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Reactive Changes in the Langerhans' Cells of Human Skin Caused by Occlusion with Water and Sodium Lauryl Sulphate

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Human skin was patch tested with sodium lauryl sulphate or with water only for 48 h and biopsied immediately and after 24 h, then analyzed by immunocytochemistry and electron microscopy. Sodium lauryl sulphate produced a decrease in the number of epidermal Langerhans' cells and an increase in dermal Langerhans' cells, with individual variations. The 48-h water occlusion controls showed only slight reactions. Unexpectedly, quite pronounced reactive changes were seen 24 h after termination of water occlusion. Thus, dermal Langerhans' cells were commonly increased and epidermal Langerhans' cells tended to decrease in number. The results indicate that the 24-h interval is not a period of recovery but a period in which more pronounced reactive changes occur. Hydration over 48 h followed by dehydration leading to temporary damage to the epidermal barrier may explain the present findings. Some of the reactive changes observed after sodium lauryl sulphate exposure probably represent the additive effects of occlusion and sodium lauryl sulphate treatment. ***Key words: Contact dermatitis; Birbeck granules; Electron microscopy; Immunocytochemistry.***

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Observations on the numbers of T6+ cells in epidermis and dermis in contact dermatitis (CD) have made it increasingly clear that the Langerhans' cells (LC) not only are immunocompetent cells (for ref see 1) but are involved in irritant CD (ICD) as well (2, 3). Ultrastructural studies in this laboratory, based on the analysis of serial sections, have revealed several new LC reactions which occur in the initial stages (5–8 h) of ICD and it has been proposed that the LC is a cell with a versatile defence function (3, 4). To find out more about such reactions we patch tested human skin with sodium lauryl sulphate (SLS) for 48 h followed by analysis with electron microscopy (EM) and light microscopic immunocytochemistry. Unexpectedly, it became evident that water occlusion per se can strongly influence human LC.

MATERIALS AND METHODS

In the first part of the study (Jan. 1989) three healthy male volunteers (code No. 1,2,5), age range 34–43, were submitted to patch testing (volar forearm skin) with 1% aqueous solution of SLS for 48 h. The 12 mm Finn chambers were filled with 50 μl SLS and a second set of chambers was applied after 24 h. Both adjacent water occluded and contralateral normal skin were used as controls; one 3 mm punch biopsy was taken upon removal of the chambers without anesthesia and processed for EM.

In the second part of the study (May 1989), two of the previously tested subjects and two additional healthy male volunteers (No. 1–4), age range 25–63, were submitted to patch testing with 1% SLS (different batch from the SLS used in the first study) or with distilled water only. Two 12 mm Finn chambers with 50 μl aqueous solution of SLS and two chambers with 50 μl water were applied to the volar forearm skin. A second set of chambers was applied after 24 h. All chambers were removed after 48 h and two punch biopsies (3 mm) were immediately taken without anesthesia from one SLS and one water test area. The remaining test areas were biopsied 24 h later. Normal skin was obtained contralaterally. The biopsies were processed for EM and immunocytochemistry, respectively.

Electron microscopy

The biopsies were immediately immersed in 2.5% glutaraldehyde in cacodylate buffer (+ 4°C) isoosmolar to blood and were further processed as previously described (4). Three, or more if needed (see Results), series of ultrathin sections (containing 20–100 consecutive sections) were prepared from three different levels of each biopsy.

Immunocytochemistry

A monoclonal antibody, anti-T6 (DAKO-T6, Dakopatts), directed against specific cell surface antigens (MW approx. 45 kD) of the LC was used. The antigen-antibody complex was visualized with an avidin-biotin-immunoperoxidase assay (Vectastain ABC Kit, Vector Lab.). Processing was as described by Christensen et al. (5) except that phosphate buffered saline was used and that the sections (8 μm) were placed on chrome alum coated slides.

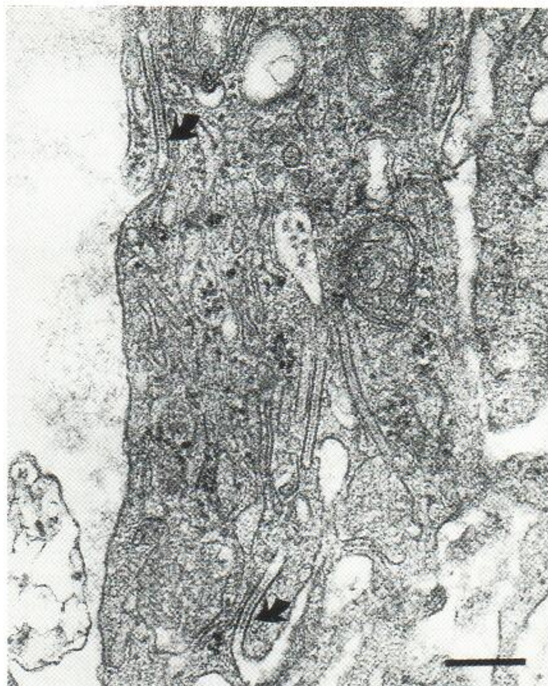

Fig. 1. A dermal LC showing numerous BG in their formative stage. Two of these (indicated by arrows) are seen in this part of the cell cytoplasm. Bar = 210 nm.

A calibrated eyepiece at 312x magnification was used to count 0.9 linear mm of the interfollicular epidermal surface of at least 7 sections. Only dendritic cells with a visible nucleus were counted. The number of LC was expressed as LC/0.9 mm. The statistical significance of differences was calculated by means of a one-way analysis of variance (ANOVA with post-hoc, Newman-Keuls test). Significance was set at $p < 0.05$.

RESULTS

Electron microscopy

All biopsies from unchallenged skin appeared normal.

Occlusion with water for 48 h caused very slight changes in epidermal morphology. In contrast to normal skin, where activated LC are few, most of the LC (a total of 52) showed one or several of the following signs that could be ascribed to enhanced activation: dilated rough endoplasmic reticulum, slightly enlarged perinuclear space, prominent Golgi fields, increased number of vesicles (coated and non-coated), lysosome-like structures and cytomembrane folds. LC dendrites terminating within the granular layer were common in two biopsies.

Scattered mononuclear cells were found 24 h after water occlusion except for one case (No. 3) which displayed mild spongiosis and an abundancy of such cells. Only one LC was seen in apposition to a mononuclear cell; the contact had a specialized character as reported earlier (3, 4), with a small and uniform intermembrane distance and enhanced electron density of the apposing membranes. A total of 34 LC were observed in the four biopsies; most showed signs of enhanced activation; in two cases there were a few dendrites penetrating into the granular layer. It was remarkable that no fewer than 7 LC were present in the dermis in one biopsy (No. 3). In another biopsy there was one dermal LC containing a large number of Birbeck granules (BG) in the formative stage i. e. still attached to the cytomembrane (Fig. 1).

Biopsies of challenged skin from the subjects in the first part of the study showed general or focal

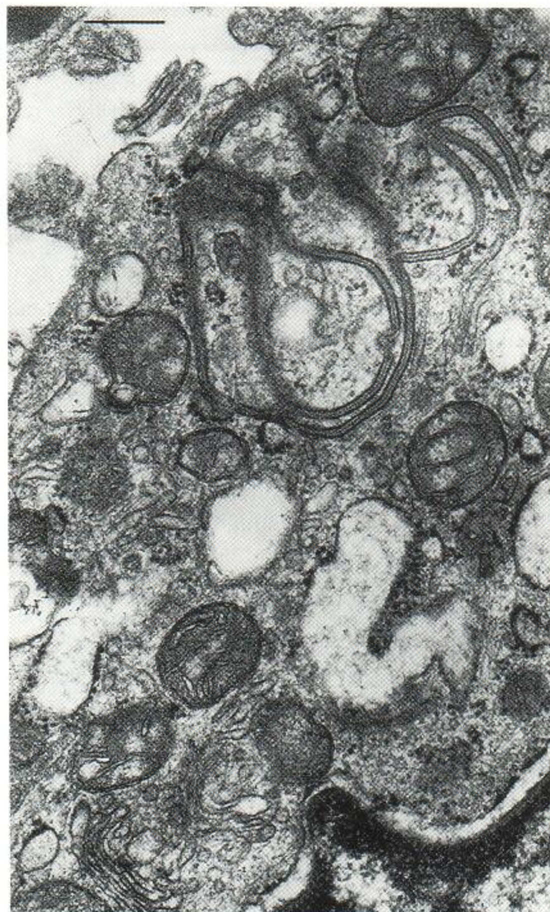

Fig. 2. An epidermal LC containing BG with an atypical shape resulting from intensified "cytomembrane-sandwiching". Bar = 300 nm.

Table I. *Number and distribution of T6+ cells in SLS patch tested skin biopsies.*

Sub ject	Normal skin	48h SLS test			48h SLS test+24h		
	T6+/0.9 mm epidermis	T6+/0.9 mm epidermis	Position in epidermis	T6+ cells in dermis	T6+/0.9 mm epidermis	Position in epidermis	T6+ cells in dermis
1	10.4	10.4	normal		10.5	high-levelled	+
2	10.1	10.1	high-levelled and basal	+	2.4*	high-levelled and basal	++
3	10.1	1.8*	high-levelled and basal	+	0	0	++
4	9.9	12.7	high-levelled		11.7	high-levelled and basal	+

Table II. *Number and distribution of T6+ cells in water patch tested skin biopsies.*

Sub-ject	Normal skin	48h water test			48h water test+24h		
	T6+/0.9 mm epidermis	T6+/0.9 mm epidermis	Position in epidermis	T6+ cells in dermis	T6+/0.9 mm epidermis	Position in epidermis	T6+ cells in dermis
1	10.4	9.4	normal		8.3	normal	+
2	10.1	7.9	normal		6.6*	normal	+
3	10.1	9.0	normal		7.9	high-levelled	+
4	9.9	6.9	normal		7.9	normal	+

* $p<0.05$ vs. controls.
Estimated slight increase of T6+ cells in the dermis is indicated by +, clear increase by ++

spongiosis and numerous mononuclear cells, some breaking the basal lamina. The epidermal LC (a total of 34) showed one or several signs of enhanced activation and 3 LC had a specialized cytomembrane contact with a mononuclear cell as described above. Two LC were breaking the basal lamina.

In the upper dermis several LC, identified from their content of BG, were found occurring both singly and in the perivascular infiltrates. These cells (in total 8 cell bodies + 8 dendritic parts located so far apart from each other that they were judged to belong to different LC) often had specialized cytomembrane contacts with other cells in the dermal infiltrate. Some of the dermal LC contained large numbers of coated vesicles and cytoplasmic filaments.

Challenged skin of the subjects (No. 1–4) tested in the second part of the study showed great variations of the intercellular spaces (ICS), from slightly widened basal interstices to focal or general spongiosis. Mononuclear cells were usually present in small numbers in the innermost layers of the epidermis, but were numerous in one biopsy (No. 1) and sometimes seen to break the basal lamina. Specialized contact with an LC was observed in one instance only.

Most of the 55 LC observed (one breaking the basal lamina) showed one or several of the signs ascribable to enhanced activity (see above). One LC (No. 3) contained BG with an atypical shape (curved and undulating and often unusually long) in a discrete part of the cell body (Fig. 2). It has been suggested that such granules arise from an intensified superimposing between complicated cytomembrane folds (6).

Numerous dermal LC (in total 17 + 18 dendritic parts; all identified from their content of BG) were seen in the biopsies taken from three individuals (No. 1,2,3). Most of these cells were present in the dermal infiltrate and there were numerous specific cytomembrane contacts with other cells. Some of the cells contained numerous coated vesicles, many more than did epidermal LC. One LC in the upper dermis showed an intensified formation of BG in a limited area of the cytoplasm similar to the phenomenon illustrated in Fig. 1.

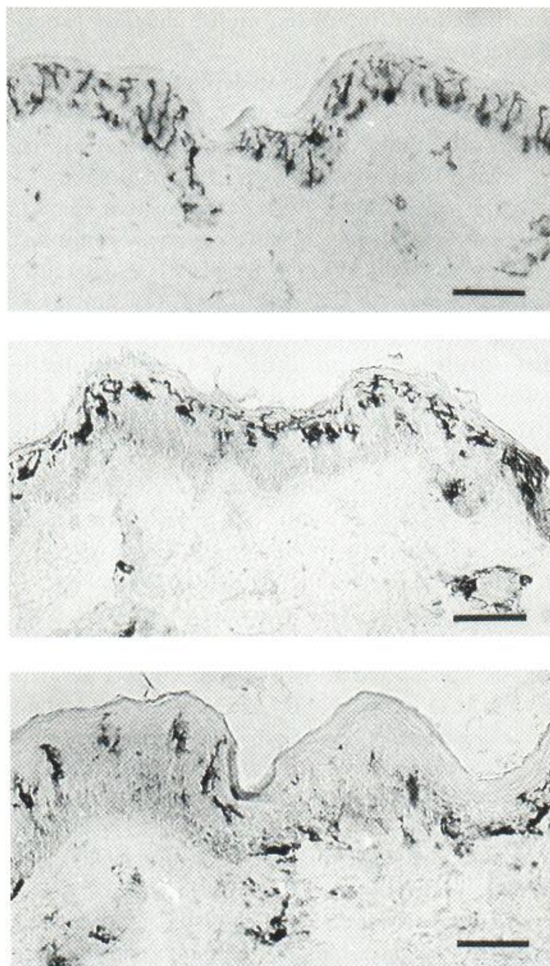

Fig. 3. T6+ cells. (a) Evenly distributed T6+ cells in normal skin biopsy. (b) High-level epidermal- and increased dermal T6+ cells 48h after 48h exposure to SLS. (c) Reduced numbers of epidermal, and increased numbers of dermal T6+ cells 24h after the removal of the SLS patch. Bar = 50 μm.

Twenty-four h after removal of the chambers, one biopsy (No. 3) showed slightly distended ICS in the innermost layers while the spinous layer contained a number of melanocytes that had become detached (this effect of SLS was recently reported by Warfvinge et al. 7). The other biopsies showed slight focal spongiosis or normal ICS. A few mitotic basal keratinocytes were found in one biopsy. Only few mononuclear cells were present, one having a specialized cytomembrane contact to an LC.

No epidermal LC were seen in subject No. 3 (this finding was confirmed in nine further section series). On the other hand there were numerous dermal LC (totally 9 + 3 dendritic parts). Biopsy No. 2 showed only few epidermal LC and 3 dermal LC, one exhibiting intensified BG formation, similar to that shown in Fig. 1. A total of 33 activated epidermal LC were seen in the remaining biopsies.

Immunocytochemistry

The densities and distribution of T6+ cells in the skin biopsies are illustrated in Tables I and II.

The epidermal T6+ cells showed an even distribution in normal skin (Fig. 3a). The dermis showed only rare T6+ cells.

A common finding following water occlusion for 48 h was that all or almost all T6+ cells had their dendrites directed towards the surface. One biopsy contained normal-looking epidermis. Two biopsies (No. 2 and 4) taken 24 h later had a normal epidermis. There was a decrease of T6+ cells in one biopsy and several T6+ cells occupied a high-level position in the fourth (No. 3) and their dendritic trees were poorly developed. There was a slight increase in the number of rounded T6+ dermal cells in connection with perivascular infiltrate in three biopsies but they appeared singly as well in one biopsy.

A 48-h exposure to SLS led to changes in intraepidermal T6+ cell distribution more pronounced than those after water occlusion only (see also Fig. 3b). Two biopsies showed a slight increase in dermal T6+ cells (both rounded and dendritic) in the perivascular infiltrates and, in one case, scattered T6+ cells were also seen just below the basal lamina of the epidermis. All biopsies showed an increase of T6+ dermal cells 24 h after termination of the SLS test. The cells occurred singly in the upper dermis or in perivascular infiltrates. Confirming the findings from EM section series, case No. 3 showed no epidermal T6+ cells. In two cases (No. 2 and 4) the dendritic trees of the epidermal T6+ cells were reduced (Fig. 3c).

Control reactions with normal ascites fluid instead of anti-T6 antibody revealed no positive staining of cells in the epidermis or dermis, and no cells with endogenous peroxidase were observed.

DISCUSSION

Occlusion per se can cause cellular changes in epidermal cells (8, 9, 10). Information regarding cellular and subcellular reactions of epidermal LC to water occlusion is sparse and this applies not least to the test model with a 48 h topical application of a

substance in aqueous solution followed by biopsy 24 h later which is often used in studies of the LC. A pronounced effect on the keratinocytes, but undisturbed integrity of the LC has been reported (9). We found that the 72 h controls yielded the unexpected finding of pronounced and functionally important reactive events in the LC system. These events included individually varying degrees of a tendency to, or a significant decrease in LC numbers, the occurrence of LC dendrites penetrating into the granular layer, ultrastructural signs of LC activation and, remarkably, an increase in dermal LC. There was in one case a pronounced redistribution of the epidermal LC combined with loss of dendrites. It is evident that such reactive phenomena must be taken into account when the effects of the tested substance itself are to be interpreted. This is amply demonstrated by the possibility revealed on comparison of Tables I and II, that reactive events observed after the topical application of SLS may well originate from the summed effects of water occlusion and SLS. We have also demonstrated that the LC reactions seen with 48 h of occlusion with water are modest and generally restricted to activation of the cells. Thus, the 24 h interval after removal of the chambers is not a period of recovery but one that leads to more pronounced reactive changes in the LC. A reason for this could be that 48 h of hydration followed by drying further damages the epidermal barrier, thereby affecting the LC.

The combination of EM and T6 immunocytochemistry revealed that SLS in aqueous solution caused the following main reactive changes in the LC: in epidermal LC: a decrease in number, change of position of the cells and altered extension of the dendritic tree; in epidermal as well as in dermal LC: increased BG formation, the appearance of contacts with exocytic cells and, for dermal LC, an increase in number.

There have been several attempts to enumerate the epidermal LC at various stages of ICD but the results to date are conflicting and of little help in understanding LC functions. The numbers of epidermal T6+ cells found after exposure to SLS (11, 12, 13) also vary widely. There are two obvious reasons for this: the well-known individual variation in susceptibility and the differences in experimental models used. Individual susceptibility to SLS was amply evidenced in the present experiments by the changes in number of epidermal T6+ cells. The number of such cells was unchanged in some individuals and greatly reduced in others, under identical experimental conditions.

The cause of the fall in epidermal LC numbers is not known. Ferguson et al. (13) suggested that the LC could be more vulnerable than the keratinocytes to chemical injury of the skin. This suggestion was recently substantiated by the finding that the LC have functionally very reactive cytomembranes and can be extremely sensitive targets for exogenously applied substances, including SLS (4, 14). This sensitivity is due to a peculiar cytomembrane mechanism that involves superimposition of cytomembrane parts, "cytomembrane-sandwiching (CMS)" (see Fig. 1). The CMS can become extensive, and some LC succumb because more cytomembrane is consumed than is compatible with cell survival. Another possibility would be that the cells escape from a toxic milieu and withdraw to the dermis. This possibility, in turn, could explain the increase in dermal LC.

Several authors have reported that, while T6+ cells are few or non-existent in normal dermis (13, 15), they are found in both allergic contact dermatitis and ICD (2, 12, 13, 16, 17) as well as in certain other diseases (for refs see 18, 19). Uncertainty prevails regarding the identity of these cells. Electron microscopy performed during the first days of CD has either failed to reveal BG (2, 20, 21) in such cells, or revealed BG in only a few of the cells (18), and it has been suggested that the absence of BG in T6+ dermal cells is due to their being indeterminate cells (15). In contrast to this we found a considerable number of BG-containing dermal cells. The difference in results may indicate that relatively extensive section series such as used in this study are sometimes necessary to reveal the presence of BG. The simultaneous increase in T6+ cells and BG-containing cells observed here leaves little doubt that many of the T6+ cells in fact are LC. Moreover, the present findings and the fact that it is now known that indeterminate cells do not exist in normal human epidermis (22) justify some caution in identifying indeterminate cells.

The reactive events now reported to occur in the LC system in response to simple water occlusion and to SLS exposure support our earlier suggestion that the LC not only are immunocompetent cells but seem to be alerted in presumably non-immunoreactive situations as well (3).

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REFERENCES

1. Wolff K, Stingl G. The Langerhans cell. J Invest Dermatol 1983; 80: 17–21.
2. Willis CM, Young E, Brandon DR, Wilkinson JD. Immunopathological and ultrastructural findings in human allergic and irritant dermatitis. Br J Dermatol 1986; 115: 305–316.
3. Falck B, Andersson A, Bartosik J. Some new ultrastructural aspects on human epidermis and its Langerhans cells. Scand J Immunol 1985; 21: 409–416.
4. Mikulowska A, Bartosik J, Andersson A, Björnberg A, Falck B. Microfocussed distribution of reactive events in the epidermal Langerhans cell system. In: Thivolet J, Schmitt D, eds. The Langerhans Cell. Colloque INSERM/John Libbey Eurotext Ltd. 1988; 172: 285–292.
5. Christensen OB, Daniels TE, Maibach HI. Expression of OKT6 antigen by Langerhans cells in patch test reaction. Contact Dermatitis 1986; 14: 26–31.
6. Mikulowska A, Andersson A, Bartosik J, Falck B. Cytomembrane- sandwiching in human epidermal Langerhans cells: A novel reaction to an irritant. Acta Derm Venereol (Stockh) 1988; 68: 254–256.
7. Warfvinge K, Agdell J, Andersson L, Andersson A. Attachment and detachment of human epidermal melanocytes. Acta Derm Venereol (Stockh) 1990; 70: 189–193.
8. Lindberg M, Forslind B. The effect of occlusion of the skin on the Langerhans cell and the epidermal mononuclear cells. Acta Derm Venereol (Stockh) 1981; 61: 201–205.
9. Forslind B, Wahlberg JE. Assesment of chromium allergy: features of patch test reactions at electron microscopic resolution. Acta Derm Venereol (Stockh) 1977; 57: 29–35.
10. Ranki A, Kanerva L, Förström L, Konttinen Y, Mustakallio KK. T and B lymphocytes, macrophages and Langerhans' cells during the course of contact allergic and irritant reaction in man. Acta Derm Venereol (Stockh) 1983; 63: 367–383.
11. Lindberg M, Emtestam L. Dynamic changes in the epidermal OKT6 positive cells at mild irritant reaction in human skin. Acta Derm Venereol (Stockh) 1986; 66: 117–120.
12. Avnstorp C, Ralfkiaer E, Jorgensen J, Wantzin GL. Sequential immunophenotypic study of lymphoid infiltrate in allergic and irritant reactions. Contact Dermatitis 1987; 16: 239–245.
13. Ferguson J, Gibbs JH, Swanson Beck J. Lymphocyte subsets and Langerhans cells in allergic and irritant patch test reactions: histometric studies. Contact Dermatitis 1985; 13: 166–174.
14. Andersson L, Bartosik J, Bendsoe N, et al. Formation and uzipping of Birbeck granules in vitro. In: Thivolet J, Schmitt D, eds. The Langerhans Cell. Colloque INSERM/John Libbey Eurotext Ltd. 1988; 172: 185–191.
15. Murphy GF, Bahn AK, Harrist TJ, Mihm MC. In situ identification of T6-positive cells in normal human dermis by immunoelectron microscopy. Br J Dermatol 1983; 108: 423–431.
16. Gawkrodger DJ, McVittie E, Carr MM, Ross JA, Hunter JAA. Phenotypic characterization of the early cellular responses in allergic and irritant contact dermatitis. Clin Exp Immunol 1986; 66: 590–598.
17. Scheynius A, Fischer T, Forsum U, Klareskog L. Phenotypic characterization in situ of inflammatory cells in allergic and irritant contact dermatitis in man. Clin Exp Immunol 1984; 55: 81–90.
18. Berti E, Cavicchini S, Cusini M, Monti M, Parolini F, Caputo R. Heterogenity of dermal OKT6+ cells in inflammatory and neoplastic skin diseases. J Am Acad Dermatol 1985; 12: 507–514.
19. Christensen OB, Wall LM. Long term effect on epidermal dendritic cells of four different types of exogenous inflammation. Acta Derm Venereol (Stockh) 1987; 67: 305–309.
20. Carr MM, Botham PA, Gawkrodger DJ, et al. Early cellular reactions induced by dinitrochlorobenzene in sensitized human skin. Br J Dermatol 1984; 110: 637–641.
21. Gawkrodger DJ, Haftek M, Botham PA, et al. The hapten in contact hypersensitivity to dinitrochlorobenzene: immunoelectron microscopic and immunofluorescent studies. Dermatologica 1989; 178: 126–130.
22. Andersson A, Sjöborg S, Elofsson R, Falck B. The epidermal indeterminate cell – a special celltype? Acta Derm Venereol (Stockh) 1981; Suppl 99: 41–48.