

Cultured psoriatic fibroblasts from involved and uninvolved sites have a partial but not absolute resistance to the proliferation-inhibition activity of 1,25-dihydroxyvitamin D₃

(psoriasis/cell proliferation/skin/1,25-dihydroxyvitamin D₃ receptor/differentiation)

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ABSTRACT We examined the responsiveness of cultured dermal fibroblasts from biopsies of uninvolved and involved areas of skin from six patients with psoriasis to the cell-proliferation-inhibition activity of 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂-D₃). Cultured fibroblasts from age-matched controls responded to 1,25-(OH)₂-D₃ (at 0.01, 1, 10, and 100 μM) in a dose-dependent fashion, whereas cultured psoriatic fibroblasts from involved or uninvolved skin showed no inhibition of proliferation when exposed to 0.01 or 1 μM of 1,25-(OH)₂-D₃. However, 1,25-(OH)₂-D₃ did inhibit proliferation of cultured psoriatic fibroblasts when the concentrations were increased to 10 and 100 μM. An analysis of the 1,25-(OH)₂-D₃ receptors in cultured psoriatic fibroblasts from uninvolved skin revealed that the K_d, n_{max}, and sedimentation coefficient were identical to the receptors found in the fibroblasts from age-matched controls. Therefore, cultured psoriatic fibroblasts from involved and uninvolved skin have a partial resistance to 1,25-(OH)₂-D₃, suggesting that there may be a biochemical defect that is inherent in the dermal fibroblasts of psoriatic patients. Recognition of this defect may provide a new approach for the evaluation of the cause and treatment of this disfiguring skin disorder.

Psoriasis is a common disease of the skin affecting about 1-3% of the population of the world (1). Although this chronic debilitating disease has been recognized for several centuries, little is known about its etiology. Psoriasis is thought to be principally a disease of the epidermis that causes a disruption in the normal events of cell maturation and proliferation (1). It is characterized by an increase in the number of basal cells in the epidermis and a reduced turnover time of the epidermis from the normal of 26-27 days to 4 days (1).

1,25-Dihydroxyvitamin D₃ (1,25-(OH)₂-D₃), the active form of vitamin D₃, is a potent inducer of differentiation and maturation of normal and tumor cells that possess cytosolic and nuclear receptors for the hormone (2-7). In addition, the skin is now recognized as a target organ for 1,25-(OH)₂-D₃. Autoradiographic studies in rats showed localization of 1,25-(OH)₂-[³H]D₃ in the cells of the root sheath of the hair follicles and in the stratum basale (8), and culture studies showed high-affinity low-capacity receptors for this hormone in human and rodent fibroblasts (9-11) and keratinocytes (12, 13). 1,25-(OH)₂-D₃ stimulates 25-hydroxyvitamin D₃-24-hydroxylase activity (11, 14) and decreases cellular proliferation (10) in a dose-dependent manner in cultured human fibroblasts that possess the 1,25-(OH)₂-D₃ receptor but has no effect on cultured fibroblasts from patients with vitamin D-dependent rickets, type II, which either lack or have a

defective 1,25-(OH)₂-D₃ receptor (10, 11, 14). 1,25-(OH)₂-D₃ also induces morphologic differentiation of cultured mouse (12) and human (13) keratinocytes. In the presence of 0.01 μM 1,25-(OH)₂-D₃, cultured human basal cells differentiate into less-dense squamous and cornified cells. In addition, this hormone increases transglutaminase activity and 25-hydroxyvitamin D₃-24-hydroxylase in these cells (13).

Because psoriasis is a disorder of differentiation and because 1,25-(OH)₂-D₃ is such a potent hormone for stimulating the maturation of dermal and epidermal cells, we examined the effect of this hormone on cultured fibroblasts from six patients with psoriasis.

METHODS

Fibroblasts were isolated from 4-mm punch biopsies from uninvolved and involved areas on the backs of six patients with psoriasis and from age-matched controls. The fibroblasts were isolated and cultured as described (10). Cultured fibroblasts (2.5 × 10⁵ cells) were exposed either to 0.0001, 0.01, 10, or 100 μM of 1,25-(OH)₂-D₃ or to ethanol vehicle (0.01%). Analyses of cell number and the 1,25-(OH)₂-D₃ receptor by sucrose density gradient, saturation binding, and DNA-cellulose chromatography were performed as described (10). Protein concentrations were determined by the method of Lowry *et al.* (15). Binding affinity (K_d) and capacity (n_{max}) were obtained from saturation-binding curves and were estimated by the method of Scatchard (16).

RESULTS

Cultured normal human fibroblasts incubated with 1,25-(OH)₂-D₃ demonstrated a dose-dependent inhibition of cell proliferation (Fig. 1A). However, when cultured fibroblasts obtained from a biopsy of an uninvolved area from a psoriatic patient were incubated with 1,25-(OH)₂-D₃ at 0.0001, 0.01, or 1 μM, no effect on cell proliferation was observed when they were compared with the control group (Fig. 1B). When these cells were exposed to 10 and 100 μM of 1,25-(OH)₂-D₃, a dose-dependent inhibition of cell proliferation was observed, suggesting that psoriatic fibroblasts have a partial but not absolute resistance to the hormone. Similar results were obtained for the cultured dermal fibroblasts from involved and uninvolved skin from the other five patients.

We examined whether the cause for the partial resistance of dermal psoriatic fibroblasts was due to a defect in the recognition or binding capacity of the cells for 1,25-(OH)₂-D₃. Similarity of binding of 1,25-(OH)₂-[³H]D₃ by a low-capacity

Abbreviation: 1,25-(OH)₂-D₃, 1,25-dihydroxyvitamin D₃.

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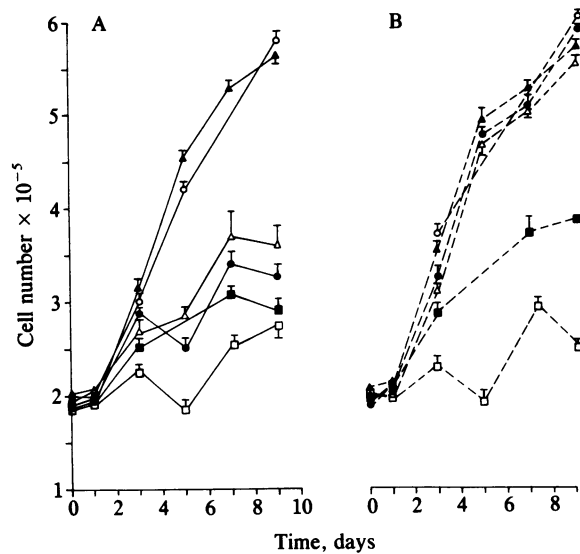


FIG. 1. Human dermal fibroblasts cultured from a skin biopsy from a normal age-matched volunteer (A) or an uninvolved area from a psoriatic patient (B) were incubated at day 0 with either EtOH (○) or 1,25-(OH)₂-D₃ at one of the following concentrations: 0.0001 (▲), 0.01 (△), 1 (●), 10 (■), or 100 (□) μM. Each point represents the mean \pm SEM of fibroblasts, plated in triplicate.

3.7S macromolecule(s) was demonstrated by analysis with sucrose density-gradient centrifugation of cytosol from fibroblasts cultured from normal (Fig. 2A) and psoriatic patients [involved (Fig. 2B) and uninvolved (Fig. 2C) skin]. In addition, DNA-cellulose chromatography of the cytosols from normal and psoriatic (involved and uninvolved) fibroblasts showed that the chromatin-binding fraction for 1,25-(OH)₂-[³H]D₃ was eluted as a single peak at 0.22 M KCl and was identical to the chicken intestinal cytosol. A Scatchard analysis of the saturation binding assays for all three cell lines revealed a single class of binding site with a K_d of $1-2 \times 10^{-10}$ M. The number of binding sites was found to be 18 and 22 fmol/mg of protein for the normal and psoriatic (uninvolved) fibroblasts (Fig. 3), whereas only about half as many binding sites (10 fmol/mg of protein) were found in the psoriatic fibroblasts from the involved area. Similar results were obtained for the other five psoriatic patients who were tested (Table 1).

Inasmuch as the dermal fibroblasts from psoriatic patients were unresponsive to 0.01 or 1 μM 1,25-(OH)₂-D₃ and yet possessed high-affinity low-capacity receptors for the hormone that were similar to the receptors in normal fibroblasts, we were concerned that 1,25-(OH)₂-D₃ may not be entering the psoriatic cells as efficiently as it does in normal fibroblasts. However, when we incubated normal and psoriatic (uninvolved) fibroblasts with 5×10^5 cpm of 1,25-(OH)₂-[³H]D₃ (specific activity, 158 Ci/mmol; 1 Ci = 37 GBq) and then isolated them 24 and 48 hr later after three separate washings, there was no difference in the uptake of 1,25-(OH)₂-[³H]D₃ by the psoriatic fibroblasts ($6.0 \pm 0.1 \times 10^4$ and $8.5 \pm 0.5 \times 10^4$ cpm for 24 and 48 hr, respectively) in comparison with normal fibroblasts ($5.0 \pm 0.7 \times 10^4$ and $7.5 \pm 0.5 \times 10^4$ cpm at 24 and 48 hr, respectively). Under similar conditions less than 4×10^2 cpm were incorporated into fibroblasts from a patient with vitamin D-dependent rickets type II.

DISCUSSION

Although it is known that psoriasis is a hyperproliferative disorder of the epidermis with a strong genetic component (1, 17), little is known about the etiology of this disfiguring

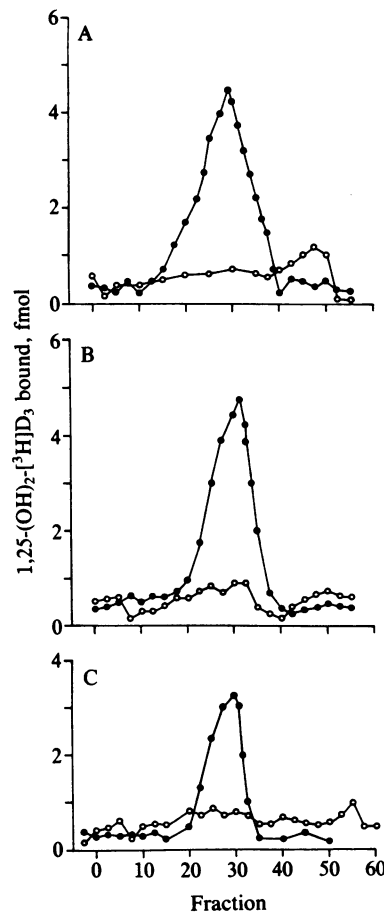


FIG. 2. Analysis by sucrose density gradient centrifugation of the binding of 1,25-(OH)₂-[³H]D₃ in high-salt cytosol extracts from cultures of normal (A), involved psoriatic (B), or uninvolved psoriatic (C) human dermal fibroblasts. Aliquots of each cytosol (0.2 ml) were incubated with 2 nm of 1,25-(OH)₂-[³H]D₃ alone or in the presence of 250 nM unlabeled hormone. Unbound hormone was removed by charcoal, and the cytosols were passed through a linear (4–20%) sucrose density gradient. Sedimentation coefficients were determined by comparison with a 3.7S ovalbumin standard.

disease. There are various treatments for this disorder including steroids, cytotoxic drugs, and psoralen in combination with exposure to ultraviolet-A radiation (PUVA), but to date there is not an effective and safe cure (1).

It has been recognized that there are several abnormalities in the dermis of patients with psoriasis. Dilated tortuous capillaries are often observed by histology (18) and there is less cross-linked collagen and a greater content of glycosaminoglycans (19, 20). Recently, Priestley and Adams (21) reported slightly quicker outgrowth of fibroblasts from psoriatic skin explants from involved and uninvolved forearm skin when compared to the outgrowth from control skin and that psoriatic fibroblasts were abnormally dependent on serum for anchorage. They concluded that their findings were most compatible with a hyperproliferative reaction of both the epidermis and dermis to extracutaneous, perhaps vascular stimulation.

Our observation that cultured fibroblasts from uninvolved sites have a partial resistance to the cell-proliferation-inhibition activity of 1,25-(OH)₂-D₃ suggests that there may be a biochemical defect that is inherent in the dermal fibroblasts of psoriatic patients. However, this defect is not due to some abnormality in the cellular uptake of 1,25-(OH)₂-D₃ or its recognition by its cytosolic or nuclear receptors. Probably, the defect is at the level of the nuclear recognition of the 1,25-(OH)₂-D₃-nuclear receptor complex; therefore, it is

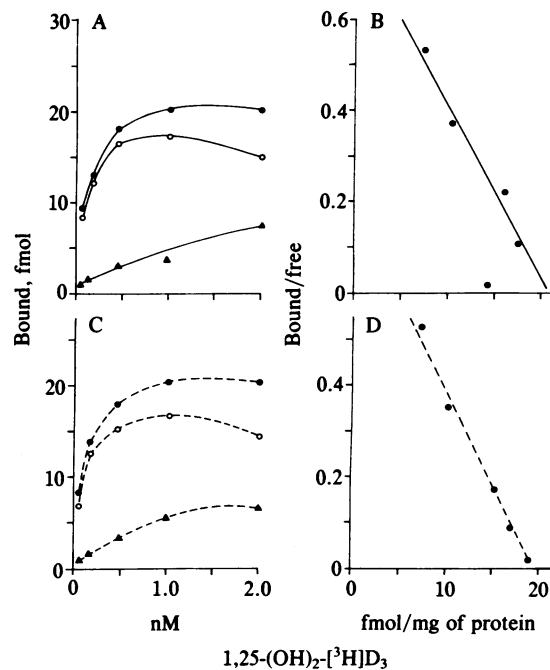


FIG. 3. Saturation analysis of binding of $1,25\text{-(OH)}_2\text{-D}_3$ in the cytosol of normal (A) and unininvolved psoriatic (C) human dermal fibroblasts. Increasing amounts of $1,25\text{-(OH)}_2\text{-[}^3\text{H]D}_3$ (0.5–3 nM) were incubated alone or in the presence of $1.0\ \mu\text{g}$ of unlabeled hormone (nonspecific binding) with $0.2\ \text{ml}$ of cytosol (2–5 mg of protein) at 0°C for 3 hr. Bound $1,25\text{-(OH)}_2\text{-[}^3\text{H]D}_3$ was recovered on DEAE filters. K_d and n_{max} for normal (B) and unininvolved psoriatic (D) fibroblasts were determined by Scatchard analysis.

unlikely that such a biochemical defect is caused by some extracutaneous mechanism.

Because fibroblasts can be obtained quickly and cultured easily from a small biopsy, we chose to evaluate only the dermal fibroblasts of the psoriatic patients. We would predict, however, that cultured psoriatic keratinocytes also would have a partial resistance to the differentiating activity of $1,25\text{-(OH)}_2\text{-D}_3$ because the receptor activity and biologic response of cultured dermal fibroblasts are predictors of the receptor activity and biologic response of cultured epidermal cells (10, 13). If our observations are confirmed and if this defect is seen in a majority of patients with psoriasis, the evaluation of the responsiveness of cultured dermal fibroblasts to $1,25\text{-(OH)}_2\text{-D}_3$ may be of great diagnostic value.

Our observation that a 1000-times-higher concentration of $1,25\text{-(OH)}_2\text{-D}_3$ was needed to inhibit proliferation of cultured

Table 1. K_d and n_{max} derived by Scatchard analysis applied to saturation analysis of $1,25\text{-(OH)}_2\text{-D}_3$ binding in the cytosol of cultured human dermal fibroblasts

Normal fibroblasts	Psoriatic-patient fibroblasts				
	Involved areas		Uninvolved areas		
n_{max}	K_d	n_{max}	K_d	n_{max}	K_d
16	1.8	10	1.1	17	1.9
20	1.3	8	1.2	20	1.5
22	1.3	5	1.0	15	1.4
14	1.7	7	0.09	15	1.1
25	1.1	12	1.1	21	1.5

Fibroblasts were obtained from skin biopsies from age-matched normal volunteers and involved and uninvolved areas from psoriatic patients. K_d s are expressed in $\text{M} \times 10^{10}$, and n_{max} s are expressed in fmol/mg of protein.

psoriatic fibroblasts when compared to normal fibroblasts may provide the basis for a new pharmacologic method of treating this disease. It is unlikely that the inhibition of psoriatic fibroblast proliferation by 1000-fold-higher concentrations of $1,25\text{-(OH)}_2\text{-D}_3$ was due to some artifact, such as a nonspecific steroid or toxic effect, because (i) cortisol at $10\ \mu\text{M}$ had no effect on cell proliferation of normal (10) or cultured psoriatic fibroblasts (data not shown), and (ii) $1,25\text{-(OH)}_2\text{-D}_3$ at 10 and $100\ \mu\text{M}$ had no effect on the proliferation of cultured fibroblasts from a patient with vitamin D-dependent rickets, type II, which are cells lacking receptor activity for the hormone (data not shown).

Whether $1,25\text{-(OH)}_2\text{-D}_3$ will be useful therapeutically in treating psoriasis remains to be determined. However, even if $1,25\text{-(OH)}_2\text{-D}_3$ does prove to be of some therapeutic value, it may not be practical to treat psoriatic patients with high doses of $1,25\text{-(OH)}_2\text{-D}_3$ in an attempt to pharmacologically restore epidermal mitotic activity to normal because this hormone is a potent calciotropic hormone that can cause hypercalciuria and hypercalcemia. However, the recent preliminary report that $\Delta 22\text{-}1\alpha,25,26\text{-trihydroxyvitamin D}_3$ (22) is able to induce maturation of tumor cell lines while having little biologic activity related to calcium metabolism may provide a novel approach to the treatment of this enigmatic disease.

Therefore, our observation that cultured fibroblasts from psoriatic skin have a partial but not absolute resistance to $1,25\text{-(OH)}_2\text{-D}_3$ offers a new evaluation of the cause of this disorder and possibly a unique approach to its treatment.

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