Immunohistochemistry of Lymphocytes and Langerhans' Cells in Long-lasting Allergic Patch Tests

LASSE KANERVA, TUULA ESTLANDER and RIITTA JOLANKI

Section of Dermatology, Institute of Occupational Health, Helsinki, Finland

Kanerva L, Estlander T, Jolanki R. Immunohistochemistry of lymphocytes and Langerhans' cells in long-lasting allergic patch tests. Acta Derm Venereol (Stockh) 1988; 68: 116–122.

A long-lasting allergic patch test is a "normal" allergic patch test that remains positive for weeks or months. An immunohistochemical study of immunocompetent cells in the skin in this rare type of patch tests was performed. Most inflammatory cells were T11 positive T-lymphocytes. The majority of these cells were of the helper/inducer phenotype (T4+), but a relative increase of T8+ cells as compared to the initial (1–2d) stages of allergic patch tests was observed. T6+ Langerhans' cells (LCs) were normal or increased in number in the epidermis, while very few dendritic cells displayed Ial antigen in the epidermis, indicating loss of Ial-staining of LCs. High to very high numbers of T6+ cells were found in the dermis. An inflammatory reaction of hair follicles with moderate numbers of T6+ cells in the peribulbar infiltrate was observed indicating that hair follicles might act as shunt pathways for allergens. A defect in down regulation of the contact hypersensitivity reaction and/or a constant antigen stimulation could be responsible for the long-lasting allergic patch tests. *Key words: Occupational contact dermatitis; Chromium; Hair follicle; Anti-Tac positive cells; Immunology.* (Received August 7, 1987.)

L. Kanerva, Institute of Occupational Health, Topeliuksenkatu 41 a A, SF-00250 Helsinki, Finland.

The distribution of immunocompetent cells during the initial stages (1, 2), in fully developed allergic patch tests (3–9) and in flare up reactions (10) have been well characterized during the last few years. It is known that most inflammatory cells are T-lymphocytes and macrophages, the B-cells being usually absent (1, 3, 5). The dominating cell is the helper/inducer T-lymphocyte (T4+), while the suppressor/cytotoxic T cells (T8+) represent the minority of inflammatory T cells. The Langerhans' cell has a central role in these events (11).

In normal patch test reactions the clinical signs of the positive reaction disappear in 5–10 days, but in rare cases the allergic patch tests persist for weeks or months. Six cases of these long-lasting allergic patch tests have been observed by us during the last 4.5 years and the results of the immunohistochemical study of biopsies from these reactions are reported in the present paper.

MATERIAL AND METHODS

Epicutaneous tests and biopsies

Epicutaneous tests using the Finn chamber technique and immunohistochemistry were carried out as previously described (2, 9). Occlusion time of the allergen was 24 hours in all cases. The patch tests showed a palpable erythema at the time of biopsy.

Biopsies

Seven biopsies from 6 patients have been analysed. The days from application of allergen to biopsy, and the allergens are seen in Table I. All biopsies were taken from the back of the patients. Half of the punch biopsy specimen was immediately frozen in liquid nitrogen or in isopentane cooled by liquid

nitrogen, stored at −70°C, and processed for immunohistochemical examination. The other half was fixed in glutaraldehyde and processed for electron microscopy.

Immunohistochemistry

We chose the following monoclonal antibodies (Ortho Pharmaceutical Corp.) to characterize the lymphocyte subclasses and Langerhans' cells: OKT11 for T-lymphocytes, OKT4 for helper/inducer T-lymphocytes, OKT8 for suppressor/cytotoxic T-lymphocytes, OKT6 and OKIal (anti HLA-DR) for Langerhans' cells (LCs) and anti-Tac (courtesy T. Waldmann, NIH, Bethesda, Maryland, USA) for activated cells bearing the interleukin-2 receptor. The biopsy specimens were sectioned using a cryomicrotome and fixed in acetone. The slides were then incubated with the monoclonal antibodies and stained using the avidin-biotin-peroxidase method as described earlier (2, 9). The avidin and biotin-labelled reagents were purchased from Vector Laboratories (Burlingham, Calif.). The reaction was visualized with aminoethylcarbazole (Sigma Chemical Co.) (2, 9). Controls included slides incubated with normal mouse ascites fluid instead of antibody.

Quantitation of immunocompetent cells

The counting of separate cells in the dermis in cryostat sections (1) was not considered to give more accurate results than a semiquantitative estimate. Accordingly, the numbers of positively stained cells are given as relative figures compared with healthy control skin and an earlier study of 3–7 day allergic patch tests (9), where identical biopsy and tissue processing techniques had been used (see Table II). The Ia-positive cells and the T6+ cells in the epidermis were counted for three different areas of each tissue section using a calibrated eyepiece reticle at 400× magnification and expressed as number of cells per linear millimeter of epidermis. Each dendritic cell with a positive staining and a distinguishable nucleus was included in the count.

Table I. *Allergen, time of biopsy, number of epidermal T6 and Ial positive cells and Ial staining of keratinocytes in six patients (seven biopsies) of long-lasting allergic patch tests*

Patient	Patch test substance and concentration	Biopsy (days)	T6/ mm of epid.	Ial/ mm of epid.	Ial+ keratinocytes
1	Para-phenylenediamine, 1.0%	15	18.3	13.3	+
2	Hexavalent chromium ($K_2 Cr_2 0_7$), 0.5%	15	18.7	11.0	+
3	Trivalent chromium ($CrCl_3$), 5%	35	40.0	6.7	−
4	Fragrance mix (8%) or p-tert-butylphenol formaldehyde resin (1%)	42	21.7	4.0	+
5	Fragrance mix (8%) and hexavalent chromium 0.5% (2 positive reactions)	42 42	30.0 33.0	6.0 7.3	− +
6	Hexavalent chromium 0.5%	75	20.0	3.3	−
	Normal control skin		18–22	11–15	−

Table II. *Immunocompetent cells in controls, in 3–7 day allergic patch tests (9), and in long-lasting allergic patch tests (15–75 days)*

+++++, large number; +++, moderate; +, small; −, absent (occasional positively stained cell can be observed). Plus in parenthesis means intermediate value. E = epidermis, D = dermis

		OKT11	OKT4	OKT8	OKT6	OKIal
E	Control	−	−	−	+++	++
	3–7 days	++	+	+	++	+
	15–75 days	++	+	+	+++ (+)	(+)
D	Control	−	−	−	+	++
	3–7 days	++++	++ (+)	++	+++	+++++
	15–75 days	++++	+++ (+)	++ (+)	++++	+++++

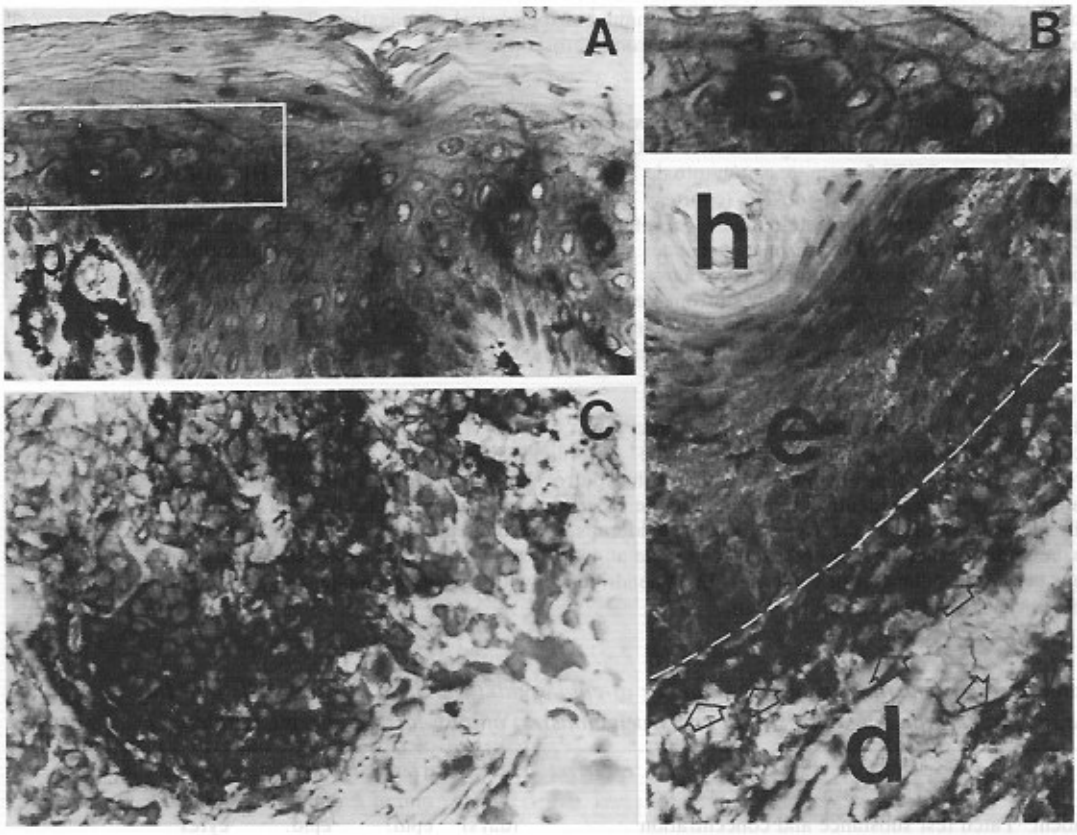

Fig. 1. OKT6-staining. Long-lasting allergic patch test (75 days). (A) A normal number of OKT6+ cells are seen in the epidermis. Note the perivascular cells in the dermal papilla (*p*). (B) Higher magnification of inset in Fig. 1A. Note the OKT6-staining in the intercellular space and/or the membranes of the keratinocytes (*arrow*). (C) Large collection of OKT6+ cells in the dermis. Part of the cells are small, non-dendritic. (D) High numbers of OKT6+ cells (*arrow*) around follicular epidermis (*e*). *d* = dermis, *h* = hair.

RESULTS

General

The epidermis showed focal parakeratosis (Fig. 2A) and mild acanthosis. A moderate to strong infiltrate of inflammatory cells was observed in the upper dermis and foci of inflammatory cells were seen in the deeper layers of the dermis. The infiltrate of the upper dermis was either band-like or perivascular.

Lymphocyte subsets

The results are summarized in Table II and compared to 3–7 day allergic patch tests (9). The infiltrate in the dermis was accentuated as compared to 3–7 d patch tests (9), but qualitatively there was a good correlation to 3–7 d patch tests except a slightly increased relative amount of T8+ cells. Most inflammatory cells stained with T11. One third to one half of the cells stained with T8. T4+ cells and T8+ were randomly mixed in the diffuse interstitial infiltrate or perivascularly. Only few T-lymphocytes were observed in the epidermis. Neutrophils with endogenous peroxidase activity were not encountered.

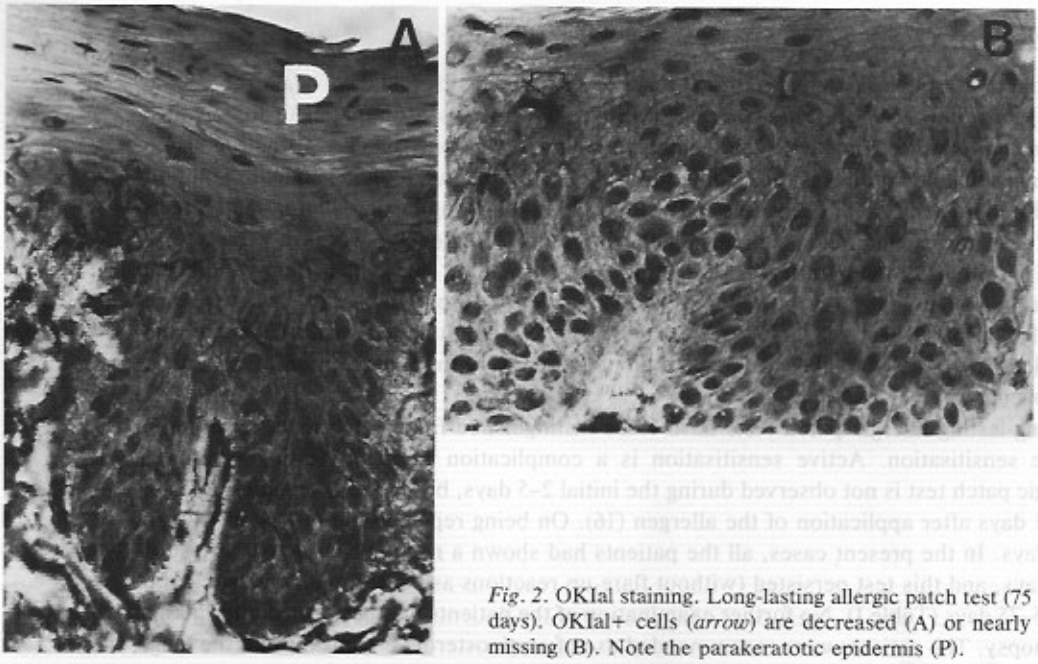

Fig. 2. OKIal staining. Long-lasting allergic patch test (75 days). OKIal+ cells (*arrow*) are decreased (A) or nearly missing (B). Note the parakeratotic epidermis (P).

Anti-Tac positive cells

Anti-Tac identifies activated T cells, which bear the interleukin-2 receptor (12). Three biopsies were studied. Few anti-Tac+ cells were found in the epidermis. Less than 10% of the dermal cells showed a strong staining reaction with anti-Tac antibody and about half of the inflammatory cells showed a weak reaction. This finding corresponds to normal, fully developed allergic patch tests (9).

T6 positive cells

In four samples the number of T6+ cells in the interfollicular epidermis was normal (18–22 cells/linear mm of epidermis) (Fig. 1 A, B) and in three increased (30–40 cells/mm; Table I). A moderate to strong increase of T6+ cells was observed in the dermis (Fig. 1 C). T6+ cells were especially numerous around and in the upper follicle epidermis. Perivascular clusters (Fig. 1 A) and single T6+ cells were observed. Single T6+ cells were seen in the deeper dermis. In some clusters of inflammatory cells, the T6+ staining seemed to locate on small, non-dendritic cells corresponding in appearance to lymphocytes (Fig. 1 C). Prominent staining was focally seen on the keratinocyte membranes or in the intercellular space of the epidermis (Fig. 1 A, B).

Ial-staining

A prominent feature was the clear decrease of Ial+ dendritic cells from the epidermis (Fig. 2 A; Table I), the cells being absent for long distances (Fig. 2 B). Most inflammatory cells and the endothelial cells were stained in the dermis. Keratinocytes showed membrane activity in four samples, but this activity was missing from three samples (35 day, 42 day, 75 day; Table I). Ia staining on keratinocytes was seen focally and not throughout the epidermis.

DISCUSSION

Hunziker (13) mentions that patch test reactions may persist for 40 days or more in some cases and Guin (14) presented a case positive at 270 days after patch testing with a *Parthenium* plant belonging to the Compositae family. The only patch test that persisted for longer than a month was, according to Fischer (15), that due to 0.5% aqueous solution of gold chloride in a gold-sensitive individual. Otherwise long-lasting allergic patch tests have been mentioned very seldomly in the literature. Previously, we have seen a case positive still at 8 months after patch testing with chromium, but at that time we did not take a biopsy. In the present paper we also included 2 cases positive at 15 days, since usually the patch test reactions are already disappearing after one week. Clinically the reactions are usually weaker at 7 days than at 3–5 days, with the exception of neomycin and bacitracin.

Long-lasting allergic patch tests have to be distinguished from patch tests resulting in active sensitisation. Active sensitisation is a complication of patch testing where an allergic patch test is not observed during the initial 2–5 days, but the test becomes positive 10–21 days after application of the allergen (16). On being repeated the test is positive in 2–5 days. In the present cases, all the patients had shown a normal allergic patch test at 2–5 days, and this test persisted (without flare up reactions as based on anamnestic data) for 15–75 days (Table I). No further examination of the patients was performed after taking the biopsy. The patients were recommended use of corticosteroid creams to treat the long-lasting allergic patch test sites after the biopsy.

No attempts have been made to get information on the frequency of long-lasting allergic patch tests. The present cases were collected from patients during a follow-up visit examination or because patients had noticed the persistence of the patch test reactions. It is known that certain substances, e.g. gold, persist in the skin for long periods and are able to give this type of reaction for long periods (15). It is also probable that chromium persists in the skin for long periods (17), but in most cases the patch test reaction fades within the normal 5–10 days. For some unknown reason the allergic patch test reactions to usual allergens persist in some cases for weeks or months as seen in the present cases.

The group of reactions presented here are for natural reasons (it took 4.5 years to collect the material) very heterogeneous both in terms of allergen and in terms of when the biopsy has been done (from 15 to 75 days). The allergens causing the long-lasting allergic patch tests were chromium in 4 patients out of 6, PPD in one case and fragrance mix in one case. In one case the patient had shown two allergic patch tests at the 4d reading. At 42 days one had persisted and one disappeared. It could not be concluded whether the positive reaction had been from fragrance mix or hexavalent chromium (case 5, Table I). We have not been able to retest these patients and thus, we do not know whether these reactions would be long-lasting when repeating the tests.

Immunohistology showed the same kind of reaction as is seen in the initial stages (1–2 days) (1, 2) and in the fully developed allergic patch tests (3–9). An interesting difference from our previous "normal" allergic patch tests (1, 2, 9) was the strong increase of T6+ cells in the dermis. Increased numbers of T6+ cells in the dermis have been reported by several authors in allergic patch tests, atopic eczema (5, 18, 19) and other dermatoses, but we have not seen as many T6+ cells as in the present cases in normal allergic patch tests or diseases that we have studied. The number of T6+ cells was especially high in the 35, 42 and 75 day patch tests. The significance of the high number of T6+ cells in the dermis is not known. Since the number remains high in the epidermis, it is evident that T6+ cells are mainly derived from lymph nodes and/or blood. Mitotic LCs have not been detected in the dermis by us, using electron microscopy.

It seems well established that LCs play a crucial role as antigen presenting cells in delayed hypersensitivity (11), but their kinetics in allergic and irritant patch tests are still poorly understood (20–27). The present study shows that there are changes in the surface markers of LCs or change in LC subpopulation during the course of allergic patch tests, e.g. loss of Ia antigen, making countings after certain stainings unreliable. So far, firm proof of loss of LCs from the epidermis is lacking. Our electron microscopic studies indicate that LCs are always found in the epidermis during allergic patch tests (28). Contrary to the observations of Willis et al. (24) in normal allergic patch tests we (29) detected LCs with Birbeck granules in the dermis in long-lasting allergic patch tests. This apparently reflected the much greater number of LCs in the dermis in long-lasting allergic patch tests.

Anti-Tac identifies activated T-cells, which bear the interleukin-2 receptor (12). No significant changes in anti-Tac+ cells as compared to normal 3–7 day patch tests (9) were observed indicating that activated T-cells persist in the long-lasting allergic patch tests but are not much accentuated.

A defect in down regulation of the contact hypersensitivity reaction and/or constant antigen stimulation could be responsible for the long-lasting allergic patch tests. The constant antigen stimulation could be locally caused by the allergen persisting in the skin or could be due to an exogenous antigen causing a constant "flare-up" of the test site.

REFERENCES

1. 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 skin reactions in man. Acta Derm Venereol (Stockh) 1983; 63: 376–383.
2. Kanerva L, Ranki A, Lauharanta J. Lymphocytes and Langerhans' cells in patch tests. An immunohistochemical and electron microscopic study. Contact Dermatitis 1984; 11: 150–155.
3. Reitamo S, Tolvanen E, Konttinen YT, Käyhkö K, Förström L, Salo OP. Allergic and toxic contact dermatitis: inflammatory cell subtypes in epicutaneous test reactions. Br J Dermatol 1981; 105: 521–527.
4. Bruynzeel DP, Nieboer C, Boorsma DM, Scheper RJ, van Ketel WG. Allergic reactions, "spillover" reactions, and T-cell subsets. Arch Dermatol Res 1983; 275: 80–85.
5. Ralfkiaer E, Wantzin GL. In situ immunological characterization of the infiltrating cells in positive patch tests. Br J Dermatol 1984; 111: 13–22.
6. 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.
7. Wood GS, Volterra AS, Abel EA, Nickoloff BJ, Adams RM. Allergic contact dermatitis: Novel immunohistologic features. J Invest Dermatol 1986; 87: 688–693.
8. Avnstorp C, Ralfkiaer E, Jørgensen J, Lange Wantzin G. Sequential immunophenotypic study of lymphoid infiltrate in allergic and irritant reactions. Contact Dermatitis 1987; 16: 239–245.
9. Kanerva L, Estlander T, Ranki A. Lymphocytes and Langerhans' cells in allergic patch tests. Dermatosen 1987; 35: 16–19.
10. Tosti A, Varotti C, Minghetti F, Bardazzi S, Pileri MT, Rivano, Varonesi S. Immunologic characterization of infiltrating cells in flare-up reactions. Contact Dermatitis 1986; 14: 62.
11. Wolff K, Stingl G. The Langerhans' cell. J Invest Dermatol 1983; 80: 17–21.
12. Platt JL, Grant BW, Eddy AA, Michael AF. Immune cell populations in cutaneous delayed type hypersensitivity. J Exp Med 1983; 158: 1227–1242.
13. Hunziker N. A propos de l'eczéma de contact. Praxis 1970 (7); 59: 238–242.
14. Guin J. A case of persistent patch test reaction: the 270-day reading. "Short report" presented at the North American Contact Dermatitis Group Meeting, Las Vegas, December 12, 1985.
15. Fisher AA. Contact dermatitis. 3rd ed. Philadelphia: Lea & Febiger, 1986; 16: 729–732.
16. Kanerva L, Estlander T, Jolanki R. Sensitisation to patch test acrylates. Contact dermatitis 1987 (in press).
17. Mali JWH, van Kooten WJ, van Neer FCJ, Spruit D. Quantitative aspects of chromium sensitization. Acta Derm Venereol (Stockh) 1964; 44: 44–48.

18. Christensen OB, Troy ED, Maibach HI. Expression of OKT6 antigen by Langerhans' cells in patch test reactions. Contact Dermatitis 1986; 14: 26–31.
19. Reitamo S, Visa K, Kähkönen K, Käyhkö K, Stubb S, Salo OP. Eczematous reactions in atopic patients caused by epicutaneous testing with inhalant allergens. Br J Dermatol 1986; 114: 303–309.
20. Bergstresser PR, Toews GB, Streilend JW. Natural and perturbed distributions of Langerhans' cells: Responses to ultraviolet light, heterotopic skin grafting, and dinitrofluorobenzene sensitization. J Invest Dermatol 1980; 75: 73–77.
21. Suitters AJ, Lampert IA. Expression of Ia antigen on epidermal keratinocytes is a consequence of cellular immunity. Br J Exp Pathol 1982; 63: 207–213.
22. Carr MM, Botham PA, Gawkrodger DJ, McVittie E, Ross JA, Stewart IC, Hunter JAA. Early cellular reaction induced by dinitrochlorobenzene in sensitized human skin. Br J Dermatol 1984; 110: 637–641.
23. MacKie RM, Turbitt ML. Quantitation of dendritic cells in normal and abnormal human epidermis using monoclonal antibodies directed against Ia and HTA antigens. J Invest Dermatol 1983; 81: 216–220.
24. Willis CM, Young E, Brandoun DR, Wilkinson JD. Immunopathological and ultrastructural findings in human allergic and irritant contact dermatitis. Br J Dermatol 1986; 115: 305–316.
25. 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; 67: 590–598.
26. Gengoux P, Lachapelle J-M, Gohy J-M, Miller X. Étude quantitative du nombre de cellules de Langerhans par unité de surface dans l'eczéma de contact allergique. Ann Dermatol Venereol 1985; 112: 799–802.
27. Lindberg M, Emtestam L. Dynamic changes in the epidermal OKT6 positive cells at mild irritant reactions in human skin. Acta Derm Venereol (Stockh) 1986; 66: 117–120.
28. Kanerva L, Ranki A, Mustakallio KK, Lauharanta J. Langerhans' cell—mononuclear cell contacts are not specific for allergy in patch tests. Br J Dermatol 1983; 109, Suppl 25: 64–67.
29. Kanerva L, Estlander T, Jolanki R. Immunohistochemistry and electron microscopy of long-lasting allergic patch tests. In: Society for Cutaneous Ultrastructure Research 13th Annual Meeting & European Society for Comparative Skin Biology, Paris, May 28–31, 1986; 48.