperplasia of psoriatic skin, but cause hyperplasia when applied to normal mouse skin.[16,17] Thus, vitamin D seems to have

opposite effects on healthy and diseased skin. The effect of vitamin D on the proliferation of epidermal cells from healthy human skin has not been determined. To elucidate whether calcipotriol had a differential effect on the proliferation of epidermal cells from psoriatic and healthy skin, we analysed their proliferative capacity using flow cytometry. We found that calcipotriol significantly inhibited the proliferation of epidermal cells from psoriatic skin after only 7 days. In contrast, there was no such effect on healthy skin. When we more specifically stained for CD29+ cells from psoriatic skin, which contain the basal hyperproliferative keratinocytes of psoriatic skin, we found an even more significant reduction in their proliferative capacity. There was no detectable change in the proliferation of CD29+ cells from healthy volunteers, neither a reduction similar to that found for psoriatic skin nor an increase as might have been expected from the murine *in vivo* studies. In agreement with our results, others have found a reduction in the epidermal cell DNA content and in the number of actively cycling epidermal cells after treatment of psoriatic skin with calcipotriol. However, in these studies, the treatment periods were 8 and 2 weeks, respectively.[14,15]

From *in vitro* studies in which different extracellular calcium concentrations were added to keratinocyte cultures, it has been suggested that vitamin D analogues induce proliferation of keratinocytes that are highly differentiated, but inhibit proliferation of basal undifferentiated keratinocytes.[17] Based on this interesting observation, it could be imagined that a balance exists in normal skin between the number of differentiated transit-amplifying cells and the number of basal undifferentiated cells, whereby vitamin D analogues would have opposite effects on these two cell types. This would lead to no total effect on the proliferation of epidermal cells in normal skin. In contrast, psoriatic skin is characterized by an imbalance between the two cell types, because the basal keratinocytes hyperproliferate. The dominant action of vitamin D analogues in psoriatic skin would therefore be an inhibition of the proliferation of basal hyperproliferating keratinocytes. Further studies will be needed to determine whether calcipotriol acts on such different cell types in diseased vs. healthy human skin.

In conclusion, we have shown that calcipotriol does not alter either the function or the number of epidermal antigen-presenting cells in psoriatic skin, and that the primary mechanism of action of calcipotriol *in vivo* therefore does not appear to be modulation of T-cell activation through antigen presentation. Instead, one of the primary mechanisms of action of calcipotriol treatment *in vivo* is inhibition of the proliferation of CD29+ basal keratinocytes in psoriatic skin. This is highly relevant because the keratinocytes responsible for the hyperproliferative state in psoriatic epidermis are present within the CD29+ population.

Acknowledgments

This work was supported by grants from the Danish Medical Research Council, the Danish Cancer Society and the Danish Psoriasis Research Foundation. The calcipotriol and placebo ointments were provided by Leo Pharmaceutical Products, Ballerup, Denmark. We are grateful to Jette Christensen for technical assistance.

References

1 Gupta AK, Baadsgaard O, Ellis CN *et al.* Lymphocytes and macrophages of the epidermis and dermis in lesional psoriatic skin, but not epidermal Langerhans cells, are depleted by treatment with cyclosporin A. *Arch Dermatol Res* 1989; 281: 219–26.

2 Valdimarsson H, Baker BS, Jónsdóttir I, Fry L. Psoriasis: a disease of abnormal keratinocyte proliferation induced by T lymphocytes. *Immunol Today* 1986; 7: 256–9.

3 Strange P, Cooper KD, Hansen ER *et al.* T-lymphocyte clones initiated from lesional psoriatic skin release growth factors that induce keratinocyte proliferation. *J Invest Dermatol* 1993; 101: 695–700.

4 Binderup L, Bramm E. Effects of a novel vitamin D analogue MC903 on cell proliferation and differentiation *in vitro* and on calcium metabolism *in vivo*. *Biochem Pharmacol* 1988; 37: 889–95.

5 Kragballe K, Wildfang IL. Calcipotriol (MC903), a novel vitamin D_3 analogue stimulates terminal differentiation and inhibits proliferation of cultured human keratinocytes. *Arch Dermatol Res* 1990; 282: 164–7.

6 Rigby WFC, Stacy T, Fanger MW. Inhibition of T-lymphocyte mitogenesis by 1,25-dihydroxyvitamin D_3 (calcitriol). *J Clin Invest* 1984; 74: 1451–5.

7 Gniadecki R. Stimulation versus inhibition of keratinocyte growth by 1,25-dihydroxyvitmin D_3: dependence on cell culture conditions. *J Invest Dermatol* 1996; 106: 510–6.

8 Bagot M, Charue D, Lescs M-C *et al.* Immunosuppressive effects of 1,25-dihydroxyvitamin D_3 and its analogue calcipotriol on epidermal cells. *Br J Dermatol* 1994; 130: 424–31.

9 Hansen ER, Baadsgaard O, Lisby S *et al.* Cutaneous T-cell lymphoma lesional epidermal cells activate autologous CD4+ T lymphocytes: involvement of both CD1+OKM5+ and CD1+OKM5$^-$ antigen-presenting cells. *J Invest Dermatol* 1990; 94: 485–91.

10 Strange P, Skov L, Baadsgaard O. Interferon gamma-treated keratinocytes activate T cells in the presence of superantigens: involvement of major histocompatibility complex class II molecules. *J Invest Dermatol* 1994; 102: 150–4.

11 Bata-Csorgo Z, Hammerberg C, Vorhees JJ, Cooper KD. Flow cytometric identification of proliferative subpopulations within normal human epidermis and the localization of the primary hyperproliferative population in psoriasis. *J Exp Med* 1993; 178: 1271–81.

12 Dam TN, Møller B, Hindkjœr J, Kragballe K. The vitamin D_3 analogue calcipotriol suppresses the number and antigen-presenting function of Langerhans cells in normal human skin. *J Invest Dermatol Symp Proc* 1996; 1: 72–7.
13 Ashworth J, Booker J, Breathnach SM. Effects of topical corticosteroid therapy on Langerhans cell antigen presenting function in human skin. *Br J Dermatol* 1987; 118: 457–70.
14 de Jong EMGJ, de Kerkhof PCM. Simultaneous assessment of inflammation and epidermal proliferation in psoriatic plaques during long-term treatment with the vitamin D_3 analogue MC903: modulations and interrelations. *Br J Dermatol* 1991; 124: 221–9.
15 Glade CP, van Erp PEJ, de Kerkhof PCM. Epidermal cell DNA content and intermediate filaments keratin 10 and vimentin after treatment of psoriasis with calcipotriol cream once daily, twice daily and in combination with clobetasone 17-butyrate cream or betamethasone 17-valerate cream: a comparative flow cytometric study. *Br J Dermatol* 1996; 135: 379–84.
16 Lützow-Holm C, De Angelis P, Grøsvik H, Clausen OPF. 1,25-dihydroxyvitamin D3 and the vitamin D analogue KH1060 induce hyperproliferation in normal mouse epidermis. *Exp Dermatol* 1993; 2: 113–20.
17 Gniadecki R, Gniadecka M, Serup J. The effects of KH 1060, a potent 20-epi analogue of the vitamin D_3 hormone, on hairless mouse skin *in vivo*. *Br J Dermatol* 1995; 132: 841–52.
18 Prens EP, Benne K, van Joost T, Benner R. The autologous mixed epidermal cell–T lymphocyte reaction is elevated in psoriasis: a crucial role for epidermal HLA-DR+/CD1a$^-$ antigen-presenting cells. *J Invest Dermatol* 1991; 96: 880–7.
19 Schopf RE, Hoffmann A, Jung M *et al.* Stimulation of T cells by autologous mononuclear leukocytes and epidermal cells in psoriasis. *Arch Dermatol Res* 1986; 279: 89–94.