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INCREASED MEMBRANE-ASSOCIATED TRANSGLUTAMINASE ACTIVITY IN PSORIASIS

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SUMMARY: Terminal differentiation of human skin involves the formation of an insoluble cross-linked protein envelope (CLE), which functions as an external barrier. To characterize terminal differentiation in the skin disease psoriasis, we have measured 1) membrane-associated transglutaminase (mTGase) activity, the rate limiting enzyme in the formation of CLE, and 2) the number of CLE in biopsies from normal and psoriatic skin. mTGase activity was increased 5-fold ($p<0.0001$) in psoriatic versus normal skin. Kinetic analysis revealed that the increased activity was due to an elevation in the V_{max} of the enzyme. In addition, the number of CLE was 10-fold greater in psoriatic compared to normal skin. The increase in mTGase and CLE in psoriasis is in contrast to the decrease in other markers of terminal differentiation in skin, such as synthesis of specific intermediate filaments, observed in this disease.

The upper compartment of human skin, the epidermis, is composed of 10-12 cell layers. The predominant cell type in the epidermis is the keratinocyte, which undergoes maturation as it migrates upward through the epidermis from the lower proliferative cell layer (basal layer) to the upper terminally differentiated cell layer (cornified layer). A prominent feature of keratinocyte terminal differentiation is the formation of a cross-linked protein envelope (CLE), which lies beneath the cell surface, and provides an insoluble barrier of protection from the external environment. Formation of the CLE in keratinocytes is catalyzed by membrane-associated transglutaminase (mTGase), which covalently cross-links polypeptide chains with ϵ-(γ-glutamyl)lysine-bonds (1). The primary substrates for this enzyme in humans is believed to be loricrin (2), involucrin (3) and keratolinin (4). Epidermal transglutaminase activity has been shown, with histochemical (5,6,7) and monoclonal antibody (8,9) staining techniques, to be confined almost exclusively to the upper, more differentiated layer of normal human skin and therefore serves as a marker of epidermal terminal differentiation (10). Keratinocytes and most other cell types also contain soluble transglutaminase activity. This enzyme is distinct from epidermal

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ABBREVIATIONS: CLE, Cross-linked envelope; EDTA, ethylenediamine tetraacetic acid; mTGase, membrane-associated transglutaminase; SDS, sodium dodecylsulfate; TCA, trichloracetic acid; Tris, Tris(hydroxymethyl) aminomethane; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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transglutaminase and does not participate in the formation of CLE (10). No specific function for soluble transglutaminase has yet been established.

Psoriasis is a common skin disease characterized by keratinocyte hyperproliferation and defective differentiation. mTGase and involucrin, appear by immunocytochemistry to be aberrantly distributed throughout most cell layers in psoriatic skin (6,9,11). In contrast, other markers of epidermal terminal differentiation, such as certain specific intermediate filaments (keratins) are decreased in psoriatic epidermis (12,13). Thus, the normal programmed sequence for keratinocyte maturation is altered in psoriasis.

Based on its diffuse staining pattern, it has been suggested that mTGase activity may actually be increased in psoriatic epidermis (9,11). Direct biochemical evidence for this, however, is lacking. To address this issue we have biochemically quantified soluble and membrane-associated transglutaminase activities and the number of CLE, the product of membrane-associated transglutaminase activity, in biopsies of normal (i.e. without disease) and psoriatic skin.

MATERIALS AND METHODS

Materials. Trizma-base, EDTA, dithiothreitol, casein and Triton X-100 were obtained from Sigma Chemical Co. (St. Louis, MO). Protosol and [2,3-^{3}H]putrescine (30-40 mCi/mmol) were from New England Nuclear (Boston, MA). All other chemicals were of at least reagent grade.

Epidermal samples and preparation of transglutaminase. Samples of human epidermis were obtained from 1) active psoriatic lesions **(psoriatic involved)**, 2) the normal appearing skin of persons with psoriasis **(psoriatic uninvolved)** and 3) healthy skin from persons with no history of any skin disease **(normal)**. The biopsies were taken from the lower back and buttocks with an electric keratome. The psoriatic patients received no medication or topical treatment 2 weeks prior to biopsy. Psoriatic involved skin was keratomed at a depth of 0.4mm, while normal and uninvolved skin was keratomed at 0.2mm. All subjects provided informed consent and all procedures were conducted with the approval of the University of Michigan Institutional Review Board. Epidermis was ground in liquid nitrogen with a mortar and pestle and the resulting powder suspended in 10mM Tris, 10mM dithiothreitol, 0.5mM EDTA, (pH 7.4) and further homogenized in a glass homogenizer. Samples were centrifuged at 150,000 x g for 40 minutes at 4°C. The supernatant was decanted and kept on ice. The pellet was resuspended in the homogenizing buffer containing 1% Triton X-100 and incubated for 5 minutes at 37°C to solubilize the membrane-associated transglutaminase. The samples were centrifuged as above to obtain a solubilized fraction.

Measurement of Transglutaminase. Transglutaminase was measured by a modification of the method of Yuspa et al. (14) Typical reactions contained 20μl of sample, 20μl of 500mM sodium borate (pH 9.5), 10μl of 100mM $CaCl_2$, 10μl of 10mM EDTA (pH 7.5), 20μl of casein (20mg/ml), 10μl of [2,3-^{3}H]putrescine and 110μl of distilled water to a final volume of 200μl. Reactions were conducted at 37°C for 15 minutes and terminated by addition of 1ml of ice cold 10% TCA containing 1% putrescine. Precipitated protein was collected on Whatman GF/C glass fiber filters. The filters were rinsed with 10ml 10% TCA containing 1% putrescine, dissolved in 0.5 ml Protosol and counted in 10ml scintillation fluid. For the kinetic studies the [^{3}H]putrescine content in the reaction mixture was increased to 30μl and unlabelled putrescine (0.025 - 1mM) was added.

Quantitation of cross-linked envelopes. Paired 4mm diameter punch biopsies from uninvolved and involved skin were obtained from 6 psoriatic individuals for measurement of cross-linked envelopes. Biopsies were boiled for 15-30 minutes in 6M urea, 1.0% SDS and 5mM β-mercaptoethanol. Envelopes were visualized in a dark field microscope and counted in a hemocytometer.

Miscellaneous assays. Protein was measured by the method of Bradford with bovine gammaglobulin as standard (15). DNA was assayed by the method of Burton (16).

Statistical analysis. Differences in group means of transglutaminase activity in normal, uninvolved and involved psoriatic skin were analyzed by Scheffe's method of multiple comparisons. Analysis of the number of cross-linked envelopes in uninvolved and involved psoriatic epidermis was by pairwise t-test. A 2 tailed hypothesis was employed to interpret the data.

RESULTS AND DISCUSSION: mTGase in Normal and Psoriatic Skin- The time course of mTGase activity in paired biopsies of psoriatic involved and uninvolved skin from two individuals is shown in Figure 1A. Activity from both types of skin was nearly linear over 30 minutes. Psoriatic involved skin, from both individuals, contained approximately 3 fold more activity than uninvolved skin at all time points examined. The increased mTGase activity in psoriatic involved skin, compared to uninvolved skin, was also observed over a wide range of sample concentrations (Fig. 1B). These data suggest that mTGase activity is elevated in psoriatic involved skin compared to uninvolved skin. Furthermore, this apparent elevation is not due to differences, in time or sample concentration dependence, of mTGase from involved and uninvolved psoriatic skin.

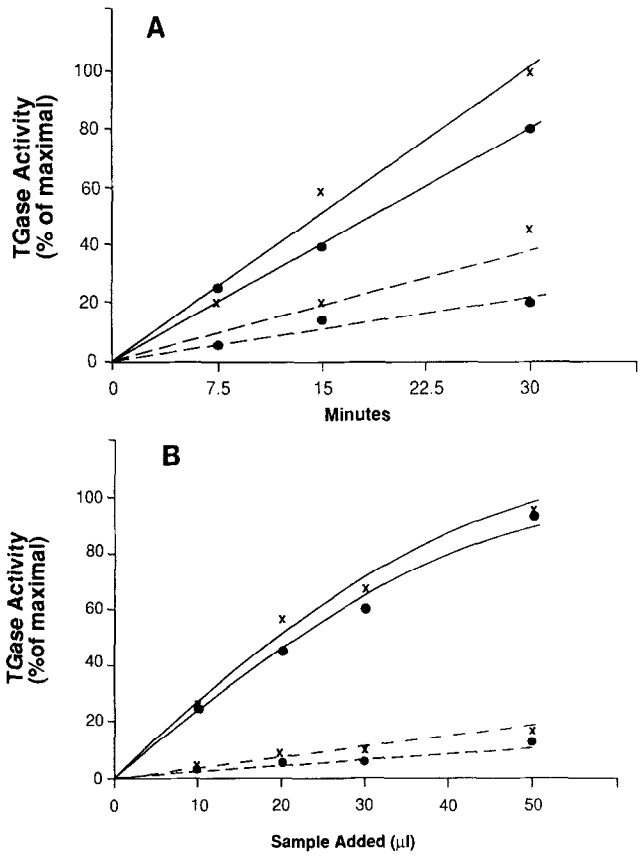

Figure 1. Time course and enzyme dependence of membrane-associated transglutaminase in uninvolved and involved psoriatic skin. (A) Membrane-associated transglutaminase was prepared from keratome biopsies of uninvolved and involved psoriatic skin from two individuals. Transglutaminase was assayed for the indicated times as described in "Methods". (●) patient 1 and (x) patient 2. Solid lines, involved skin; dashed lines, uninvolved skin. (B) The indicated amounts of the membrane-associated transglutaminase preparations described in A were assayed. Symbols the same as in A.

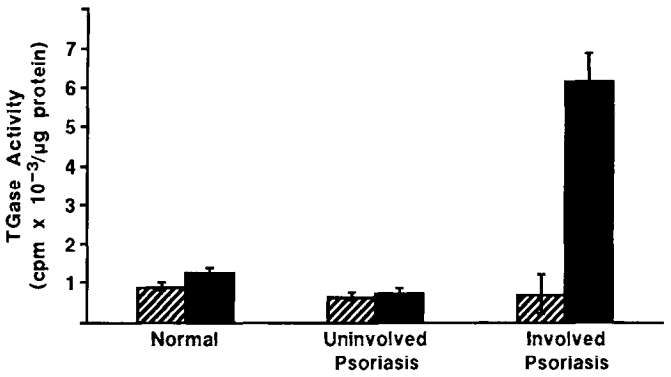

Figure 2. Increased membrane-associated transglutaminase activity in psoriatic involved versus uninvolved and normal skin. Soluble and membrane-associated transglutaminase activities were determined in biopsies from normal (n=11), psoriatic uninvolved (n=11) and psoriatic involved (n=10) skin. ▨, soluble transglutaminase, ■ membrane-associated transglutaminase. * Involved vs. uninvolved or normal $p<0.0001$.

We next quantified soluble and membrane-associated transglutaminase activity in 11 normal, 11 psoriatic uninvolved and 10 psoriatic involved skin biopsies. mTGase activity, was increased approximately five fold in psoriatic involved epidermis compared to uninvolved and normal epidermis ($p<0.0001$) (Fig. 2). There was no difference in mTGase activity between normal and psoriatic uninvolved skin. These quantitative biochemical data are consistent with qualitative histochemical and immunocytochemical studies indicating a more diffuse staining of transglutaminase throughout psoriatic involved skin, compared to normal skin. Soluble transglutaminase activity, which is a distinct enzyme from mTGase (8,17), did not differ among normal, psoriatic involved, or psoriatic uninvolved skin, indicating that the observed elevation in mTGase was specific.

Under the conditions employed to assay mTGase activity, the putrescine concentration (1μM) is well below the reported K_m (50μM) of the enzyme from cultured murine keratinocytes (17). The observed increase in mTGase activity of psoriatic involved skin could therefore reflect an elevated V_{max}, decreased K_m for putrescine or both, compared to mTGase activity in uninvolved skin. We therefore assayed mTGase activity from paired samples of involved and uninvolved psoriatic skin over a wide range of putrescine concentrations (Fig. 3A). A Lineweaver-Burk plot of the data (fig. 3B) indicated that the V_{max} was elevated 4-fold (25.6 vs 6.2 nmol/min/mg prot.) and the K_m for putrescine was increased 2-fold (0.116 vs 0.052 mM) in involved compared to uninvolved psoriatic skin.

These data are consistent with there being a greater amount of mTGase in involved epidermis, although other explanations are possible. The K_m for putrescine of mTGase in psoriatic uninvolved skin is similar to that for in cultured murine keratinocytes (17). The finding of an increased K_m for putrescine of mTGase in involved versus uninvolved psoriatic skin suggests that the regulation of the enzyme may be altered in psoriasis. It must be remembered, however, that putrescine is not the physiological substrate.

Cross-Linked Envelopes in Psoriatic Skin- In vivo, membrane-associated transglutaminase catalyzes formation of CLE; therefore, if the demonstrated increase in transglutaminase activity

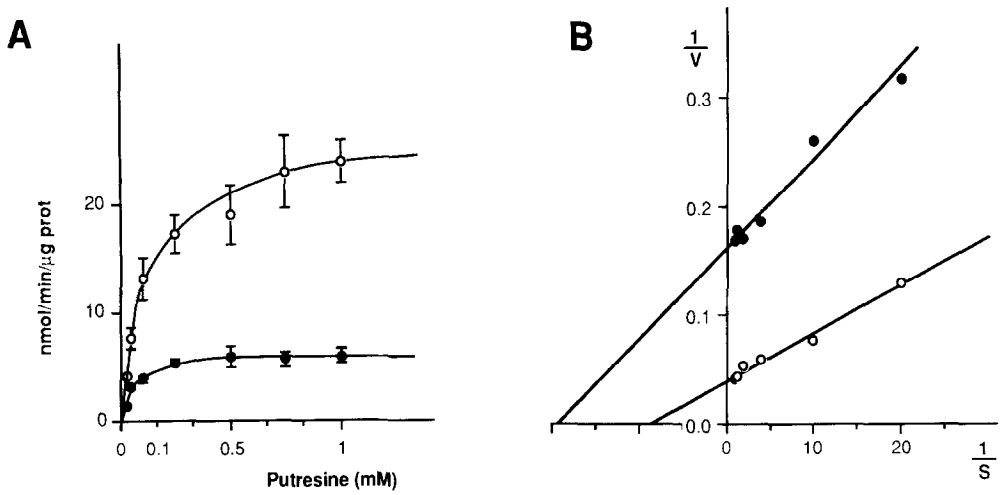

Figure 3. Putrescine dependence of membrane-associated transglutaminase activity in uninvolved and involved psoriatic skin. (A) Activity was assayed as described in "Methods" with the exception that unlabelled putrescine was added to give the final indicated concentrations. (●) uninvolved psoriatic skin (n=4), (o) involved psoriatic skin (n=4). (B) Lineweaver-Burk plot of the data in fig. 3A. V_{max} and K_m were calculated by linear regression. V, velocity, S, putrescine concentration, other symbols as in 3A.

is functionally active in vivo, one would expect increased CLE in psoriatic involved skin. To examine this, we determined the number of CLE in paired biopsies of involved and uninvolved skin from 6 individuals. The number of CLE in psoriatic involved skin was found to be approximately 10 fold greater than in uninvolved skin ($p<0.0001$) (fig. 4).

Recent evidence by Michel et al. (18) indicates that the protein composition of CLE in psoriatic involved skin differs from that of normal skin. This difference may in part be due to the observed increased mTGase activity in psoriatic skin, which may promote the cross-linking of proteins not usually incorporated into CLE.

The above results demonstrate that terminal differentiation, as defined by increased mTGase activity and CLE formation is increased in psoriatic involved skin. The increase in these

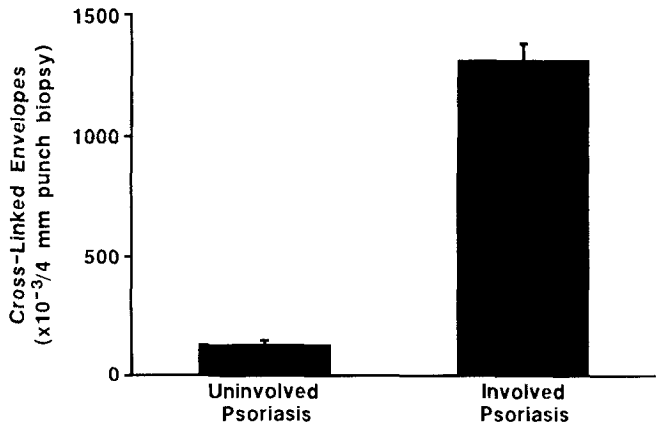

Figure 4. Cross-linked envelopes in involved and uninvolved psoriatic skin. Paired 4 mm diameter punch biopsies were obtained from 6 individuals and the number of cross-linked envelopes determined as described in "Methods". *Involved vs. uninvolved, $p<0.0001$.

two markers of terminal differentiation is in contrast to previous findings of decreased accumulation of terminal differentiation specific intermediate filaments K1, K10 and K11 (13) in psoriatic skin. The finding that two prominent features of terminal differentiation, CLE formation and keratin synthesis, are differentially expressed in psoriatic involved skin suggests that these processes are independently regulated.

In cultured human (19, 20, 21) and murine keratinocytes (22) activators of protein kinase C, such as TPA, 1,2-diacylglycerol and phospholipase C, induce mTGase activity and stimulate formation of CLE. We have previously reported that phospholipase C and 1,2-diacylglycerol are elevated in psoriatic skin and have suggested that this may lead to chronic activation and concomitant down regulation of protein kinase C (23,24). We propose therefore that the increased mTGase activity in psoriatic skin may result from activation of protein kinase C. The molecular mechanism(s) by which activation of protein kinase C leads to increased mTGase activity and CLE formation remains to the determined.

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