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Secretion of Urokinase and Tissue-plasminogen Activator by Epidermal Cells in the Presence of Psoriatic Fibroblast-conditioned Medium

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The present study examined secretion of urokinase and tissue-plasminogen activator by epidermal cells in the presence of psoriatic or uninvolved skin fibroblast-conditioned medium. Using zymographic analyses, a 54kD lysis band and a small 110kD band derived from urokinase could be detected in the harvest fluid from keratinocytes treated with both psoriatic and uninvolved fibroblast-conditioned medium, as well as very weak lysis bands of 63kD and 120kD derived from tissue-plasminogen activator in the harvest fluid treated with psoriatic fibroblast-conditioned medium, but not with uninvolved fibroblast-conditioned medium. ***Key words: Psoriasis; Keratinocytes.***

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Since Fräki et al. (1) demonstrated that epidermal plasminogen activator (PA) activity is correlated with psoriasis activity, considerable attention has been directed to PAs, which convert plasminogen into the proteolytic enzyme plasmin (2). A previous report (3) indicated that psoriatic fibroblasts (FBs) induce hyperproliferative activity in normal keratinocytes (KCs). However, there is little understanding of the relationship between the epidermal PA activities and the role of FBs in psoriasis. The present study was conducted to examine the secretion of urokinase and tissue-plasminogen activator by epidermal cells in the presence of psoriatic and uninvolved skin FB-conditioned medium.

MATERIALS AND METHODS

Fibroblast culture

Skin FBs from an explant culture (slices of epidermis including the upper dermis cut into 2 mm cubes) of involved skin from 3 adult patients (P1, P2, P3) with psoriasis (plaque lesions) and the uninvolved skin of two (N2, N3) of these patients were grown in Eagle's minimal essential medium (MEM), supplemented with 10% heat-inactivated fetal calf serum (FCS, Gibco), 2 mM L-glutamine in a humidified atmosphere of 5% CO_2: 95% air. Third to fifth passaged cells were used throughout the experiments. Serum-free conditioned medium (10 ml serum-free MEM per 25 cm^2 culture flask) was obtained from confluent cultures after conditioning for 48 h.

Keratinocyte culture

KCs from newborn foreskin in a primary culture were grown in MEM supplemented with 5% heat-inactivated FCS, 2 mM L-glutamine, 0.4 μg/ml hydrocortisone, 10 ng/ml choleratoxin, and 10 ng/ml epidermal growth factor (Sigma) (KC medium) as described previously (4). A 1.5 ml suspension (20×10^5 cells) of KCs, about 3 cm in diameter and concentrically covering the dish, was seeded on the center of the dish, and after 1 day of incubation, the media were replaced with 5 ml of complete KC medium, which were subsequently changed twice a week. After reaching the KC sheet (not confluent) in about 2 weeks, the cells were washed twice in PBS (phosphate buffered salt solution, pH 7.4), and the serum-free FB-conditioned medium (Filtered through 0.45 μm, Gelman) from psoriatic and uninvolved FB samples was replaced on a KC sheet, and incubated for 48 h. KC culture fluid was harvested. The number of KCs harvested from each culture sample was $280\times10^4\pm30\times10^4$/dish.

SDS-PAGE and zymography

Samples (80 μl of each conditioned medium in a lane) were electrophoresed with sodium dodecyl sulphate (SDS) through a 10–15% gradient polyacrylamide as previously described (5). Fibrin-agar overlays were applied on both sides of the gel as previously reported (5). Plasminogen-enriched (10 μg/ml) fibrin-agar overlays were used to detect PAs, and reverse fibrin-agar overlays containing plasminogen (12.5 μg/ml) and 0.18 IU/ml UK (urokinase, Leo Labo.) to detect PA inhibitors.

Anti-UK and anti-tPA antibody indicator gels

Polyclonal antibodies against UK (human urinary urokinase, Leo Labo.) and tPA (tissue-PA, obtained from Bowes melanoma-conditioned medium) raised in rabbits were purified through protein A-sepharose. A positive fibrin-agar indicator gel mixed with anti-UK IgG serum, and anti-tPA IgG serum-mixed indicator gel were applied to the gel made by the electrophoresis of the KC culture fluid treated with psoriatic P1 FB-conditioned medium.

RESULTS

All serum-free conditioned media from psoriatic and uninvolved FBs showed the 57kD inhibitor (6) band on SDS-PAGE and reverse zymography. No activator lysis bands could be found. As shown in Fig. 1, the KC culture fluid treated with

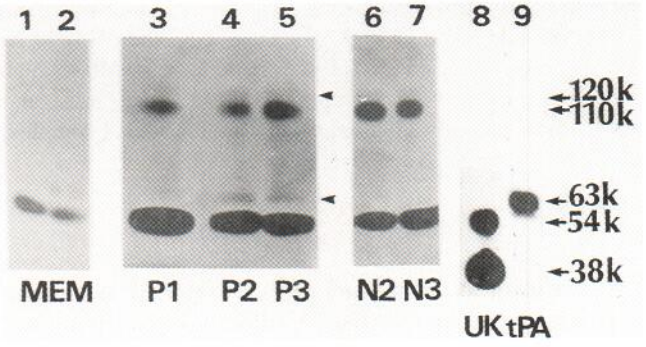

Fig. 1. Zymography analysis of KC culture fluid. *Lanes 1, 2.* Only 54kD lysis band detected in serum-free MEM treated sample. *Lanes 3, 4, 5.* A large 54kD lysis band, a small 110kD band, and very weak lysis bands of 63kD and 120kD (arrow heads) detected in harvest fluid treated with psoriatic FB-conditioned medium (P1, P2, P3). *Lanes 6, 7.* 54kD and 110kD lysis bands detected in samples treated with uninvolved FB-conditioned medium (N2, N3). *Lane 8.* High and low molecular weight urokinase. *Lane 9.* Tissue-plasminogen activator.

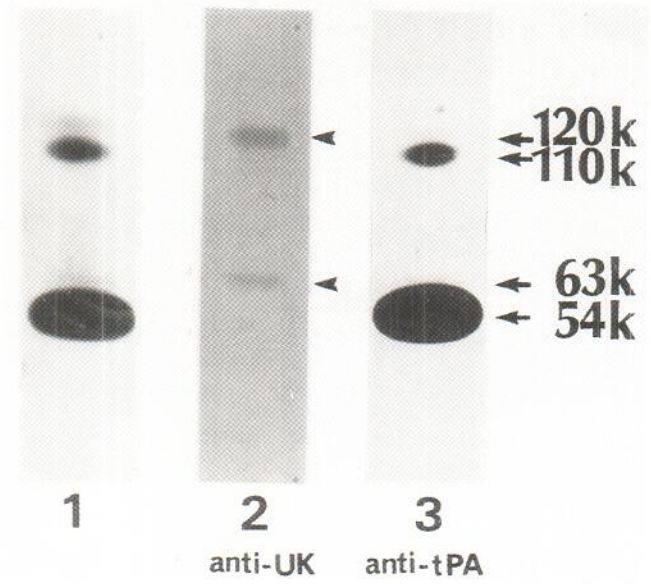

Fig. 2. Results of analysis of indicator gel mixed with anti-UK or anti-tPA antibodies. *Lane 1.* Original KC culture fluid treated with psoriatic P1 FB-conditioned medium. *Lane 2.* Following treatment with indicator of anti-UK antibody, 54kD and 110kD lysis bands disappeared. *Lane 3.* Following treatment with indicator of anti-tPA antibody, 63kD and 120kD weak lysis bands disappeared.

serum-free MEM gave rise to only small 54kD lysis bands (Lanes 1, 2). A large 54kD lysis band, a small 110kD band, and very weak lysis bands at 63kD and 120kD (Lanes 3, 4, 5) could be detected in the harvest fluid treated with psoriatic FB-conditioned medium (P1, P2, P3). Those treated with uninvolved FB-conditioned medium (N2, N3) showed a rather small lysis band of 54kD and 110kD (Lanes 6, 7). A KC culture fluid treated with serum-free MEM or uninvolved FB-conditioned medium gave rise to neither 63kD nor 120kD lysis bands.

As evident from Fig. 2, after treating fibrin-agar indicator gel mixed with anti-UK serum, both 54kD and 110kD lysis bands of KC culture fluid treated with psoriatic P1 FB-conditioned medium disappeared on SDS-PAGE and zymography, and after treating the indicator gel mixed with anti-tPA serum, the 63kD and 120kD lysis bands disappeared. The 54kD band is thus considered to be high molecular UK, the 63kD to be tPA, the high molecular 110kD lysis band to be possibly a high molecular inhibitor-UK complex, and the high molecular 120kD to be possibly a high molecular inhibitor-tPA complex, which may have been reactivated by SDS (6).

DISCUSSION

The proteolytic enzyme plasminogen activators (PAs), known as urokinase (UK) and tissue-PA (tPA), take part in normal epidermal proliferating processes; UK may play a role in keratinocyte migration, and tPA has been detected in shed KCs more often than in adherent cells (7). Both UK and tPA have been found intracellularly as well as extracellularly in cell culture systems. Several types of tumor cells secrete tPA, particularly cell lines derived from Bowes melanoma cells (8). In the present study, both the psoriatic and uninvolved FB-conditioned medium were found capable of regulating the secretion of UK from epidermal cells. It is of particular intererst that very weak lysis bands of 63kD and 120kD derived from tPA were detected in harvest fluid treated with psoriatic FB-conditioned medium, but not with uninvolved FB-conditioned medium. Since the method used in this study is only semi-quantitative and the amounts of the tPA secreted were small, it is possible that faster growing psoriatic FBs simply produce more stimulants and that the levels produced by the media from FBs derived from uninvolved skin are too low for detection. The difference in our results is thus quantitative rather than qualitative.

PA activity has been shown to be possibly present in the scales and epidermis of psoriatic skin (1). Immunohistochemical studies confirm that the basal KCs in suprapapillary areas stain positively with the anti-UK antibody, and superficial keratinizing cells and parakeratotic layers stain positively with the anti-tPA antibody (2). Both these findings support our in vitro results. Hansen et al. (2) noted that KCs in uninvolved and normal skin did not stain with antibodies for PAs. Uninvolved FB-conditioned medium was found capable of regulating UK in epidermal cells. Saiag et al. (3) observed that psoriatic FBs induce hyperproliferative activity in normal KCs. Psoriatic FBs may thus be involved in the pathomechanism of psoriasis. The regulating mechanism of psoriasis by activation of epidermal PAs should be further investigated.

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