Clinical and laboratory study

CUTANEOUS MERKEL CELLS IN PORCINE AND HUMAN TISSUE EXPANSION

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ABSTRACT

Merkel cells (MC) act as mechanical transducers and paracrine cells of the skin. MC were identified in human and porcine skin by immunoperoxidase technique with monoclonal antibody Cam 5.2. We compared the epidermal MC frequency in expanded and non-expanded skin since tissue expansion causes a change of mechanical forces. For human skin, MC density in non-expanded epidermis was 21.3 ± 19.9 per square mm for transversal sections and 7.5 ± 17.5 per square mm for upsidedown preparations of epidermal sheets. The values for expanded skin were 14.4 ± 21.5 and 12.7 ± 10.4 per square mm skin surface ($p > 0.05$). In porcine epidermal sheets 5.5 ± 5.5 MC per square mm skin surface were estimated, in the expanded samples 1.6 ± 2.1 ($p > 0.05$). Skin surface area, however, was markedly enlarged.

Within the anagen hair follicles, MC were present in the outer hair root sheath. MC were concentrated in the bulb region, the bulge area and pars infundibularis. The distribution pattern of hair follicle MC remained unaffected by tissue expansion. These findings suggest a de novo formation of epidermal MC during tissue expansion of human and porcine skin.

KEY WORDS

Cutaneous Merkel cells, skin expansion, density

INTRODUCTION

Tissue expansion has become a widely used method in plastic and reconstructive surgery (for overview see: Mackay et al., 1989). A silicon prosthesis is implanted under the skin. One or two weeks later the expansion is started by injection of saline solution. The dermal tissue becomes rearranged under stretching conditions. The epidermis responds with a

mitotic burst (Austad et al., 1986). Temporarily the papillary layer is thinned and the length of rete ridges is decreased (Leighton et. al., 1988). Wound healing seems to be accelerated (Timmenga et. al, 1991).

Merkel cells (MC) are of epithelial origin. This facilitates the light microscopic identification with monoclonal antibodies against simple-type keratins (Moll et al., 1984; Saurat et al., 1984). In the epidermis, MC are localized at the bottom of the rete ridges and in the outer hair root sheath. MC function as mechanical transducers and paracrine cells of skin (Mahrle and Orfanos, 1974).

One would expect a reduction of the number of epidermal MC due to stretching of the skin. On the other hand, there is evidence for a de novo formation of epidermal MC in wound healing and skin grafts (Compton et al., 1990). Their number is even increased in certain hypertrophic skin diseases (Gould et al., 1985); Wollina and Karsten, 1988; Mérot and Mooy, 1989).

Therefore, an involvement of MC in the epidermal response to tissue expansion seems possible. To verify the thesis, we investigated the MC in normal and expanded skin of humans and pigs.

MATERIAL AND METHODS

Human tissue samples

Twelve patients (four males, eight females) who underwent tissue expansion were involved in this study. The patients' age was 4 to 49 years (mean 32.5 years). Details of the method have been reported elsewhere (Berger and Hyckel, 1989; Berger et al., 1991). Briefly, the Radovan-type expanders were filled up to a maximum volume of 400 ml with 15 to 50 ml NaCI solution per week (Maximum: 9 weeks). To avoid tissue necrosis the procedure was monitored by a simultaneous measuring of transcutaneous pO_2 and expander pressure (Berger and Hyckel, 1989). A total of 21 human biopsy specimen from the head and the trunk were available. In 8 cases two samples were taken from the same patient at the same region of the body, one from the centre of expanded skin and one from the neighbouring non-expanded skin. In additional 3 cases, expanded skin was investigated. Samples from the same body site were available from two additional cases. The tissue samples were snap frozen in liquid nitrogen and cut at 5 µm. Sections were acetone-fixed for 10 min and used for immunoperoxidase staining.

From 14 samples out of the 12 patients, epidermal sheets have also been prepared. Briefly, the skin samples were incubated in 4N sodium bromide at 37°C for 30 min. The epidermal sheets were carefully slipped upsidedown on glass slides and washed with phosphate buffered saline, pH 7.2.

Porcine tissue samples

Tissue expanders were placed under the thoracic and pelvic skin in 13 Minilewe pigs and filled during 6 to 9 weeks. The final expander volumes were 350 to 500 ccm. For further details see Berger et al. (1992) and Wollina et al. (1991). A total of 16 tissue samples were obtained within one week after the last expansion to circumvent any influence of the delay phenomenon on MC counting. Epidermal sheets were prepared as described above with a prolonged incubation time of one hour.

Antibodies and immunoperoxidase staining

The primary antibody Cam 5.2 (Becton-Dickinson) is a mouse monoclonal IgG1 antibody against simple-type keratins (nos. 8, 18, 19). These keratins are not presented in interfollicular keratinocytes after birth but can be identified in the epidermal MC and in eccrine glands. Peroxidase-conjugated swine antirabbit or rabbit antimouse immunoglobulins were purchased from Dako (Hamburg, FRG). Their working dilution was 1:30. Peroxidase was developed with 3-amino-9-ethylcarbazole (AEC; Sigma, St. Louis/MO, USA).

Unlabeled immunoperoxidase staining followed the protocol given by Sternberger et al. (1970) with minor modifications as described recently (Wollina, 1991). Briefly, methanol/H_2O_2 was used to block the endogeneous peroxidase activity. Then, serial sections were incubated for 30 min with normal serum to prevent nonspecific staining followed by addition of the primary antibody for one hour. Thereafter, the secondary antibody was added for 30 min and the color reaction was developed with AEC for further 10 to 20 min. Slides were counterstained with toxyline. All incubation steps were performed at room temperature and the slides were washed twice in between successive steps. Negative control was performed by ommission of the primary antibody.

Merkel cell counting

MC were identified by positive staining with monoclonal antibody Cam 5.2 against simple-type keratins in the basal cell layer of both the interfollicular epidermis and the outer hair root sheath. Dermal MC were counted within the papillary layer only. A rough estimation of epidermal interfollicular MC density related to the skin surface area was made according to Moll et al. (1991): Since the mean diameter of a Merkel cell is about 10 µm, a given MC would only be estimated once in 5 µm-thick sections, when every second or third section was used. The 5 µm-sections investigated were considered to be representative for the total volume of skin and projected directly to the unit area of skin.

In epidermal sheet preparations, the MC number was simply related to the surface area of the sheets.

RESULTS

In non-expanded skin, Merkel cells were identified at the bottom of the rete ridges, in the basal layer of the outer hair rooth sheath and in the papillary, dermal layer. The majority of Cam 5.2-positive cells was found within the epithelium (Fig. 1, 2). The frequency of dermal MC was to low for reasonable statistical analysis (total number: 7 MC) but dermal MC seemed evenly distributed among expanded and non-expanded samples.

Table 1. Merkel cell density in Minilewe pigs (in MC par square mm skin surface area); n.d., not done.

No.	Non-expanded skin	Expanded skin
Thoracic region		
1.	1.8 ± 0.7	0.8 ± 1.0
2.	7.7 ± 1.8	6.6 ± 0.9
3.	5.1 ± 1.2	0.3 ± 0.7
4.	2.3 ± 0.9	1.6 ± 0.8
5.	2.2 ± 0.9	1.2 ± 1.0
6.	10.3 ± 7.3	n.d.
7.	n.d.	3.8 ± 0.6
8.	n.d.	0.2 ± 0.8
9.	23.1 ± 9.3	n.d.
10.	5.8 ± 1.6	n.d.
Pelvic region		
11.	1.9 ± 0.7	1.0 ± 1.2
12.	2.3 ± 0.6	0.7 ± 0.5
13.	3.8 ± 1.5	< 0.1
14.	7.7 ± 2.4	n.d.
15.	2.6 ± 1.3	n.d.
16.	3.8 ± 0.8	n.d.

Table 2. Epidermal Merkel cells (in number per square mm surface area) in expanded and control skin

Expanded skin	Controls	P
I. Human skin		
A Transverse Sections [1]		
14.4 ± 21.5	21.3 ± 19.9	> 0.05
B Upside down preparations [2]		
12.7 ± 10.4	7.5 ± 17.5	< 0.05
II. Porcine skin		
A Transverse Sections [1]		
1.6 ± 2.1	5.5 ± 5.5	> 0.05

1) Estimation according to Moll et al (1991)

2) The differences of MC counts between transverse sections and upsidedown preparations are not significant. The lower values in the latter, however, seem to be due to underestimating the infundibular Merkel cells.

In expanded skin, the distribution of Merkel cells was in general not different from non-expanded samples. A minor variation was the tendency to cluster formation of interfollicular MC. The MC density estimated by the method of Moll et al. (1991) provided slightly higher values for MC density of human skin than those obtained from epidermal sheets, but the difference reached no significance. To be sure for what we found in human samples, mainly paired specimen of porcine origin were investigated. The details are given in Table 1. As one can see, MC density of both human and porcine samples remained almost unchanged after tissue expansion (Table 2). The finding is surprising, since skin surface area was markedly enlarged (Up to 160 %).

Since Cam 5.2-positive cell also occur within the bulbar region, not related to MC, only the outer root sheath was considered in this study. In human skin MC were found in the basal layer of the outer hair root sheath from the bulb to the

infundibulum, but a local concentration of MC was observed in anagen hairs at three locations: in the infundibulum, the bulge area, and the bulbar region. Up to ten MC were seen forming clusters (Fig. 2). In late anagen, the number of bulbar Cam 5.2-positive cells seemed to increase during anagen.

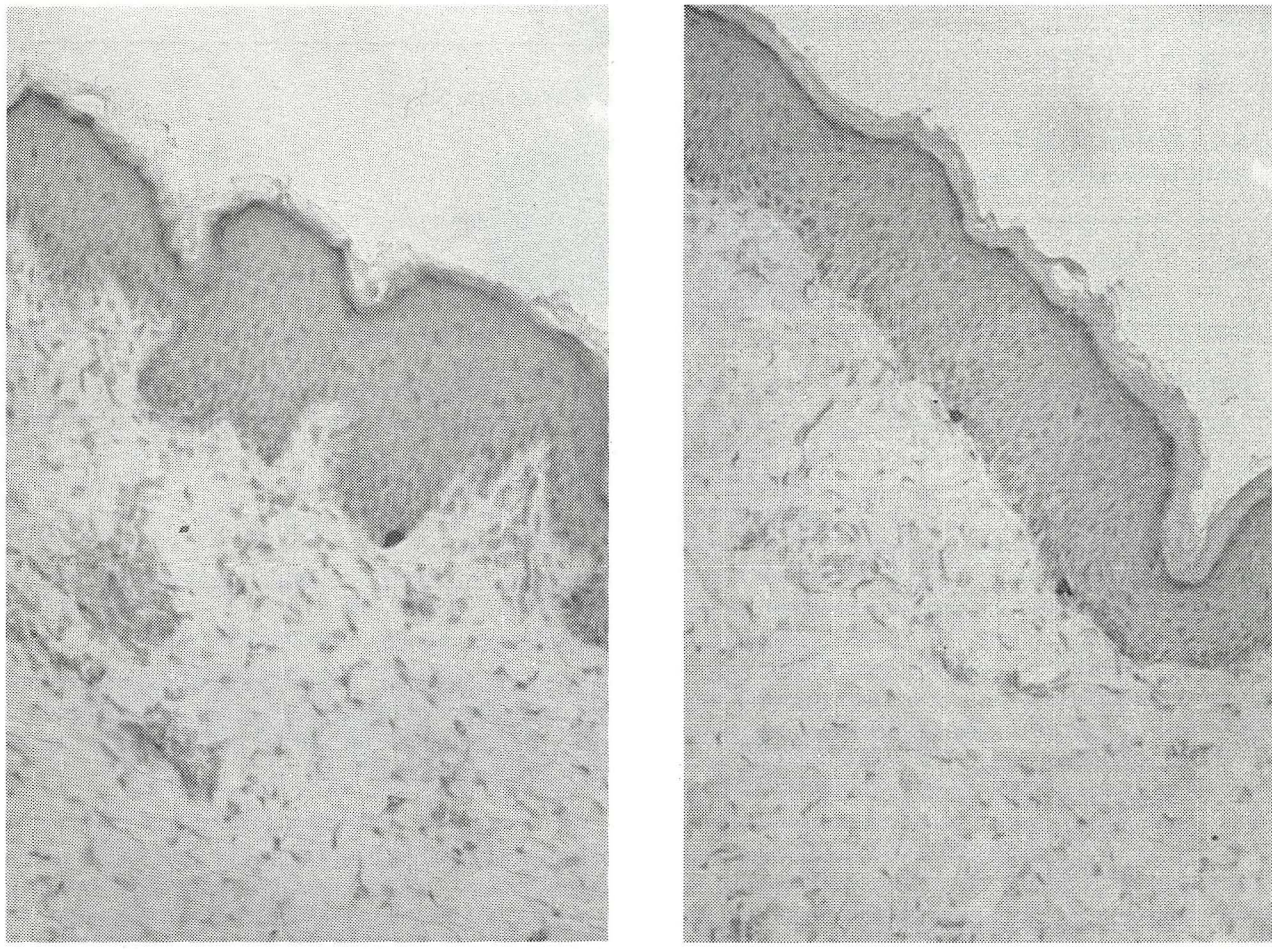


Fig. 1. Identification of epidermal Merkel cells with monoclonal antibody Cam 5.2: (a) non-expanded, (b) expanded skin (peroxidase technique, x 250).

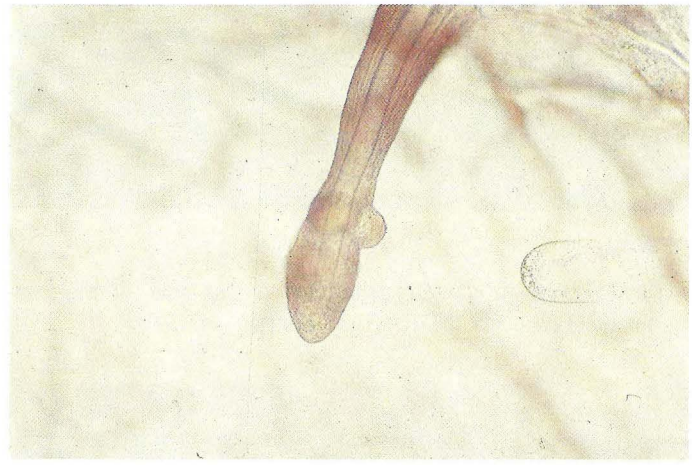
In porcine skin, follicular MC were more sparsely distributed within the outer hair root sheet (data not shown).

DISCUSSION

MC are paracrine epithelial cells, which express simple-type keratins and various neuronal markers including neuropeptides (for overview see: Gould et al., 1985; Hartschuh et al., 1989). By use of monoclonal antibody Cam 5.2, MC were identified in normal and expanded skin. A possible involvement of MC in tissue response to expansion is suggestable because of the following assumptions and observations: (i) MC function as mechano-receptors, especially those in the outer hair root sheath (Mahrle and Orfanos, 1974). (ii) MC produce and secrete neuropeptides which act as paracrines (Gould et al., 1985; Hartschuh et al., 1989; Wollina and Mahrle, 1992). (iii) MC are found in some hypertrophic skin diseases (Gould et al., 1985; Wollina and Karsten, 1988; Mérot and Mooy, 1989). (iv) Tissue expansion induces a rearrangement of the dermis and subcutaneous tissue (Austad et al., 1986; Leighton et al., 1988). (v) Tissue expansion leads to a thinning of the papillary layer (Austad et al., 1986; Leighton et al., 1988) and (vi) a loosening of the dermis accompanied by a loss of factor XIIIa (Penneys et al.,

a.

b.



c.

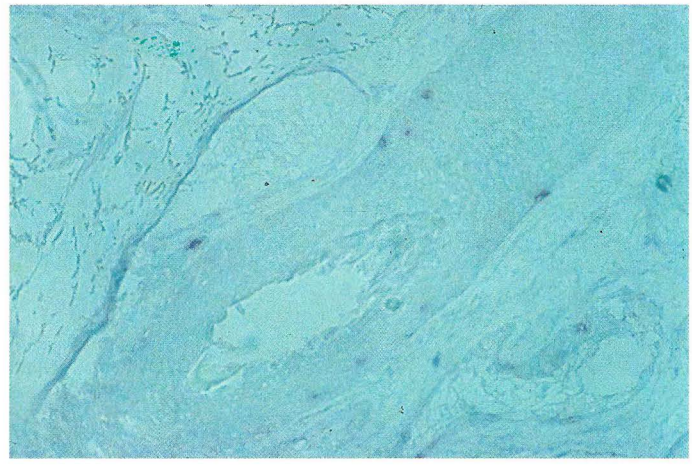


Fig. 2. Identification of Merkel cells in the pilosebaceous unit with monoclonal antibody Cam 5.2: (a) outer hair root sheath, supraseboglandular; (b) bulge area (Peroxidase technique, x 250), (c) hair bulb in an epidermal sheet preparation (Peroxidase technique with Cam 5.2).

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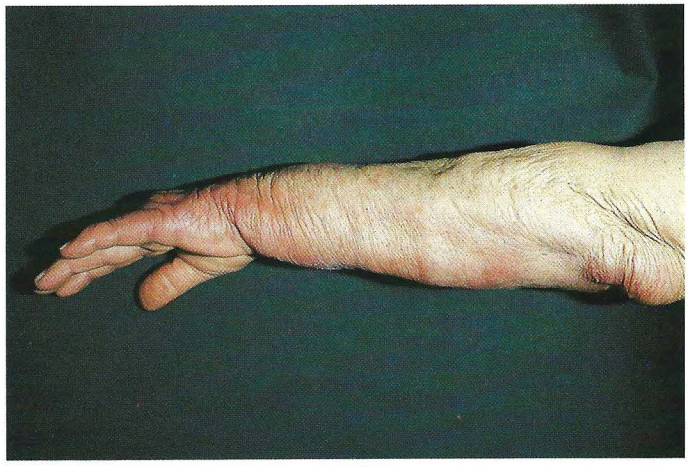

Figure 1: 55 year-old woman with acrodermatitis chronica atrophicans on the ulnar aspect of the distal left forearm and hand.

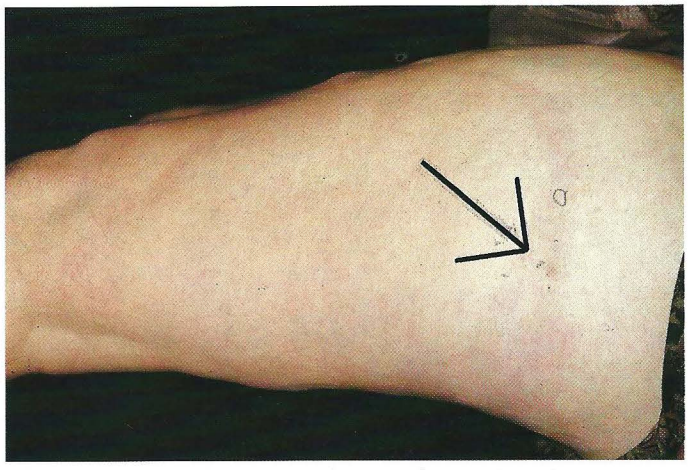

Figure 3: Two years later, the patient presented with erythema migrans. Interestingly, the site of the tick bite is located within the "active" border of the lesion (arrow).

1991). Thus, it seems conceivable that tissue expansion could have an impact on MC quantity and/or functional activity.

In the present study, the frequency and density of MC in non-expanded and expanded human and porcine skin was carefully investigated. MC in normal and expanded skin were identified at the bottom of the rete ridges, the outer hair root sheath and very sparsely within the papillary layer (Fig. 1). Since tissue expansion causes a stretching of the skin, one might expect a reduction of the frequency and density of epidermal MC. Surprisingly, epidermal MC density was not significantly changed both in humans and pigs. We only could find a significance in humans when using upsidedown preparations of epidermal sheets. In this particular case, the MC density was almost double as high in expanded vs. non-expanded samples. A limitation of our human study was, that the tissue samples were from different body sites and from patients of various ages. So we cannot exclude an underestimation of numerical variations (cf. Moll et al., 1991). On the other hand., our MC densities were within the range reported by Moll et al. (1991) for normal human skin.

In order to avoid that coincidence overriding facts, a standardized animal study was performed in mini pigs. The porcine samples provide lower values of epidermal MC but substantiated our results for humans (Tables 1 and 2).

Recently, Berger et al. (1991) reported on an increase of both hair diameter and hair follicle density in expanded human scalp skin. Therefore MC distribution in the pilosebaceous unit was of interest. Single cells expressing simple-type keratins have been observed in the bulb region and the pars infundibularis of anagen hairs (Heid et al., 1988; Wollina, 1992). Yet not described, we also found a local concentration of MC (up to ten cells) in the bulge area (Wulst) (Fig. 2). The bulge marks the lower end of the "permanent" follicle. It is located just beneath the opening of the sebaceous gland where the musculus arrector pili attaches (Madsen, 1965). Cotsarelis et. al. (1990) defined label-retaining cells inside the bulge of mouse hair follicles probably serving as stem cells. Rapidly proliferating matrix cells are thought to correspond to transient amplifying cells. In vitro studies have shown, that outgrowing keratinocytes of the outer hair root sheath do not express simple-type keratins. But hair plucking left basal cells of the bulge area in situ (Schaart et al., 1990). Therefore, a relationship between bulge stem cells and the formation of hair follicle MC is possible. If MC of the bulge move to the matrix and if they might serve as growth modulators of matrix cells remains to be elucidated. On the other hand, there is experimental evidence for the involvement of neurohumoral factors in the formation of hair follicles (cf. Jones and Munger, 1987; Paus).

The exact enumeration of MC density in hair follicles is hampered by the complex structure of the pilosebaceous unit. So we cannot exclude that tissue expansion interferes with MC density of the outer hair root sheath.

In conclusion, tissue expansion is a highly valuable method to obtain a physiological wound covering in plastic and aesthetic surgery with a regular epithelial differentiation (Wollina et al., 1991). The present findings suggest an increase of epidermal MC during the procedure. If MC contribute to the mitotic activity of epidermal keratinocytes by secretion of (neuro-) peptides is under investigation.

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