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Cell Membrane Polypeptides on Keratinocytes of Normal and Psoriatic Skin

L. M. DiCicco[1,2], J. E. Fraki[1,2], and J. N. Mansbridge[1]

[1] Department of Dermatology, Stanford University, Stanford, California, USA
[2] Psoriasis Research Institute Palo Alto, California, USA

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The idea that proteins are ubiquitous plasma membrane components and display cell-type-specific patterns stemmed from the classical studies of proteins in erythrocyte and bacterial plasma membranes. These proteins lie on the surface of the plasma membrane or traverse it. Many changes occur in cell surface membrane proteins as a result of infectious and inflammatory processes, malignant transformation and during proliferation and differentiation. As an example of such changes in the epidermis, bullous pemphigoid antigen is expressed by basal cells and pemphigus antiges by maturing keratinocytes (Katz 1984). As keratinocytes from patients with psoriasis appear to follow an alternative pathway to maturation (Mansbridge 1984), it seems possible that protein changes on the plasma membrane would reflect this new pathway.

There have many reports on alterations of the keratinocyte plasma membrane in psoriasis; however, none of these have shown changes that are specific to psoriasis. To study the nature of protein changes we have chosen radioiodination by the lactoperoxidase technique, which labels the surface of viable cells (Morrison 1974), in order to look at the pattern of cell surface proteins of cultured keratinocytes from psoriatic patients and controls.

For preparation of human epidermal keratinocytes, adult facelift skin was placed in 0.9% saline, sliced at 0.3 mm using a Storz Castroviejo keratotome, and the epidermal portions placed overnight in Dulbecco's minimal essential medium (DMEM) supplemented with 200 units/ml penicillin, 200 μg/ml streptomycin and 100 units/ml mycostatin. The skin sections were floated in 0.25% trypsin, 0.1% ethylenediaminetetraacetic acid (EDTA) in Hanks' balanced salt solution (BSS) at 37° C for 30 min. The epidermis was then peeled from the dermis and epidermal cells were dispersed and resuspended in DMEM containing 10% fetal calf serum (FCS) and stored on ice until used. Cultured cells were incubated for approximately 2 weeks on 3T3-fibroblasts monolayers, followed by subculturing onto collagen-coated petri dishes according to the technique of Liu and Parsons (1983). Epidermal cells from psoriasis patients were obtained by punch biopsy and treated in a similar fashion. Following the primary culture period of 2 weeks, all of the cultures from psoriatic epidermis were stored in liquid nitrogen. Approximately 5 days following the subculture of keratinocytes taken from liquid nitrogen onto collagen, at a time when the flask appeared confluent, the cells were incubated for 5 min with 0.25% trypsin and 0.1% EDTA in Hanks' BSS at 37° C, washed with medium containing FCS followed by phosphate-buffered saline (PBS) to remove FCS proteins and resuspended to a concentration of $5 \times 10^6/0.5$ ml in PBS.

Cells were then radioiodinated by the lactoperoxidase technique (Morrison 1974). The viability of cells following iodination was generally 60% – 80% by ethidium bromide staining. Labeled cells were placed on a Ficoll-Hypaque gradient (sp. gr. 1.09 g/cm^3; Pharmacia, Uppsala, Sweden) and centrifuged for 20 min at 5,600 rpm in a Beckman L265B ultracentrifuge (SW 50.1 rotor) at 15° C. Following centrifugation, all material excluding the pellet was removed, diluted 10 times and the cells collected by centrifugation. The cells were then washed twice with extraction buffer A (PBS containing 10 μg/ml RNase and DNase, 1 mM phenyl methane sulfonyl fluoride, 1 mM n-methyl maleimide and 5 mM $MgSO_4$ and

Offprint requests to: Linda M. DiCicco, Department of Oncology (R-71), University of Miami School of Medicine, P.O. Box 016960, Miami, FL 33101, USA

collected by centrifugation at 4°C. We found that we could increase the percentage of viable cells, for example, from 60% to 85% or from 70% to 95% using Ficoll-Hypaque fractionation. However, if the viability prior to Ficoll-Hypaque fractionation was less than 50%, the procedure usually resulted in little improvement. In these experiments only preparations of cells showing at least 90% viability were used. Overall, the recovery of cells following both iodination and Ficoll-Hypaque separation was usually 10% – 30%. It is possible that we are losing a subgroup of keratinocytes; however, by morphological appearance we found both small round and large flat keratinocytes. The importance of selecting only viable cells is that we reduced as far as possible contamination by labeled intracellular proteins.The whole cell preparation was extracted with 1% Triton X-100 in buffer A to obtain triton-soluble proteins, followed by extraction of the pellet after centrifugation with buffer B (10 mM tris, 1% SDS, 1% 2-mercaptoethanol) to extract triton-insoluble proteins. Protein concentration was estimated by the method of Lowry et al. (1951), using bovine serum albumin (BSA) as a standard, and radioactivity by trichloroacetic acid (TCA) precipitation and liquid scintillation spectrometry. The fractions were further analyzed by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli (1970). The gel lanes were equalized for radioactivity. After staining with Coomassie blue, gels were vacuum-dried and developed with X-OMat-AR film plus an enhancing screen at – 70°C.

In some experiments, the lactoperoxidase technique was modified for radiolabeling adherent epidermal cells in vitro before trypsinization (Bystryn and Smalley 1977). Following iodination directly on the tissue culture flask, the adherent cells were successively extracted with 1% Triton X-100 in buffer A followed by buffer B. Thus, both Triton-soluble and triton-insoluble proteins were obtained. A comparison of the two iodination techniques allowed evaluation of the effects of trypsin; however, using this technique on adherent cells resulted in a population of cells that was usually only 60% – 80% viable. As the iodination procedure also labels intracellular proteins in nonviable cells, it is not possible to conclude that the labeled polypeptides are derived solely from plasma membranes.

In determining the effects of trypsin, we compared keratinocytes iodinated as adherent cultures with cells iodinated after trypsinization as described earlier. We observed more protein bands following trypsinization (Fig. 1). We have considered two possible reasons for this. The first is the production of trypsin digestion products of surface proteins and the second is exposure of iodinatable proteins obscured in adherent cultures. The tryptic polypeptide pattern did not appear to depend upon trypsinization conditions and was reproducible from one gel to another. We also obtained the same patterns from cells cultured for 24 h or 7 days after subculturing, indicating the stability of

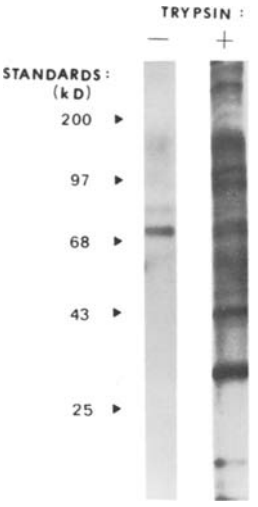

Fig. 1. Effect of trypsin on the yield of iodinated, triton-soluble protein bands on SDS-PAGE. Cultured normal keratinocytes were labeled with ^{125}I by the lactoperoxidase method directly on the flask (*left lane*) or following dispersion by trypsinization (*right lane*). The standard proteins are indicated on the left by *arrows*. One of three similar experiments is shown

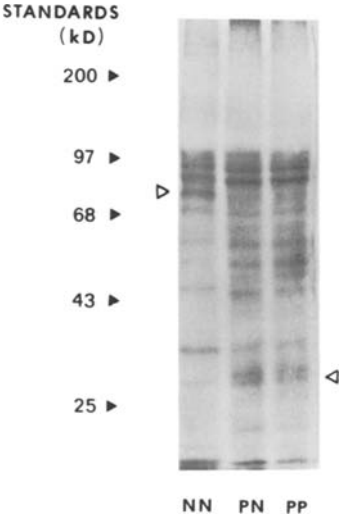

Fig. 2. Comparison of plasma membrane polypeptides from normal and psoriatic keratinocytes. Cultured keratinocytes from normal (*NN*), psoriatic non-lesional (*PN*) and lesional (*PP*) epidermis were harvested with trypsin and iodinated. Triton extractable proteins were separated by SDS-PAGE. ▲ indicates the protein standards; △ indicates the 80 kilodalton (kD) band on the left and the 30 kD band on the right

their protein phenotypes. Furthermore, in our studies, keratinocytes from lesional or non-lesional skin from an individual gave the same pattern (Fig. 2). This technique thus provides cell surface markers by which different individuals can be compared.

A comparison of preparations from different individuals showed a number of apparent polymorphisms. Figure 2 shows a comparison of ^{125}I-labeled polypeptides from keratinocytes from normal and psoriatic individuals. Of note is an extra band at an apparent M_r of 30,000, seen in this gel in the psoriatic sample. However, this band did not occur consistently in preparations from psoriatic individuals. We found it in four of six psoriatic samples and in two of seven normals. In a similar fashion, a band of an apparent M_r of 80,000 was found in six of seven normal samples but was missing or reduced in three of six psoriatic samples.

A possible cause of extra bands on the gel is partial degradation by proteolytic enzymes present in the cultures. We regard this as unlikely, firstly because we include inhibitors of two major classes of keratinocyte proteinases, the serine and the cysteine, in the extraction buffers. However, metallo- and aspartic proteinases may be present, but it is unlikey that the latter are active at the alkaline pH of epidermal cell cultures. Secondly, there is no evidence of differences in the proteinases present in second-passage keratinocytes cultured in vitro from normal or psoriatic skin.

Thus we have shown the existence of markers which are reproducible and characteristic of the individual from which the cells were obtained. The distribution of markers does not depend on whether the keratinocytes were originally obtained from a psoriatic plaque or symptomless skin and, indeed, showed no absolute association with the disease. Establishing increased incidence of a marker in psoriasis caused, for instance, by linkage disequilibrium, would require a far larger survey.

Lactoperoxidase-catalyzed iodination of cultured keratinocytes from involved and uninvolved psoriatic epidermis has not been previously used to investigate cell surface polypeptides. This technique is relatively simple and, when used in conjunction with Ficoll-Hypaque for isolation of viable radioiodinated cells, is superior to most techniques currently available for analysis of cell surface polypeptides of viable cells. One advantage is that small numbers of cells can be assayed ($2-3\times10^6$). In comparison to iodination of adherent cells, this technique allows analysis of whole cell fractions. Zhu et al. (1984) have shown that trypsinization of adherent cells following iodination removes 90% of the label. We and others (Roelfzema and van Erp 1983; Van Beek et al. 1973, 1977) show only a small loss of iodinatable proteins by prior trypsinization and, in fact, more polypeptides are detected following trypsin treatment. However, our experience indicates that the major variant bands of cell surface proteins are related to the individual polymorphism rather than psoriasis.

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