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Localization of immuno-analogues of erythrocyte protein 4.1 and spectrin in epidermis of psoriasis vulgaris

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Abstract The presence and localization of immuno-analogues of human erythrocyte protein 4.1 and spectrin were examined in the epidermis of psoriasis vulgaris. Immunoblot analysis with antibodies against human erythrocyte protein 4.1 revealed that psoriatic epidermis contains a 4.1-like protein of 80 kDa, and also minor immunoreactive polypeptides, including a 45-kDa polypeptide. The 45-kDa band was not detected in non-lesional epidermis. Lesional epidermis of psoriasis contains spectrin-like proteins of 240 kDa. Analysis with immunofluorescence microscopy revealed that 4.1-like proteins were detected mainly in the cytoplasm of the suprabasal cells in lesional epidermis and in the peripheral cytoplasm of the basal cells in non-lesional epidermis. On the other hand, spectrin-like proteins were localized to the peripheral cytoplasm of basal keratinocytes in both lesional and non-lesional psoriatic epidermis. The present results indicate that proteins related to protein 4.1 and spectrin are consistently detected within epidermal cells of psoriasis, a chronic skin disease characterized by epidermal hyperplasia; the expression and distribution of protein 4.1 in lesional epidermis of psoriasis differs from that in non-lesional epidermis. These membrane skeletal proteins may be of significance in the hyperproliferative epidermis of psoriasis.

Introduction

Protein 4.1 and spectrin are major components of the membrane skeleton which laminates the cytoplasmic face of the erythrocyte membrane (Bennett 1985, 1990). The membrane skeleton together with the membrane bilayer is responsible for controlling the shape and structural integrity of mature erythrocytes (Bennett 1985; Elgsaeter et al. 1986). Some roles have been established of protein 4.1 and spectrin in the erythrocyte membrane skeleton. Protein 4.1 functions in stabilizing spectrin binding to actin filaments and in linking the spectrin-actin network to transmembrane glycoproteins (Bennett 1985, 1990). Protein 4.1 binds with high affinity to one or more integral components of the membrane, such as glycophorin C and band 3 (Elliott and Ralston 1984; Danilov et al. 1990). These membrane skeletal proteins were first characterized as components of the erythroid membrane skeleton, but are now known to be expressed in many nonerythroid tissues as well (Burridge et al. 1982; Bennett 1985).

Previously, we have demonstrated the presence of proteins immunologically related to erythrocyte protein 4.1 in human skin and in cultured human epidermal keratinocytes (Shimizu et al. 1991). cDNA clones of protein 4.1 were isolated from a human keratinocyte library (Mutha et al. 1991). Spectrin-like protein (fodrin) in epidermal keratinocytes has also been identified by immunochemical methods (Kaiser et al. 1989; Shimizu et al. 1990; Yoneda et al. 1990). Recently, adducin, which associates with the spectrin-based membrane skeleton (Gardner and Bennett 1987; Bennett et al. 1988), was also reported to be present in epidermis (Kaiser et al. 1993). These studies have led us to suggest that the membrane skeletal lattice of keratinocytes has a similar constitution to that of erythrocytes.

Defects or deficiency in erythrocyte membrane skeletal proteins result in fragile red blood cells and hereditary hemolytic anemia (Tchernia et al. 1981; Agre et al. 1982).These studies indicate that the membrane skeleton is essential for normal survival of erythrocytes in the circulation and that defects in the skeletal proteins may be the basis for disease (Palek and Lambert 1990; Palek and Sahr 1992). Psoriasis, a chronic skin disease of widespread occurrence, is characterized by epidermal hyperplasia of unknown etiology and pathogenesis. Lesional

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epidermis shows a rapid turnover of keratinocytes and a defect in keratinization. Whether or not the epidermis is the site that is primarily affected in psoriasis cannot be ascertained; however it is unquestionable that epidermal symptoms are very precocious during the development of a skin lesion (Parent et al. 1990). Since psoriasis may reflect cell membrane modifications not only in skin but also in erythrocytes (Kumar et al. 1983; Peserico et al. 1988; Semplicini et al. 1988), we sought to elucidate the presence and localization of immuno-analogues of protein 4.1 and spectrin within keratinocytes of lesional psoriatic epidermis. We further compared the distribution of 4.1-like and spectrin-like proteins between lesional epidermis and non-lesional epidermis.

Materials and methods

Preparation of antibodies against protein 4.1 and spectrin

Protein 4.1 and spectrin were purified from human erythrocytes according to the method of Tyler et al. (1979, 1980). Affinity-purified polyclonal antibodies against spectrin and protein 4.1 were prepared as described previously (Shimizu et al. 1990, 1991).

Preparation of tissues

Skin biopsies were obtained from lesional, perilesional and non-lesional skin of five patients with psoariasis vulgaris (age 20–76 years, mean age 52.6 years). Psoriasis patients had not received systemic therapy or topical treatment at the biopsy sites for at least 10 days. For immunoblotting, skin slices were treated with 1000 U/ml of dispase in RPMI 1640 medium (Gibco) for 30 min at 37° C, and the pure epidermal sheets were peeled off with sharp forceps. Epidermal sheets were then homogenized in a solution of 10 m*M* phosphate-buffered saline (10 m*M* $NaHPO_4$, 150 m*M* NaCl; PBS) containing 5 m*M* EDTA, 5 m*M* diisopropylfluorophosphate (DFP), and 20 μg/ml of leupeptin, pH 7.4. In a related experiment, epidermal sheets of non-lesional psoriasis were homogenized in PBS without protease inhibitors and incubated overnight at 4° C. For immunofluorescence microscopy, skin slices were snap-frozen in liquid nitrogen, embedded in OCT compound (Tissue Tek; Miles, Napesville, Mich., USA) and stored at –80° C until use. White ghosts of erythrocytes were prepared by lysing and washing human erythrocytes with a hypotonic buffer of 5 m*M* $NaHPO_4$, 1 m*M* EDTA, and 1 m*M* DFP, pH 7.4 until membranes became white.

Immunoblot analysis

The samples of total human erythrocyte membranes (white ghosts), homogenates of lesional psoriatic epidermis, or non-lesional psoriatic epidermis were treated with four volumes of SDS-reducing buffer (60 m*M* TRIS/HCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 0.025% bromophenol blue), and subjected to SDS-polyacrylamide gel electrophoresis (PAGE; 10%), as described by Laemmli (1970). Proteins on the gel were transferred electrophoretically to a nitrocellulose membrane by the procedure of Towbin et al. (1979). The membrane was first incubated with rabbit anti-protein 4.1 antibodies or anti-spectrin antibodies. After washing, the membrane was incubated with peroxidase-conjugated goat anti-rabbit IgG. Positive bands were visualized by the 4-chloro-1-naphthol reaction.

Immunofluorescence study

Skin sections of psoriasis (4 μm in thickness) mounted on slides were air-dried and fixed in –20° C cold acetone. Samples of skin sections were first exposed to anti-protein 4.1 antibodies, anti-spectrin antibodies, or non-immunized rabbit IgG by incubation for 1 h. After rinsing in PBS, samples were incubated in fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG for 30 min. A fluorescence microscope (Nikon Tokyo, Japan) was used for immunohistochemical observation.

Results

Immunoblot analysis

Immunoblot analysis with affinity-purified antibodies against human erythrocyte protein 4.1 is shown in Fig. 1. Protein 4.1a of 80 kDa and 4.1b of 78 kDa, as well as minor immunoreactive polypeptides were detected when total erythrocyte membrane proteins (white ghosts) were analyzed (Fig. 1, lane 1). These polypeptides were previously shown to be protein 4.1 sequence-related proteins (Goodman et al. 1984). Immunoblotting of non-lesional epidermis of psoriasis revealed an 80-kDa 4.1-like protein as well as minor immunoreactive polypeptides (Fig. 1, lane 2). These proteins were also observed in lesional epidermis of psoriasis (Fig. 1, lane 3). The results of the immunoblotting corresponded to the previous analysis of normal human epidermis (Shimizu et al. 1991). Interestingly, in psoriatic epidermis, strong staining of 45 kDa polypeptides was observed, which was not detected in non-lesional epidermis of psoriasis. Immunoblot analysis of non-lesional epidermis without protease inhibitors revealed an increase in numbers of various low molecular weight polypeptides. However, the 45-kDa polypeptide was not conspicuous by this treatment (Fig. 1, lane 4).

Affinity purified erythrocyte spectrin antibody reacted with spectrin dimers of 240 kDa and 220 kDa (Fig. 1, lane 5). Non-lesional epidermis of psoriasis was positive for the polypeptide of 240 kDa (Fig. 1, lane 6). We also detected the polypeptide of spectrin-like protein of 240 kDa in lesional epidermis of psoriasis (Fig. 1, lane 7). Low molecular weight polypeptide of 150 kDa was also observed. This 150-kDa polypeptide was sometimes observed when the immunoblotting was performed with non-lesional epidermis of psoriasis (data not shown). We therefore examined whether the 150-kDa polypeptide was a proteolytic product of the keratinocyte spectrin of 240 kDa. Proteolytic treatment clearly showed the 150-kDa polypeptide in non-lesional epidermis of psoriasis (Fig. 1, lane 8).

Analysis by immunofluorescence microscopy

To examine the localization of 4.1-like protein(s) in psoriatic epidermis, we utilized immunofluorescence microscopy with anti-protein 4.1 antibodies. Non-lesional epidermis showed strong positive staining in the periphery of the basal cells and moderate staining of spinous cells (Fig. 2a). This result is consistent with the localization of 4.1-like proteins in normal human epidermis (Shimizu et al. 1991). In contrast to non-lesional epidermis, spinous

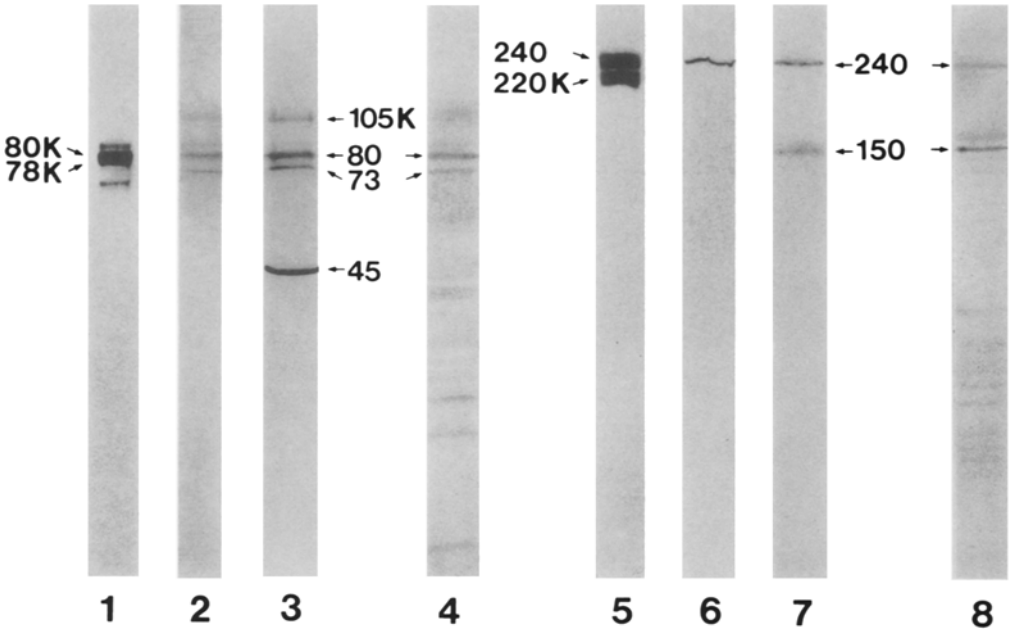

Fig. 1 SDS-polyacrylamide gel electrophoresis (PAGE; 10%) was carried out with total erythrocyte membranes, non-lesional psoriatic epidermis, and lesional psoriatic epidermis. Immunoblotting of erythrocyte membranes (*lanes 1, 5*), non-lesional psoriatic epidermis (with protease inhibitors; *lanes 2, 6*), lesional psoriatic epidermis (*lanes 3, 7*) and non-lesional psoriatic epidermis (incubated overnight at 4° C without protease inhibitors; *lanes 4, 8*) was carried out using rabbit anti-erythrocyte protein 4.1 antibodies (*lanes 1-4*) or rabbit anti-erythrocyte spectrin antibodies (*lanes 5–8*). Protein molecular weights are given in kDa

cells of lesional epidermis of psoriatic skin were intensely stained (Fig. 2c). Staining was strong in the cytoplasm, around the nuclear space; lesional basal cells were weakly stained. In the border areas between lesional and non-lesional skin, there was transitional staining; intense cytoplasmic staining in the middle of the epidermis (arrows) and peripheral staining of the basal cells (Fig. 2b).

Immunofluorescence analysis with anti-spectrin antibodies showed strong positive staining in the periphery of the basal cells in non-lesional epidermis and weak staining of the spinous cells (Fig. 3a). Psoriatic lesional epidermis also displayed intense peripheral staining of the basal cells, and moderate staining of the spinous cells (Fig. 3c). Border areas between the lesional and non-lesional skin showed strong staining not only in the basal layer, but also in several rows of suprabasal cells (Fig. 3b). In contrast to anti-spectrin or anti-protein 4.1 antibodies, non-immunized IgG showed no immunoreaction (data not shown).

Discussion

In the present study, we have demonstrated the presence of proteins immunologically related to erythrocyte protein 4.1 and spectrin in psoriatic epidermis. Lesional and non-lesional psoriatic epidermis were found to contain a 4.1-like protein of 80 kDa and also minor immunoreactive polypeptides of 4.1 related proteins (Goodman et al. 1984). However, a major polypeptide of 45 kDa was found only in lesional psoriatic epidermis. The result of our experiment on proteolytic breakdown of the protein supports the idea that this 45-kDa polypeptide is unlikely to be a proteolytic product. It has been reported that the major protein 4.1 isoform in human erythrocytes is an 80-kDa doublet. However, avian erythrocytes and mammalian nonerythroid tissues exhibit a remarkably diverse collection of immunoreactive 4.1 polypeptides ranging in size from 30 to 210 kDa (Granger and Lazarides 1984, 1985; Anderson et al. 1988). Abnormal erythroblasts, such as Friend erythroleukemia cells, have been shown to contain a protein 4.1-doublet and two minor bands of 105 kDa and 43 kDa that cross-react with anti-human protein 4.1 IgG (Benabdallah et al. 1991). Furthermore, abnormal expression of protein 4.1 in teratospermia (abnormal spermatozoa) has been reported (Rousseaux-Prévost et al. 1994).

Recently, Conboy et al. (1991) claimed that splicing switches may account for the considerable development-specific and tissue-specific heterogeneity in protein 4.1 expression by demonstrating the abundance of alternative splicing events in erythroid 4.1 mRNAs. It is interesting to note that immunofluorescence studies revealed a pronounced difference in protein 4.1 staining in the lesional epidermis of psoriatic skin compared to non-lesional epidermis. The prominent cytoplasmic staining of suprabasal cells suggests that the suprabasal epidermal cells in psoriasis might be abnormal in their ability to regulate the expression of the protein 4.1. The decreased fluorescence intensity in basal cells might be caused by preferential staining of spinous cells containing the 45-kDa isoform. Therefore, 80 kDa 4.1-like protein was considered to be expressed relatively weakly in the basal cell layer.

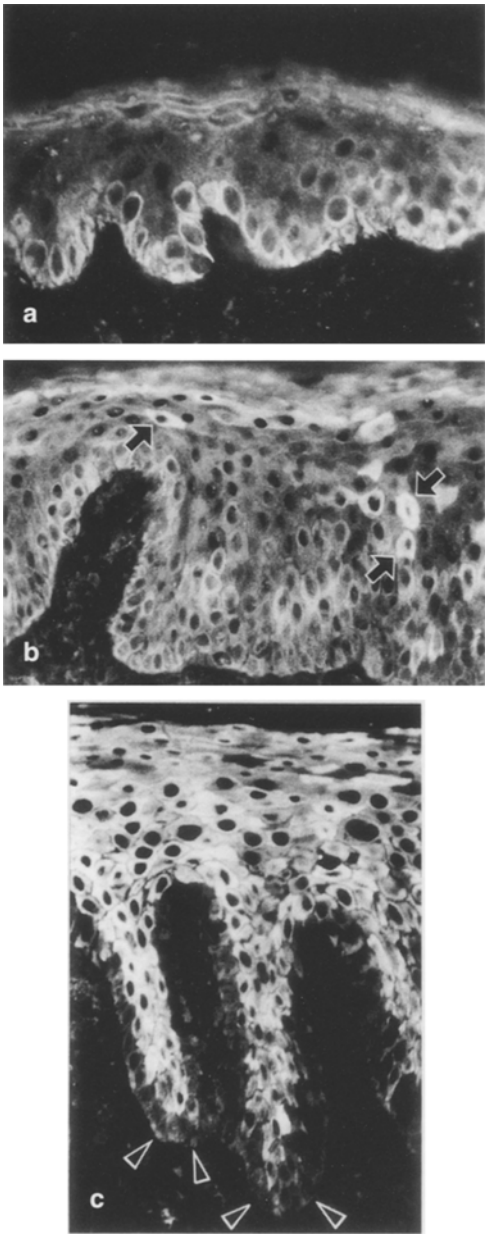

Fig. 2 Distribution of immunoreactive protein 4.1 in non-lesional epidermis (**a**), the border areas between the lesional and non-lesional epidermis (**b**), and lesional epidermis of psoriatic skin (**c**). Sections of psoriatic skin were subjected to the immunolocalization procedure as described in the Materials and methods using anti-protein 4.1 antibody. *Arrows* indicate intense cytoplasmic staining of spinous cells (**b**). *Arrowheads* show the basal membrane (**c**). **a** ×160; **b**, **c** ×100

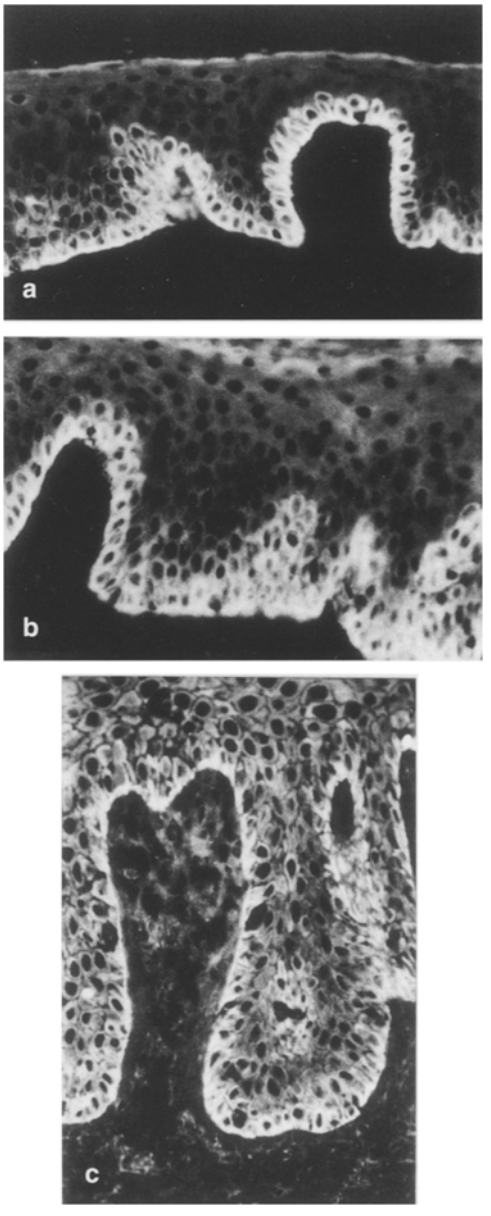

Fig. 3 Distribution of immunoreactive spectrin in non-lesional epidermis (**a**), the border areas between the lesional and non-lesional epidermis (**b**), and lesional epidermis of psoriatic skin (**c**). Sections of psoriatic skin were subjected to the immunolocalization procedure as described in the Materials and methods using anti-spectrin antibody. **a** ×160; **b**, **c** ×100

Immunoblot analysis revealed a 240-kDa spectrin-like protein in the epidermis of lesional psoriasis as well as in non-lesional psoriasis. We also detected a low molecular weight polypeptide of 150 kDa, which presumably represents a degradation product of the 240-kDa protein. The finding of a 150-kDa polypeptide has been confirmed by the results of spectrin analysis of non-lesional epidermis. The activities of several kinds of proteases are reported to be increased in lesional epidermis (Dubertret et al. 1982). Brain spectrin (fodrin) is sheared to generate proteolytic fragments of 155 kDa by proteases, mainly calcium-dependent proteases (calpains; Siman et al. 1984). Furthermore, the 150-kDa breakdown product of spectrin was also observed in erythrocytes (Repasky et al. 1982) and lenses (Truscott et al. 1989).

The distribution of spectrin-like proteins in psoriatic lesional epidermis was a little different from that of non-lesional epidermis of psoriasis and normal epidermis (Shimizu et al. 1990). Compared to weak staining of spinous cells in non-lesional epidermis, there was moderate

staining of the spinous cells in lesional epidermis. Previously, Kariniemi et al. (1984) reported the localization of spectrin-like proteins in psoriatic epidermis, using antibodies against a 230-kDa polypeptide isolated from calf lens, which cross-react witzh erythroid spectrin. Similar staining was seen in lesional and non-lesional psoriatic epidermis in their immunochemical study. The results of our immunohistochemical observation are different from those reported by Kaiser et al. (1993). These investigators showed that all layers of epidermis were strongly stained, whereas spectrin-like proteins were localized preferentially in the layer in our study. The different staining pattern may be due to differences in the source of antigen: we used human erythrocyte spectrin, whereas Kaiser et al. (1993) used pig brain as the source.

Since protein 4.1 is known to be one of the membrane skeleton components which accumulate during erythropoiesis, it has been suggested that protein 4.1 functions to stabilize the membrane skeleton during the final stages of erythroid terminal differentiation (Staufenbiel and Lazarides 1986). The 4.1-like protein(s) and the spectrin-like proteins (fodrin) in keratinocytes may play a similar function to that in erythrocytes. One possible function for these membrane skeletal proteins in keratinocytes might be the stabilization of newly synthesized cell membranes before elaboration of cell-cell interdigitations, as was suggested previously (Shimizu et al. 1991). However, the interactions of 4.1-like proteins with integral component(s) of membrane in keratinocytes remain to be elucidated. Enhanced suprabasal cytoplasmic expression was demonstrated of 45 kDa protein in psoriatic keratinocytes. The results suggests that this low molecular weight polypeptide might lack the ability to bind to the integral components of the membrane due to abnormal mRNA processing of protein 4.1 in the hyperproliferative keratinocytes of psoriasis.

Psoriasis is a chronic skin disease of unknown etiology. This disease of cell proliferation and differentiation has been recently correlated with altered expression of several cytokines, of which some are synthesized and secreted by keratinocytes themselves, and playing a key role in the regulation of the keratinocyte function (Krueger et al. 1990). Increased epidermal cell proliferation may also be explained as an initial decreased adhesiveness of keratinocytes, which in turn leads to loss of controlled cell proliferation and alteration of cell signaling (Fisher et al. 1990). Altered phosphoinositide-mediated signal transduction (for example phospholipase C activity is elevated and protein kinase C activity is decreased or down-regulated) is likely to be one of the major factors in the misregulation of epidermal-cell homeostasis seen in psoriasis. Although the physiological significance of protein phosphorylation in the regulation of cytoskeletal protein interactions in erythrocytes, remains uncertain, there is evidence suggesting that phosphorylation of membrane proteins may contribute to the deformability and mechanical strength of erythrocytes (Yuthavong and Limpaiboon 1987; Cohen and Gascard 1992). We must therefore consider the possibility that keratinocyte membrane protein phosphorylation affects the expression and distribution of 4.1-like and spectrin-like proteins in the epidermis. Further study is required of phosphorylation of the membrane skeletal proteins in the keratinocytes of normal and psoriasis.

In conclusion, 4.1-like and spectrin-like proteins were consistently detected within keratinocytes of lesional psoriatic epidermis. Our present observations of differential expression and distribution of these membrane skeletal proteins in lesional epidermis of psoriasis indicate that alterations of these proteins may be important in the pathogenesis of psoriasis.

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