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GP37 Expression in Normal and Diseased Human Epidermis: A Marker for Keratinocyte Differentiation[1]

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An antiserum prepared against a glycoprotein (GP37) extracted from the upper epidermal layers, was used to stain frozen sections of human oral mucosa, normal and abnormal skin by an indirect immunofluorescence technique. On normal human epidermis, this antiserum mainly reacted with the cytoplasm of granular cells, whereas on buccal mucosa the recognized antigen was observed as scattered dots limited to the upper epithelial layers. In epidermal diseases, alterations in the staining pattern were observed. In psoriasis, the labelling was markedly diminished; in contrast, in lichen planus it was intense and present on the 3–6 uppermost cellular layers. Basal cell epitheliomas were almost negative, except around horn cysts. In Bowen's disease dyskeratotic cells were strongly labelled. In squamous cell carcinomas a clear-cut staining was observed in squamous nests. On cultures, GP37 expression could be induced by growing epidermal cells in vitamin A-depleted medium. The biological significance of the observed staining patterns remains to be precised. Nevertheless, GP37 represents a sensitive marker of epidermal differentiation and may be useful in skin pathology and in *in vitro* studies. *Key words: Epidermal disorders; Glycoprotein; Differentiation; Epidermal cell culture; Vitamin A.* (Received October 21, 1985.)

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Glycoconjugates are believed to play a key role in many cellular functions (1). In the skin these molecules are widely involved in epidermal cell behaviour and interactions as well as in epidermal integrity and homeostasis (2). Moreover glycoconjugate distribution in normal and diseased skin has been investigated and correlated with epidermal differentiation (3, 4). Lectins, by their binding to specific carbohydrate configurations, have proved to be useful tools to pinpoint in situ differences in glycoconjugate expression (5–8). Recently, attempts have been made to characterize at the biochemical level the macromolecules labelled by lectins in situ (9–12).

In a previous study, we investigated Con-A binding glycoproteins in NP-40 extracts of normal human epidermis and various epidermal cell preparations (13). Some of these molecules appeared to be restricted to the upper epidermal layers and thus could represent glycoprotein markers of the differentiation process. A polyclonal antiserum was prepared against one major glycoprotein of apparent molecular weight 37 Kd (GP37). Immunohistochemistry using GP37 antiserum gave a cytoplasmic staining of the upper layers of the living epidermis: granular cells showed a strong reactivity whereas spinous cells were weakly labelled (14). According to these observations the GP37 antiserum was considered to define a cytoplasmic marker of normal human granular layer.

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In the present report we detailed the reactivity of GP37 antiserum on normal human and animal epidermis and oral mucosa. We also investigated GP37 expression in some epidermal proliferative and tumoral lesions. The known effect of vitamin A on the expression of epidermal antigens (15), glycoconjugate metabolism (16–17) and cellular differentiation (18), prompted us to investigate the expression of GP37 by cultured human keratinocytes, grown both in normal and in vitamin A-depleted (delipidized) medium.

MATERIALS AND METHODS

Tissue specimens

(*a*) *Normal tissues*. Human skin and buccal mucosa were obtained from healthy volunteers. Lips were obtained from monkeys (*Macaca cynomulgus*), rabbits and mice in order to have on a single specimen, areas of mucosa, parakeratotic and orthokeratotic epithelium.

(*b*) *Skin lesions*. Several biopsies from various proliferative and tumoral skin lesions were studied (see Table I). Tissue specimens were immediately divided into two parts, one snap frozen in liquid nitrogen for immunofluorescence (IF) examination, another fixed in Bouin's medium for routine histology. In addition, frozen tumor sections, obtained in sequence with sections used for IF, were stained with hematoxylin-eosin.

Epidermal cell culture

Human epidermal cell cultures were prepared from breast skin removed during plastic surgery. After storage overnight in Hank's solution lacking Ca^{++} and Mg^{++} (HBSS) and containing antibiotics, skin slices were prepared with a keratotome and epidermis was separated from dermis by incubation in trypsin (GIBCO 0.25% in HBSS) for 1 h 30 mn at 37°C. A cell suspension was prepared by agitation of the epidermal sheets. Cells were counted and seeded in culture dishes (Corning) previously coated with Type I rabbit skin collagen following established methods (19). The culture medium was Dulbecco's modified Eagle medium (GIBCO) containing 10% FCS, 100 U/ml penicillin, 100 U/ml streptomycin, 0.25 µg/ml fungizone, non-essential aminoacids (GIBCO) and 10 mM Hepes, pH 7.0.

In order to grow cells in the absence of vitamin A, some cultures were switched in medium containing FCS that had been delipidized using polar solvents according to the method of Rothblat et al. (20).

As control some cultures were grown in "delipidized medium" added with retinyl acetate 10^{-7}M (Sigma). Cultures were maintained at 37°C in 10% CO_2 and the medium was changed every two days. Conditions of vitamin A depletion or retinyl acetate supplementation were maintained at least 2 weeks before labelling experiments. In order to assure reliability of results, four different culture experiments were carried out.

Experimental antiserum

The preparation of GP37 antiserum has already been reported (14). Briefly, this was obtained by immunizing female Hartley guinea pigs with a ConA-reactive glycoprotein of apparent MW 37Kd obtained after SDS-PAGE separation. For immunization, gel sections were used following established methods (21).

Immunohistochemical procedures

(*a*) *Indirect immunofluorescence* (IIF) (performed on tissue sections and occasionally on cell cultures): four micron-thick frozen sections were cut on a cryotome, air-dried and fixed in acetone (10 min, −20°C). For IIF studies, the slides were flooded with GP37 antiserum (1 : 30), washed in PBS, pH 7.2, then incubated with fluorescein-conjugated goat anti-guinea pig IgG (Nordic 1 : 30). After washing, the slides were mounted in a polyvinylalcohol medium and examined with a Zeiss fluorescence microscope (epi-illumination, Orthoplan). Control slides were obtained by omitting the first layer antiserum and using instead normal guinea-pig serum.

(*b*) *Indirect immunoperoxidase* (performed on cell cultures): cultures were rinsed in PBS and fixed in 2% paraformaldehyde (1 h, 4°C). The reaction was then carried out as follows: after washing in PBS and incubation with GP37 antiserum, cultures were incubated with peroxidase-conjugated rabbit anti-guinea pig (Zymed 1 : 30). The reaction was revealed by 3–3′ diaminobenzidine (Sigma) added with H_2O_2 (22). The cultures were mounted under coverslips in a gelatin containing medium and viewed under a Leitz microscope. Controls were performed as above mentioned.

RESULTS

Normal tissues

On normal human epidermis, GP37 gave a strong and homogeneous labelling of the cytoplasm of the granular layer cells. A mild homogeneous fluorescence of the spinous layer was also observed. The basal cells were negative. No staining was seen in the stratum corneum (Fig. 1 A).

The acrosyringium showed a cytoplasmic staining with distinct fluorescent minute granules (Fig. 1 B). The outer root sheath, the sebaceous and eccrine duct epithelium were faintly stained, while the secretory portion of eccrine and sebaceous glands remained negative.

No specific staining of dermis or subcutaneous tissue was noted.

On human buccal mucosa, the staining was visualized as scattered dots limited to the upper epithelial layers (Fig. 1 C). On animal lip sections studied, all but the basal cell layer of the epidermal zone were labelled. The parakeratotic intermediate zone showed in rabbit and monkey a weak diffuse staining of some isolated keratinocytes. The reactivity of monkey mucosa was similar to that observed on the corresponding human tissue. No staining was observed on rodent mucosa.

Epidermal cell cultures

No staining was observed on cells cultured in normal medium or in delipidized medium added with retinyl acetate. On the contrary, when the delipidized medium was not retinyl acetate-supplemented, a specific immunolabelling was seen in most large superficial cells (more than 70%) (this was confirmed by IIF performed on frozen perpendicular sections of cell culture sheets). In these cells, well defined perinuclear vacuole-like structures of irregular size were reactive (Fig. 1 D).

Skin lesions

Psoriasis vulgaris: The lowermost 2 or 3 epidermal cell layers were negative. The keratinocytes of the stratum malpighii showed a staining similar to that observed on normal epidermis. A few scattered cells of the upper malpighian layers showed a strong cytoplasmic staining. Areas of parakeratosis revealed a punctate labelling in the horny layer (Fig. 2A).

Lichen planus: The basal and spinous layers showed a negative or a weak irregular

Table I. *Lesions studied*

Type of lesion	Number of cases
Psoriasis vulgaris	8
Lichen planus	3
Common warts	4
Actinic keratosis	2
Bowen's disease	2
Basal cell epitheliomas	
Superficial	1
Solid	7
Adenoid	2
Keratinizing	3
Squamous cell carcinomas (well-differentiated)	5

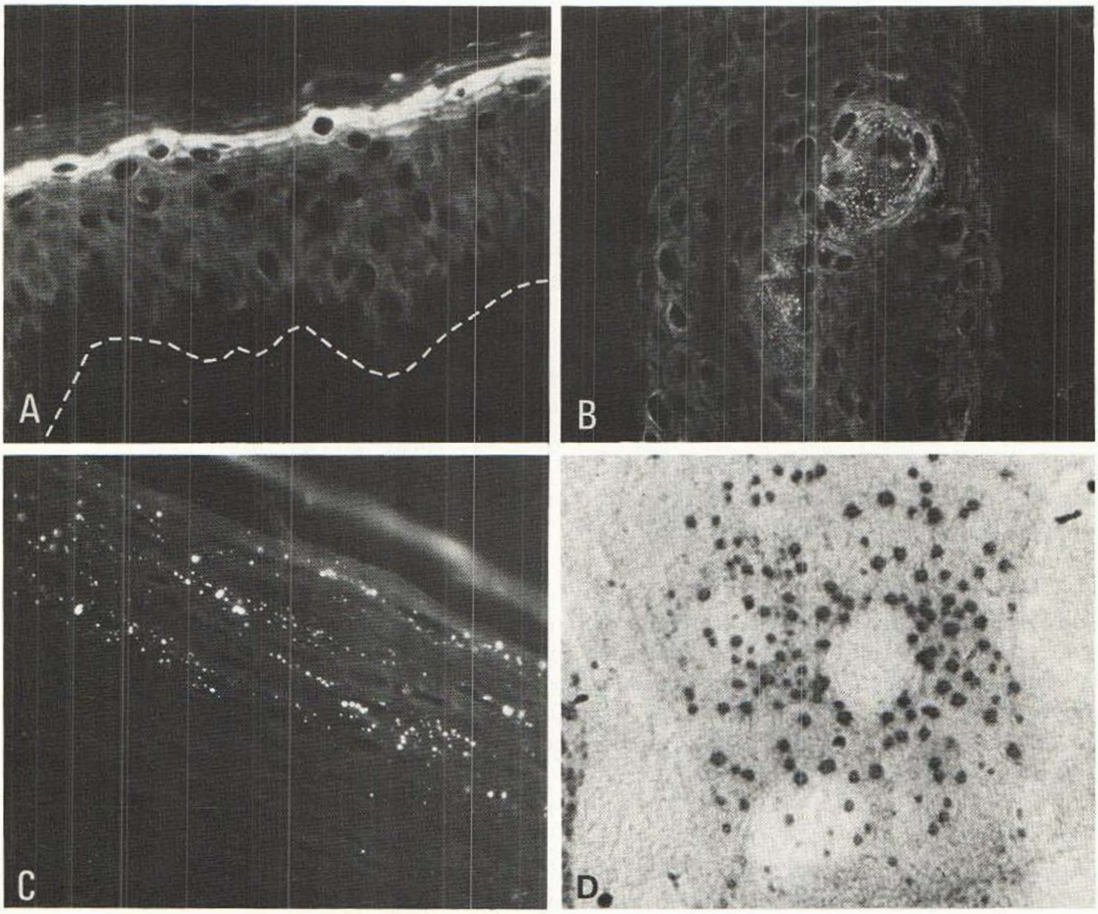

Fig. 1 A. Immunofluorescence of normal human epidermis reacting with GP37 antiserum. Note the intense cytoplasmic staining of the granular layer cells. The basal membrane is marked with a dotted line (×360).

Fig. 1 B. Labelling of the acrosyringium: minute cytoplasmic granules can be observed (×360).

Fig. 1 C. Human buccal mucosa: note the dot-like staining restricted to the upper epithelial layers (×300).

Fig. 1 D. Immunoperoxidase staining of cell cultures grown in delipidized medium. Note the positive staining of perinuclear vacuolar structures (×300).

staining. The upper epidermal layers (corresponding to the granular layer) showed a cytoplasmic staining appearing as small granules. The stratum corneum was also labelled (Fig. 2 B).

Common warts: The staining was weaker and more irregular than that of normal epidermis. Areas of parakeratosis were positive.

Actinic keratosis: A faint staining of suprabasal layers was observed. The parakeratotic horny layer was positive.

Bowen's disease: The labelling was as a rule diminished when compared to normal epidermis. However, single dyskeratotic cells were strongly stained (Fig. 2 C).

Basal cell epitheliomas: the leading feature was a greatly diminished or even absent labelling. In four cases (three of them were of the keratinizing type) circumscribed areas of

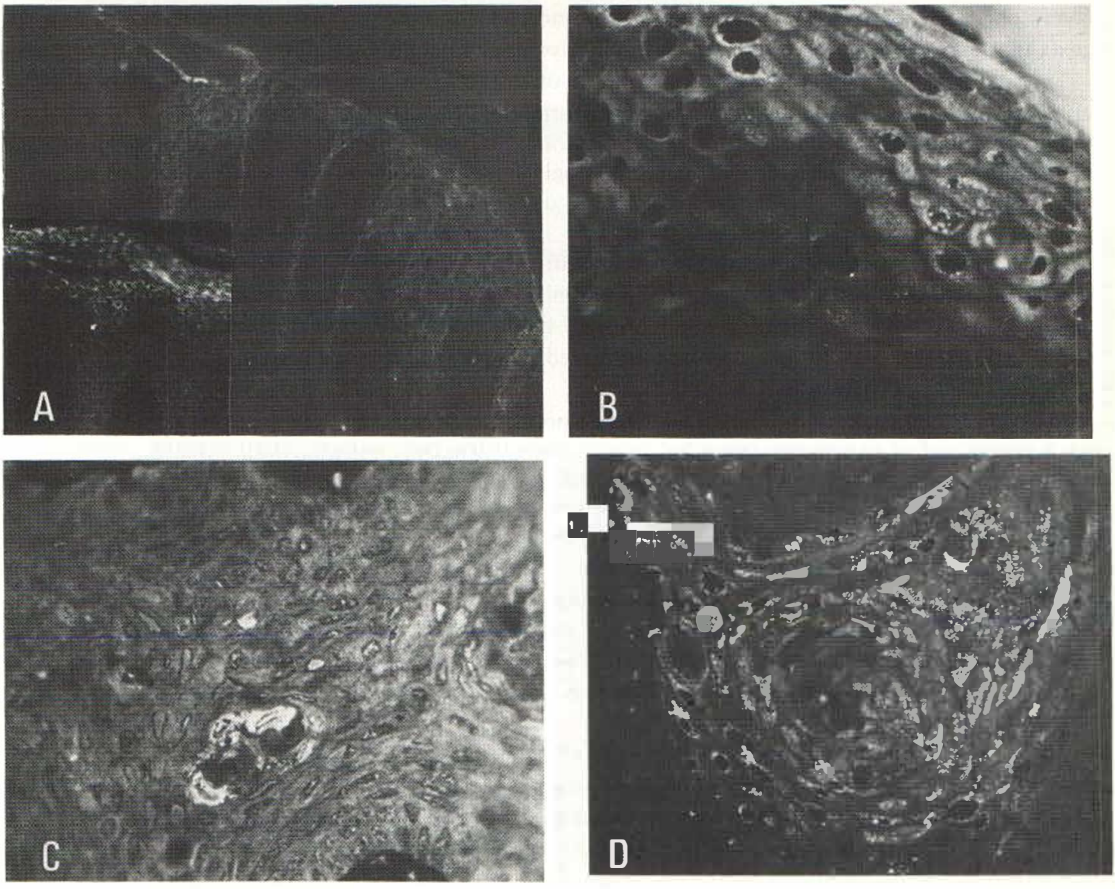

Fig. 2 A. Psoriasis: note the greatly diminished labelling of the epidermis. Inset: a punctate staining of parakeratotic areas is observed (×75).

Fig. 2 B. Lichen planus: an intense, granular staining of the upper epidermal layers is seen (×300).

Fig. 2 C. Bowen's disease: an intense, homogeneous labelling of dyskeratotic cells is observed (×185).

Fig. 2 D. Squamous cell carcinoma: an intense, granular staining of keratinocytes forming a horn pearl is seen (×185).

homogeneous cytoplasmic staining were observed, apparently around areas of horn cyst differentiation.

Squamous cell carcinomas: in tumour islands an irregular pattern of labelling was noticed. In one case, some scattered cells showed an homogeneous cytoplasmic staining while, in the four remaining cases, keratinocytes around numerous horn pearls exhibited a characteristic bright cytoplasmic granular staining (Fig. 2 D).

DISCUSSION

The results of the present study confirm that GP37 is a marker of the granular layer of normal epidemis, as previously suggested (14).

The distinct labelling pattern obtained with GP37 antiserum on the different epidermal

layers corresponds to the compartments of the normal differentiation program and suggests that GP37 may be considered as a marker for epidermal differentiation.

This antigen may be synthesized as a precursor in the lower epidermal layers; in the granular layer, it may undergo its terminal transformation, i.e. glycosylation, giving rise to the highly reactive form. Previous data, demonstrating the expression of this glycoprotein by thymic Hassall's corpuscles, whose morphologic and antigenic similarities with epidermal granular cells are well documented (23–24), underline the specificity and interest of such a marker (14).

In the present study we observed a striking difference between the staining pattern of normal human epidermis and buccal non-keratinizing mucosa, the same findings being visualized on monkey lip. The overall decreased staining observed on buccal mucosa, as compared to normal epidermis, may be interpreted as a residual presence of GP37, whose significance is as yet unknown.

In a simple culture system, epidermal cells do not achieve their complete maturation. In such a system GP37 expression could not be detected. It has been demonstrated that the F.C.S. supplement of cell culture medium contains enough vitamin A to affect the differentiation of cultured epidermal cells (18). By using vitamin A-depleted F.C.S., it is possible to induce a high maturation level, as evidenced by the presence of structural components (desmosomes, keratohyalin granules, cell envelopes) and by the synthesis of the 67 Kd keratin polypeptide specific for terminal keratinocyte differentiation (15, 18). Cultures grown in delipidized medium exhibited a strong labelling of some superficial cells, whereas GP37 expression was prevented by addition of retinyl acetate.

These findings suggest that GP37 expression requires a high degree of differentiation and depends on vitamin A level.

Among the epidermal lesions studied, several findings have to be discussed. The irregular and less discretely compartmentalized staining pattern observed in lichen planus and, to a minor degree, in psoriasis, as compared to normal epidermis, may reflect a lack of normal cellular coordination during differentiation in these epidermal proliferative disorders. The greatly diminished labelling in psoriasis is in agreement with the morphological absence of the granular layer in this disorder. In the same way the strong labelling of upper epidermal layers in lichen planus may be correlated with the hypergranulosis typical of this disease.

The almost negative staining observed in basal cell epitheliomas is not surprising since normal basal cells lack GP37 and does well agree with the generally accepted histogenesis of this tumour.

Noteworthy is the intense GP37 reactivity of squamous nests in squamous cell carcinomas as well as that of dyskeratotic cells in Bowen's disease. The presence of GP37 in these areas correlates with the differentiation of neoplastic keratinocytes and with the presence of other markers of epidermal differentiation, such as involucrin and high-molecular-weight keratins (25–26). These results further support the idea that in human epidermal neoplasms inappropriate or premature terminal differentiation occurs.

Interestingly, two different staining patterns were observed: an homogeneous and a granular one, which may respectively identify a diffuse and an aggregated form of the antigen. The particular staining of the acrosyringium provides further evidence in favour of the biological individuality of acrosyringial keratinocytes when compared to the surrounding epidermis (27–28). The labelling of buccal mucosa has already been discussed. Among skin lesions, the minute granules observed in lichen planus and the more conspicuous ones observed in squamous cell carcinomas may reflect an altered distribution of GP37 corresponding to an altered differentiation process.

The unexpected staining of the horny layer observed in areas of parakeratosis in

different diseases (psoriasis, warts, actinic keratosis) merits to be discussed. A correlation may be postulated between the persistent expression of GP37 and the pathological retention of nuclei in the horny layer. This suggests a participation of GP37 in the degradation of the nucleus in the granular layer in normal epidermis.

In a previous study, the immunoelectronmicroscopic distribution of GP37 in granular cells, visualized as discrete cytoplasmic granules differing from keratoyalin granules, was reported (14). Therefore GP37 could correspond to a lysosomal enzyme present in the granular layer. According to this view GP37 could be involved in the process of nuclear degradation occurring in the granular layer. However this hypothesis needs confirmation.

In conclusion, we feel that GP37, by virtue of its particular distribution in the epidermis and its biochemical nature (glycoprotein), represents an interesting marker of the epidermal differentiation process. As such it may be helpful in the further study of various epidermal inflammatory and proliferative disorders and in the better evaluation of drug influence on keratinocyte maturation in culture.

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