Increased Protein Kinase C Activity in Fibroblast Membranes From Psoriatic Patients

Seiji Nagao, M.D., Ph.D., Mariko Seishima, M.D., Ph.D., Shunji Mori, M.D., Ph.D., and Yoshinori Nozawa, M.D., Ph.D.
Department of Biochemistry and Department of Dermatology, Gifu University School of Medicine, Gifu, Japan

...normal and psoriatic fibroblasts. These data suggest that PKc is preferentially associated with the membrane in psoriatic fibroblasts and that such elevated PKc activity in membranes may play a role in the pathogenesis of this disease. (J Invest Dermatol 90:406–408, 1988)

Psoriasis is a common skin disease characterized by epidermal proliferation and inflammatory changes. The nature of the initial abnormality in developing skin lesions is the subject of controversy [1–3], but it has been documented that one of the initial lesions may arise in the dermal fibroblasts [4]. In addition, fibroblasts from psoriatic patients were reported to have unusual properties. Priestley and Adams [5] have demonstrated that cultured fibroblasts isolated from both involved and uninvolved psoriatic skin appear to proliferate at a significantly faster rate than normal fibroblasts. They have also reported that psoriatic fibroblasts may be inherently hyperresponsive to unidentified mitogenic factors in human serum [6]; psoriatic fibroblasts proliferate more rapidly than normal fibroblasts in psoriatic as well as normal human serum, despite similar rates of proliferation in the presence of fetal calf serum.

Phospholipid/Ca²⁺-dependent protein kinase (PKc) is thought to play an important role in the regulation of cell proliferation [7]. This idea is supported by the following observations: 1) the cascade of events leading to the activation of PKc can be induced by several growth factors [8,9]; 2) direct activators of PKc (phorbol ester tumor promoters, diacylglycerols) have mitogenic activity [10–12]; and 3) important regulators of cellular proliferation are probable substrates of PKc-dependent phosphorylation [13,14]. PKc is found both in tight association with the plasma membrane and in the cytosolic fraction of many tissue homogenates. Recently, it has been shown that PKc activity and subcellular distribution were altered according to the proliferative activity of the cells [7].

As psoriatic fibroblasts show the changes in the proliferative activity as described above, we have undertaken experiments to determine the distribution and activity of PKc in psoriatic fibroblasts.

MATERIALS AND METHODS

Materials Phosphatidylserine (PS), ATP, 1,2-diolein, trypsin, cyclic AMP, and histone (Type II-A and III-S) were purchased from Sigma. E64 N-N-([3-trans-carboxiraxan-2-carbonyl]-l-leucyl)-sagmatine was donated by Taisho Pharmaceutical Co. Eagle’s minimum essential medium (MEM) and glutamine were from Nissui Pharmaceutical Co. Fetal calf serum was from Irvine Scientific. [y-³²P]ATP was obtained from New England Nuclear. DEAE- Sephacel was from Pharmacia. All other chemicals were of reagent grade.

Fibroblast Culture Skin samples were obtained from upper back in normal healthy volunteers without any skin diseases and noncon- sanguineous psoriatic patients by excision biopsies. All the skin specimens of psoriatic patients were taken from involved skin. Each skin sample thus obtained was rinsed with three 5-ml portions of prewarmed (37°C) Hanks’ balanced salt solution. The washed skin sample was cut into small pieces and plated on a 35-mm cell culture dish. For outgrowth, the dish was cultivated in Eagle’s MEM containing penicillin (100 U/ml), streptomycin (100 μg/ml), 10% fetal calf serum, and 2 mM glutamate at 37°C in humidified 5% CO₂/95% air for 30 days. During outgrowth the medium was changed twice a week. Confluent cells were detached with 0.25% trypsin and 0.02% EDTA in phosphate-buffered saline solution and 5 × 10⁵ cells were replated in a 80 × 100-mm plastic tissue-culture dish. The medium was changed every 3 days. Confluent cells from passages 4 to 5 were used for the following experiments.

Preparation of the Fibroblast Fractions and DEAE-Sephacel Chromatography The cultured cells were washed twice with cold Hanks’ balanced salt solution and scraped from the dishes with a rubber policeman. The cell suspension was spun for 5 min at 200 g and resuspended with 25 mM Tris/HCl at pH 7.5, containing 2 mM EGTA, 5 mM dithiothreitol, 100 μg/ml phenylmethylsulfonyl fluoride, and 0.25 mM E64 (Buffer A). After sonication, homogenates were spun at 105,000 g for 1 h to yield cytosol and membrane fractions. Membrane fractions were resuspended in Buffer A, extracted with 1% Nonidet P-40 for 1 h on ice, and reconstituted at 105,000 g for 1 h. Cytosol and detergent-extract...
able membrane fractions were separately applied to a 0.3 ml DEAE-Sephael column that had previously been equilibrated with Buffer A. After washing with 1.5 ml Buffer A, the column was eluted with 0.1 M NaCl, 0.2 M NaCl, and 0.3 M NaCl in 1.2 ml of Buffer A (0.6-ml fractions were collected in tubes).

**Protein Kinase Assay** PKc activity was assayed in a reaction mixture containing, in a final volume of 0.25 ml, 20 mM Tris/HCl (pH 7.5), 5 mM Mg acetate, 0.5 mM CaCl₂, 20 μg of PS, 0.4 μg of diolein, 10 μM [γ-32P]ATP (4 × 10⁶ cpm), 50 μg histone (Type I)-S, and sample protein [15]. Cyclic AMP-dependent protein kinase (PKa) activity was assayed in a reaction mixture containing, in a final volume of 0.25 ml, 20 mM Tris/HCl (pH 7.5), 5 mM Mg acetate, 1 mM EGTA, 1 μM cyclic AMP, 10 μM [γ-32P]ATP (4 × 10⁶ cpm), 100 μg of histone (Type II-A), and sample protein [16]. The reaction was carried out for 5 min at 30°C and was halted by the addition of 1 ml of ice-cold 25% trichloroacetic acid (TCA). Precipitates were collected on nitrocellulose membrane filters, washed four times with 5 ml of 15% TCA, and then measured for radioactivity. Basal activity that was obtained in control assays without PS (PKc assay) or cyclic AMP (PKa assay) was subtracted from the experimental value. Protein kinase activities were expressed as pmol 32P incorporated/min/mg protein from cytosolic or membrane fraction. Protein concentrations were determined by the method of Bradford [17] using bovine serum albumin as a standard.

**RESULTS AND DISCUSSION**

To minimize the effects of the proliferative states of the fibroblasts on PKc activity and its subcellular distribution, confluent cells were prepared from normal persons or psoriatic patients and then the cytosolic and detergent-extractable membrane fractions were obtained as described in Materials and Methods. Psoriatic fibroblasts closely resembled normal fibroblasts with respect to protein contents in these two fractions (data not shown). Figures 1 and 2 show typical elution profiles from DEAE-Sephael columns of PKc activity in normal and psoriatic fibroblasts, respectively. In cytosolic and detergent-extractable membrane fractions from normal fibroblasts, most of the PKc activity was eluted from the column up to 0.1 M NaCl and only a small activity was found in elute of 0.2 M NaCl (Fig. 1A, B). The elution profiles of PKc activity from psoriatic fibroblasts were similar to those from normal fibroblasts (Fig. 2A, B). Notable, however, is the high amount of PKc activity present in membrane fractions from psoriatic fibroblasts compared with normal cells.

Brion et al [18] recently characterized PKa in the cytosolic fractions from normal and psoriatic fibroblasts, and showed that in normal fibroblasts two major forms of PKa (referred as type I and II) were separated by using a DEAE column. However, the psoriatic fibroblasts displayed a complete absence of type II activity and also a decrease of type I activity. To examine whether similar findings are observed in our experiments, we measured PKa activity in the eluates used for PKc assay. In the cytosol fractions from normal fibroblasts, PKa activity was detected in the eluates of 0.1 and 0.2 M NaCl. However, the PKa activity of psoriatic fibroblasts was decreased in both of the eluates. The sum of PKa activities in the eluates of 0.1 and 0.2 M NaCl from psoriatic fibroblasts was half as much as that seen with normal fibroblasts (81 ± 21 [n = 6] vs. 177 ± 56 pmol/min/mg protein [n = 5], mean ± SD; P < 0.01, Student’s t-test).

The levels of PKc activities in fibroblasts from seven normal subjects and eight psoriatic subjects were shown in Fig 3. The membrane-associated PKc activities were found to be 196 ± 42 and 333 ± 65 pmol/min/mg protein for normal and psoriatic fibroblasts, respectively (P < 0.001). Since no difference was observed in cytosolic PKc activity between two groups, the total PKc activity (cytosolic plus membrane-associated activity) and the ratio of membrane-associated/cytosol PKc activity were greater in psoriatic fibroblasts than in normal cells. In contrast to our findings, Horn et al.

---

**Figure 1.** DEAE-Sephael column chromatography of cytosolic and membrane-associated protein kinase activities of normal fibroblasts. Cytosol (A) and detergent-extractable membrane fractions (B) were prepared and chromatographed on DEAE-Sephael as described in Materials and Methods. Aliquots of the fractions were assayed for PKc (O) and PKa (●) activities. At the point indicated by the arrows, the buffer was changed to Buffer A containing 0.1 M (I), 0.2 M (II), and 0.3 M (III) NaCl in sequence.

**Figure 2.** DEAE-Sephael column chromatography of cytosolic and membrane-associated protein kinase activities of psoriatic fibroblasts. Cytosol (A) and detergent-extractable membrane fractions (B) were prepared and chromatographed on DEAE-Sephael as described in Materials and Methods. Aliquots of the fractions were assayed for PKc (O) and PKa (●) activities. At the point indicated by the arrows, the buffer was changed to Buffer A containing 0.1 M (I), 0.2 M (II), and 0.3 M (III) NaCl in sequence.

**Figure 3.** Quantitations of the PKc activities present in fibroblasts obtained from normal and psoriatic subjects. Cytosols and detergent-extractable membrane fractions were prepared from fibroblasts from seven normal (N) and eight psoriatic (P) subjects. PKc activities were measured as described in Fig 1.
[19] recently reported that total PKc activity in the epidermis of psoriatic patients was lower than in the epidermis of normal subjects and that the ratio of membrane-associated/ cytosol PKc activity did not differ significantly between normal and psoriatic epidermis. In addition, it was suggested that the decreased PKc activity may reflect an activated state of psoriatic epidermis, since translocation of PKc to the membrane is associated with subsequent decreased PKc activity (down regulation). The inconsistent alterations of PKc activity between epidermis and fibroblasts in psoriasis remains to be interpreted. However, one explanation might be that in psoriasis both proliferation and differentiation are abnormal in epidermis, whereas proliferation is abnormal in fibroblasts (hyperresponsive to unidentified mitogenic factors in human serum).

Note that an increase in the membrane-associated PKc activity without changes in the cytosolic enzyme activity was observed in rapidly growing human [20] and 3T3-L1 [21] fibroblasts, which indicates that the partitioning of PKc activity between cytosol and membrane fractions may be related to the proliferative state of fibroblasts; PKc activity is elevated in the membrane fraction in cells with higher proliferative activity. In the present experiments, similar alteration in the PKc subcellular distribution was observed in confluent psoriatic fibroblasts. Therefore, the inherently increased membrane-associated PKc activity may reflect the abnormal hyperresponsibility of psoriatic fibroblasts. However, precise significance of the PKc translocation is unknown. It remains to be determined whether the changes of PKc activity are the cause or consequence of psoriasis.

REFERENCES