

Table II. Corneal formation given as layers formed per 24 hours measured after corneal staining with 5 % dansyl chloride ointment under occlusion

Sex	Age	Corneal formation	Duration of occlusion (h)
♀	54	1.14	48
♀	63	0.96	24
♀	63	1.06	48
♀	67	1.04	48
♀	69	1.14	48
♂	41	1.49	24
♂	62	0.70	48
♂	65	1.67	24
♂	66	1.43	48
♂	77	0.86	24
Mean		1.15	

material obtained after occlusion for 24 h was compared with that at 48 h (Table I).

It may be noted that the occlusion for 48 h gave almost straight lines in the material studied up to 10 days after staining (Fig. 1). This indicates that the occlusion did not affect the proliferation rate during this interval. The incomplete staining obtained after occlusion for 24 h might be a reason for the non-linearity found in one of the male subjects (Fig. 1).

The present results which indicate a formation of about 1.15 corneal cell layers per 24 h was in the upper range of three earlier studies, 0.89, 0.72–1.08 and 1.20, utilizing the surface disappearance of the fluorescent corneal marker as the base of measurement (1, 2, 3). The range in the present study varied by a factor of 2. In future studies relating observations on cell proliferation and histology with biochemical studies, it will be necessary to include the normal formation of the horny layer in the skin region used. From the data presented it is feasible to detect at the 5% level an increase in the formation of the horny layer when it is more than 15–32% above the normal rate.

ACKNOWLEDGEMENTS

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Membrane-associated Actin in Psoriatic Epidermis

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Abstract. With immunofluorescence technique by using specific antibodies, polymerized actin (F-actin) was found to be present in the peripheral parts of the cells in all layers of epidermis from lesional skin taken from twelve psoriatics, whereas normal epidermis from the same individuals showed no such reactivity. This finding might have some bearing on the induction of these lesions.

Key words: Microfilaments; Plasma membrane

Actin is a contractile protein present in almost all eukaryotic cells. Actin constitutes part of a microfilament system which appears to be associated with the plasma membrane (13). In non-muscle cells these actin-containing filaments have been implicated in a number of cellular functions including cytokinesis, endocytosis, exocytosis, cell adhesion to substratum, cell locomotion, membrane ruffling, maintenance of cell shape and cell division. There is also evidence that the distribution and mobility of plasma membrane components is controlled by submembranous microfilaments and microtubules (1, 11). Actin may be present in a cell in at least two interconvertible states: as unpolymerized actin (G-

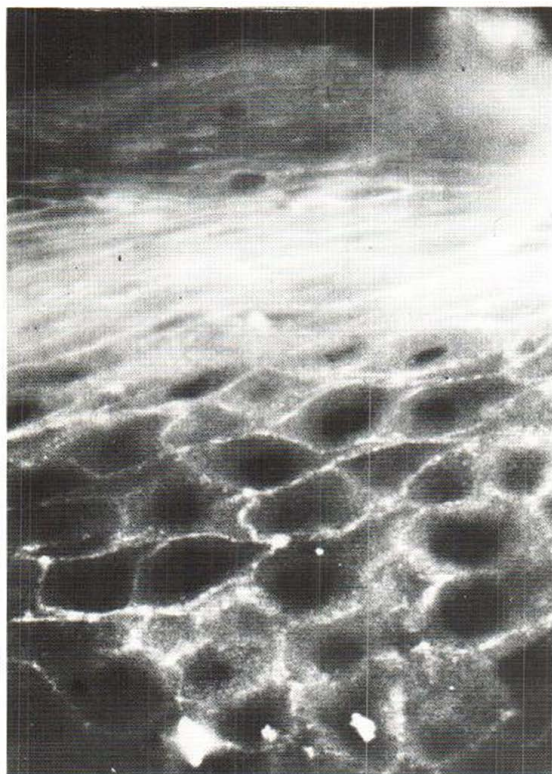


Fig. 1. Psoriatic lesional skin showing fluorescence in the peripheral part of the cells in all layers of epidermis after incubation with anti-actin antibodies.

actin), or as bundles of identifiable filaments (F-actin or polymerized actin). Antibodies to actin detect F-actin preferentially (2, 7, 9), and such antibodies can be used to monitor the presence of polymerized actin within cells.

Little is known about the involvement and significance of the microfilaments in disease. In view of the association of microfilaments with the plasma membrane and the possible role of both the microfilament system and the plasma membrane in growth control (3), one may hypothesize that in disturbances of cell growth such as psoriasis there is an alteration in the plasma membrane or in its associated cytoplasmic elements. The observation of decreased intercellular adhesion in psoriatic lesions may support the suggestion of a plasma membrane defect in this disease (10).

The present report describes a study where, by using antibodies to actin, we have investigated the presence of detectable actin in psoriatic lesions and normal skin from the same individuals.

MATERIALS AND METHODS

Punch biopsies were collected from both involved and clinically normal skin (arms or trunk) in 12 patients with psoriasis (mean age 47 years, range 23–67 years). The lesions were nummular and had been present for the last 4 months or more. The biopsies ($\varnothing$ 3 mm) were taken from the central part of the lesion under local anaesthesia induced with ethyl chloride. The patients had been using salicylic acid and/or fluorinated steroids sporadically before biopsy.

The biopsy specimens were transported in 0.9% NaCl and then frozen within one hour and kept at -70°C until sectioned. The sectioning was performed in cryostat within 4 days, and the sections fixed in dry acetone for 10 min.

Immunofluorescence

The sections were incubated for 30 min with human sera containing anti-actin antibodies, the specificity of which has been shown (7). Also used were human anti-actin sera absorbed with F-actin, as described earlier (7), and rabbit anti-actin serum. For visualization, after washings, the sections were incubated with fluorescein isothiocyanate conjugated polyvalent sheep anti-human immunoglobulin (NBL), protein content 5.7; molar F/P ratio 3.8, and sheep anti-rabbit immunoglobulin, protein content 5.2; molar F/P ratio 3.2. The sections were examined in a Leitz orthoplan fluorescence microscope. Light source was a Xenon arc lamp (SBO 75W Osram) with a filter combination of excitation filter (TAL 485, 355–505 nm), a 4 mm BG 38 X, a Calflex filter in the lamp house, mirror filters TK 495 + I 495 and a barrier filter K 910.

RESULTS

All 12 patients showed staining in lesional skin in the peripheral parts of cells from all layers of the epidermis (Fig. 1). In their normal skin, no staining was found in 8 patients (Fig. 2); 2 patients had some scattered cells with peripheral staining in the basal layer. The remaining 2 patients had slightly more abundant staining with the same pattern in the basal layer, but none was seen in the upper parts of epidermis. Sections from lesional skin treated with serum absorbed with actin displayed no staining. Rabbit anti-actin, used in 4 cases, gave the same pattern as human anti-actin. No difference in staining pattern was found between patients belonging to different blood groups.

DISCUSSION

The contractile proteins of non-muscle cells, when arranged as microfilaments, have been studied with various techniques, such as electronmicroscopy, using their ability to bind heavy meromyosin or, as

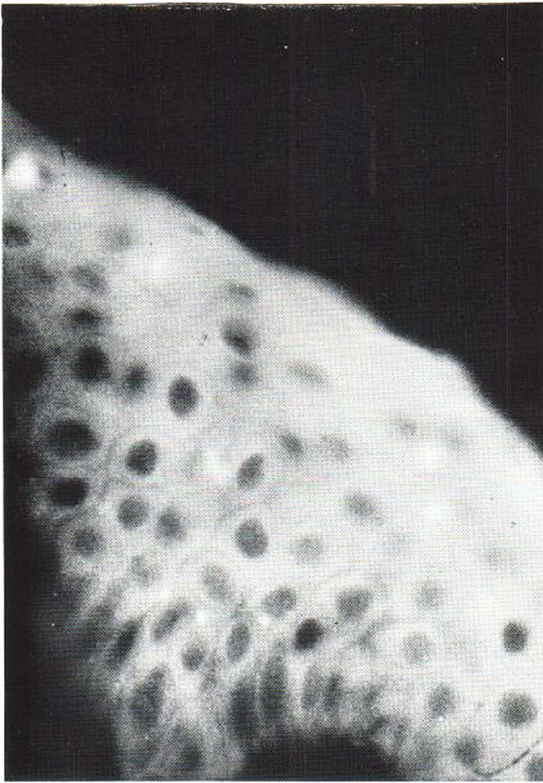


Fig. 2. Normal skin from the same individual, showing no reactivity.

in this study, by immunofluorescence. By using specific antibodies we found strong fluorescent staining of the periphery of epithelial cells in lesional skin from psoriatic patients, thus indicating the presence of F-actin. In contrast, normal epidermis generally did not show such fluorescent staining, except occasionally in some of the basal cells which finding is in agreement with those of other investigators (6). Epithelial cells growing over granulation tissue in healing wounds, and cells in basal cell carcinoma and squamous cell carcinoma have also been reported to stain with anti-actin antibodies (5, 6).

The presence of polymerized actin within cells has been correlated to active behaviour of cells, such as in locomotion (4) or tumour invasiveness (8), and may be a response of cells to stimuli affecting the plasma membrane. In view of the increased amounts of detectable actin in psoriasis demonstrated in this study, the latter point may have some bearing on the induction of exacerbations of this disease. We show in a separate paper how stimula-

tion of lymphocytes by mitogens is markedly augmented by simultaneous enhanced polymerization of submembranous actin, a process which consequently may be a prerequisite for or part of a stimulus to cell division (12).

The accumulation in psoriatic lesional skin of membrane-associated F-actin suggests the possibility of an alteration in the plasma membrane or in its associated cytoskeletal elements as being responsible for the aberrant epidermal growth in psoriasis. Psoriatic epidermal cells may, in response to external factors interacting with its plasma membrane, too easily polymerize actin, thus augmenting the response to stimuli which are suboptimal for activation of normal cells.

The finding of F-actin throughout the whole lesional epidermis may well be consistent with the finding that the intercellular space is widened and the membranes undergo microvillous transformation in the malpighian layer, which often is preserved in the horny layer (10).

The observed changes in psoriatic epidermis may imply both a change in recruitment of the cells in the growth fraction, i.e. the proportion of the germinative population that is actively progressing around the cell cycle, as well as the mobility of the cells through the epidermis.

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Failure to Detect Circulating IgG or IgM Antibodies to Basal Cell Carcinoma by Immunofluorescence

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Abstract. Immunofluorescence techniques failed to reveal evidence of anti-tumour antibody in the sera of patients with basal cell carcinoma. Although the presence of such antibodies has previously been associated with the absence of metastasis in malignant melanoma, other explanations for the low metastatic potential of basal cell carcinoma should be sought.

Key words: Basal cell carcinoma; Antibodies; Immunofluorescence

The basal cell carcinoma is the only common invasive tumour of man (outside the cranial cavity) that very rarely metastasizes. This finding remains un-

explained despite its possible considerable relevance to the problem of tumour metastasis in general. The cell-mediated immune response to basal cell carcinoma, as judged by the local inflammatory reaction observed by conventional histology, is usually mild or lacking.

The purpose of the study reported here was to determine whether or not a humoral immune response to basal cell carcinoma could be detected by using immunofluorescence techniques.

The discovery of circulating anti-tumour antibodies in malignant melanoma and their apparent association with the absence of metastatic spread (2) has led to the suggestion that serum antibodies might be involved in the control of metastasis (3). A search for similar antitumour antibodies in patients with basal cell carcinoma therefore appeared to be justified.

MATERIALS AND METHODS

After excision, basal cell carcinomas were placed in tissue culture medium 199 (Wellcome) and stored at 4°C for up to 18 hours; the varying times did not affect the results. From each patient, serum was obtained from a 10 ml clotted blood sample. From one-half of the carcinoma, 8 µm thick cryostat sections were prepared and, when possible, cell smears were made as well. Small or fragmented tumours were excluded so as not to jeopardize the routine histology performed on the remaining half of the tumour. The cell smears were prepared by scraping the surface of the tumour with a cataract knife into a drop of buffered saline on a glass slide. Haematoxylin and eosin staining revealed that this technique consistently provided tumour cells singly and in small clumps. The dried smears were frozen with isopentane in liquid nitrogen at -186°C for 5 min (2), redried and then treated in the same way as the sections.

In about half the cases studied, both fixed and unfixed preparations were used, the fixation being in dry acetone for 10 min at 4°C. As the fixation made no difference to the subsequent staining, all the subsequent preparations were processed unfixed.

The section and smears were then treated for 30 min with the serum, diluted 1:32 with 0.01 M phosphate-buffered saline, from either the same patient or another basal cell carcinoma patient. Control preparations from the same lesion were treated with 0.01 M phosphate-buffered saline only. After washing in buffered saline for 30 min all preparations were treated with fluorescein isothiocyanate-labelled goat antisera to human IgG and IgM (Behringwerke, protein concentration 0.8–1.5 g/100 ml) diluted 1:32 in 0.01 M phosphate-buffered saline. Further details of this sandwich method were published by Nairn (4).

The preparations were mounted in buffered glycerol and examined by darkground ultraviolet light with a Reichert Zetopan fluorescence microscope using excitation filter E2 and absorption filter Sp2.