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ORIGINAL ARTICLE

ALTERED KERATINOCYTE-FIBROBLAST INTERACTIONS IN PSORIASIS REVEALED BY A COMBINED CELL-CULTURE

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Abstract Psoriatic skin is composed of inflammatory epidermal and dermal tissues. We hypothesized that cell to cell interactions by means of cytokine response may be altered in psoriasis. In order to define this theory, *in vitro* experiments were carried out using the cell culture system. Psoriatic keratinocytes and fibroblasts were simultaneously co-cultured on each side of the collagen membrane in the medium of high calcium condition to identify the interactions between keratinocytes and fibroblasts. We also carried out several combined cultures of normal or psoriatic keratinocytes and fibroblasts. In the culture fluid of combined cell-culture, the level of interleukin-6 (IL-6) was significantly higher in the combination of psoriatic keratinocytes and fibroblasts compared with that using normal keratinocytes. Psoriatic keratinocytes significantly secreted larger amounts of transforming growth factor-α (TGF-α) compared with normal keratinocytes when they were solely cultured. However, TGF-α secretion of normal keratinocytes was suppressed by co-culture with psoriatic fibroblasts, by which psoriatic keratinocytes were not influenced. These results indicate that the altered interaction between keratinocytes and fibroblasts exists in the psoriatic lesion, and it might occur in abnormal keratinocyte proliferation and differentiation.

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Key words: psoriasis; keratinocyte-fibroblast interaction; IL-6; TGF-α; cell-culture.

原　著

共培養法により明らかになった乾癬における表皮細胞—線維芽細胞の相互作用の変化

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抄録　乾癬病変は炎症性表皮及び真皮から構成されている．乾癬における細胞間相互作用の変化をサイトカインの反応により確認するため，表皮細胞と線維芽細胞を高カルシウム条件下においてコラーゲン膜上で共培養した．乾癬表皮細胞，乾癬線維芽細胞を共培養したところ，培地内IL-6値は正常表皮細胞と乾癬線維芽細胞の共培養に比較して有意に高値であった．また，乾癬表皮細胞を単独で培養した場合，単独正常表皮細胞に比較して有意に高値なTGF-α分泌が認められた．しかしながら，正常表皮細胞からのTGF-α分泌は乾癬線維芽細胞と共培養することにより抑制された．これらの結果は，表皮細胞，線維芽細胞間の変化した相互作用が乾癬病変に存在し，またそれは表皮細胞の異常な増殖，分化に重要な役割を果たしている可能性を示唆している．

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Introduction

Pathogenesis of psoriasis is still unclear. Besides, hyperexpression of cytokines such as transforming growth factor (TGF)-α, interleukin (IL)-6, IL-8, IL-1, interferon-γ in the psoriatic lesion has been reported[1-7]. These cytokines are secreted by various cells of lesional epidermis and dermis, and inflammatory cells to construct the cytokine networks. They are assumed to be significant factors in the formation and maintenance of the lesion. We hypothesized that cell to cell interactions by means of cytokine response may be altered in psoriasis. In order to examine this hypothesis, we carried out combined cell-culture experiments. In these experiments, we focused on the interaction between keratinocytes and fibroblasts.

There was no report which psoriatic keratinocytes (PKs) and fibroblasts (PFs) were simultaneously cultured in high calcium (Ca^{2+}) condition. Recently, culture of PKs using a permeable collagen membrane under the high Ca^{2+} condition without feeder layer and any epidermal growth factor was established[8]. We developed the culture system, in which PKs and PFs could be separately cultured on each side of the permeable collagen membrane. Keratinocyte-fibroblast interactions were examined by this culture method, and altered cytokine secretion and their interactions were found in psoriasis.

Materials and Methods

Culture medium

For the culture of keratinocytes and fibroblasts, 1.4 mM Ca^{2+} Eagle's minimum essential medium (EMEM, Sigma Chemical Co., St Louis, MO) was supplemented with 10% heat-inactivated fetal calf serum, 50 U/ml penicillin, 50 μg/ml streptomycin (Gibco BRL, Gaithersburg, MD), and 2.5 μg/ml amphotericin B (ICN Biochemicals, Costa Mesa, CA). Any special agent such as corticosteroid or epidermal growth factor was not added as described previously[8].

Cell preparation

Psoriatic and normal skin samples were obtained from fully informed, consenting patients. PKs and PFs were prepared from the untreated chronic psoriatic plaque, which clinically and histopathologically corresponded to typical features of psoriasis vulgaris. As control, normal keratinocytes (NKs) were obtained from the normal skin lesion which had undergone surgical resection for breast cancer. Epidermis was separated from dermal tissue as described previously[8, 9]. Briefly, these skin specimens were cut into small pieces about 3 mm in width and soaked in phosphate-buffered saline (pH 7.2) containing 500 U/ml disperse (Godo-Shusei, Tokyo, Japan) for 24 hours at 4℃. The separated epidermis was placed in 0.25% trypsin (Gibco BRL) dissolved in phosphate-buffered saline (pH 7.2) for 25 minutes at 37℃. After the inactivation of the trypsin using the supplemented EMEM, the epidermis was gently agitated with a pipette to make cells individual. After 5 minutes of centrifugation at 1000 rpm, 2.4×10^6 keratinocytes were used in this study.

For the preparation of PFs, the dermal lesion, derived from the untreated chronic lesional skin of the different patients with psoriasis, was placed on the bottom of 100 mm plastic dish, and maintained in the supplemented EMEM. After being confluent, PFs were trypsinized and used for the combined cell-culture.

Combined cell-culture

As shown in Figure 1, a permeable collagen membrane (Koken Cellgen®; Koken

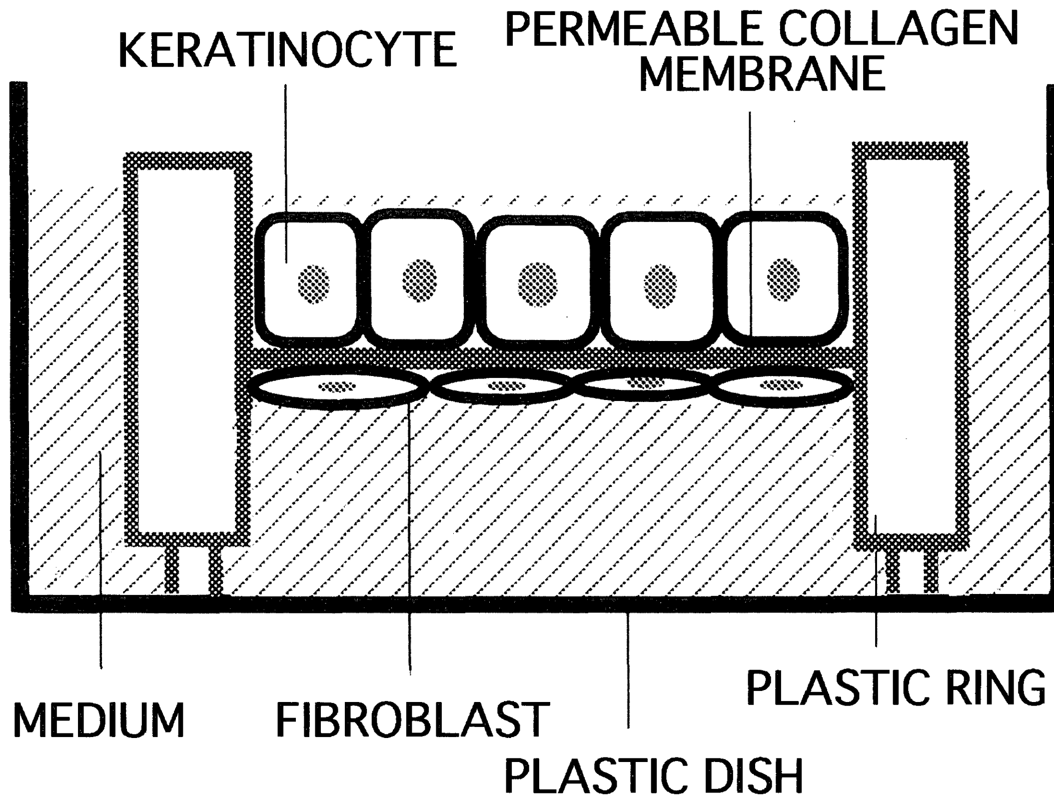

Figure 1 The scheme of culture system.

Co., Tokyo, Japan) of 33 mm in diameter was set in 60 mm dish which contained 7.0 ml medium. 1×10^5 fibroblasts were seeded on the collagen membrane and cultured for 24 hours at 37℃ in a humidified atmosphere of 5% CO_2[10]. The dish was changed to a new one and the membrane was turned upside down. Then 2.4×10^6 keratinocytes in primary culture were inoculated on the new upper surface of the membrane and we started the culture as day 1. Cultured cells were observed by a phase contrast microscope, and the medium was recovered and renewed every 4 days until day 12. Keratinocytes and fibroblasts were cultured on the upper and reverse surface of the collagen membrane, respectively. A series of experiments included sole culture of PFs, sole culture of NKs, co-culture of NKs and PFs, sole culture of PKs, and co-culture of PKs and PFs, was examined in triplicate.

Cytokine assay

The concentrations of TGF-α and IL-6 in the medium at day 4, 8, and 12 were measured using specific enzyme-linked immunosorbent assay kit according to manufacturer's (Otsuka Pharmaceutical, Tokushima, Japan, and Toray-Fuji Bionics, Tokyo, Japan, respectively).

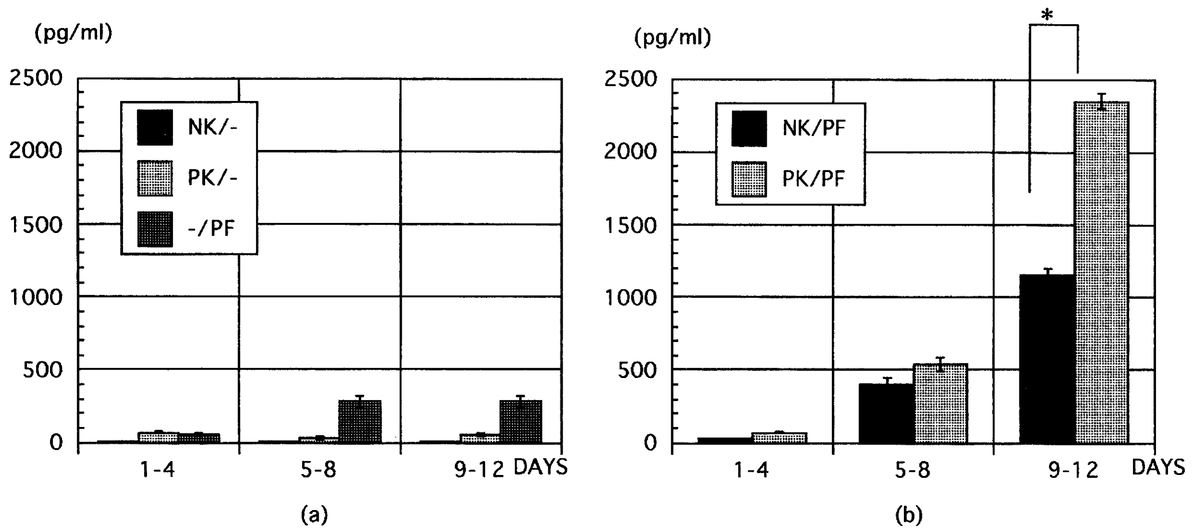

Figure 2 IL-6 concentrations in sole and combined culture.
(a) IL-6 secretion of normal (NK/-), psoriatic keratinocytes (PK/-), and psoriatic fibroblasts (-/PF) under sole culture. (b) IL-6 secretion under combined cell-culture. NK/PF; normal keratinocytes and psoriatic fibroblasts. PK/PF; psoriatic keratinocytes and psoriatic fibroblasts. Bars indicate standard deviations. *$P<0.05$.

Medium transfer experiment

After the same amount of PKs and NKs were incubated in the supplemented EMEM for 24 hours, the keratinocyte-conditioned medium (PK-CM, NK-CM, respectively) was recovered and used for the culture of PFs. As standard medium (SM), the supplemented EMEM was used. After 4 days of the incubation, the medium was recovered. Similarly, PKs and NKs were also cultured in PF-conditioned medium. The medium was used to measure the concentration of IL-6 by the commercial kit.

Statistics

Statistical significance was evaluated with Student's unpaired *t-test.*

Results

Morphological observation

As Miyauchi et al.[8] have reported previously, the morphological figure in the sole culture of PKs was similar to NKs. PKs and NKs attached on the membrane after 12 hours of seeding, and then proliferated. At the initial phase of culture, PKs proliferated more slowly, making a net-like configuration, compared with NKs, and reached a confluent state on day 10, and finally stratified. Phase contrast microscopic examination revealed no difference in the attitude of proliferation of keratinocytes between sole culture of PKs and co-culture of PKs with PFs. PFs became confluent on day 5 of the culture.

Secretion of IL-6 and TGF-α

Three series of independent experiments were examined using different sources of keratinocytes and fibroblasts. As shown in Figure 2, IL-6 secretion of sole culture and combined culture was detected in every 4 days. In the sole culture of NKs or PKs, the

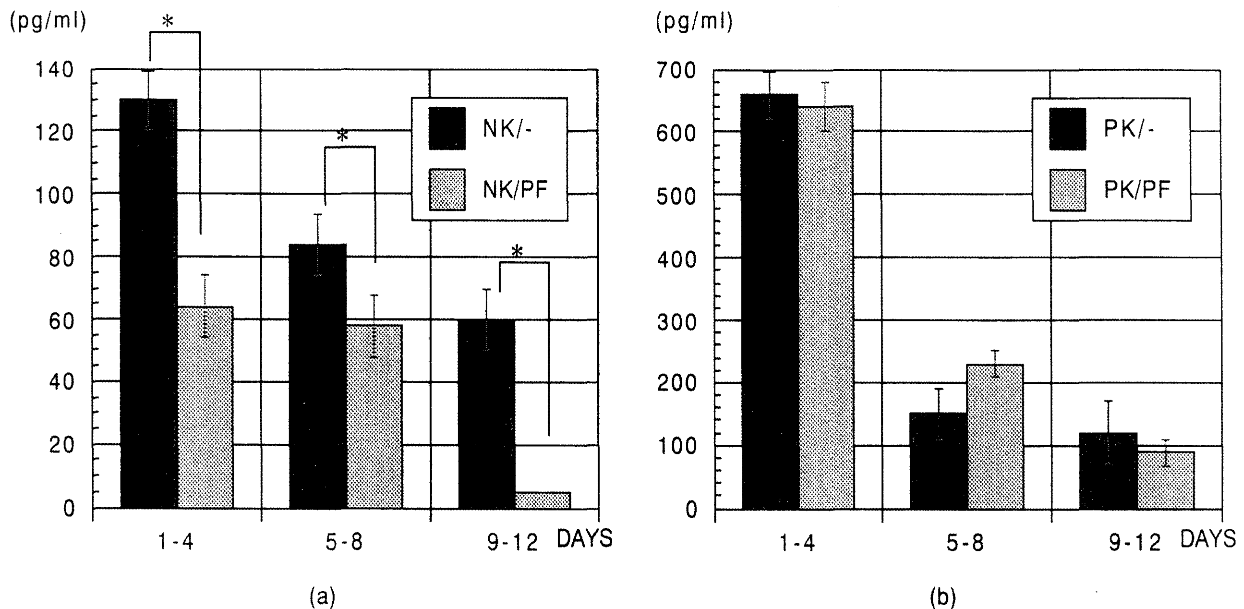

Figure 3 TGF-α concentrations in sole and combined culture.
(a) TGF-α secretion of sole culture of NKs (NK/-), combined culture of NKs and PFs (NK/PF).
(b) TGF-α secretion of sole culture of PKs (PK/-), combined culture of PKs and PFs (PK/PF).
Note: scale in (a) is different from that in (b). Bars indicate standard deviations. *$P<0.05$.

significant increase of IL-6 secretion was not shown in the period of 5 to 12 days (Figure 2a), however, the concentration of IL-6 increased in the co-culture of NKs and PFs (NK/PF) in the time-dependent manner. Co-culture of PKs and PFs (PK/PF) similarly provided the large amount of IL-6 in the medium, and the concentration of IL-6 was significantly higher than that of NK/PF on 9-12 days (Figure 2b).

As shown in Figure 3, TGF-α secretion of NKs (NK/-) and PKs (PK/-) in the sole culture clearly decreased in the time-dependent manner. In any periods, PK/- significantly secreted higher amounts of TGF-α compared with NK/-. When NKs were cultured together with PFs (NK/PF), TGF-α secretion was suppressed in contrast to that of NK/- in any culture periods (Figure 3a). On the other hand, PKs in the combined culture (PK/PF) did not show any significant decrease of TGF-α secretion compared with PK/- (Figure 3b).

IL-6 secretion by PFs cultured in the keratinocyte-conditioned medium

In order to determine the source of IL-6 in the co-culture, medium transfer experiment was examined using the cultured PFs and the medium, in which PKs or NKs were cultured for one day. The concentrations of IL-6 in the medium of PK-CM and NK-CM were below the detectable level. After PFs were cultured in PK-CM, NK-CM, or SM for 4 days, the concentrations of IL-6 in PK-CM and NK-CM increased 39- and 20-folds as high as the control (SM), respectively. Especially, PFs cultured in PK-CM significantly secreted

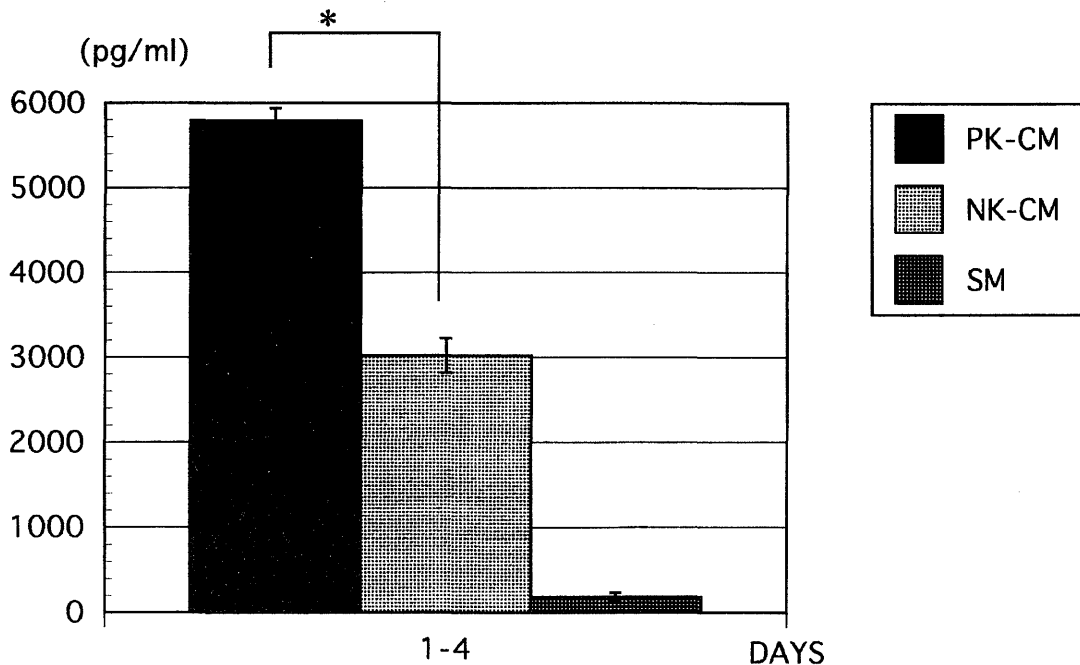

Figure 4 IL-6 concentration secreted from psoriatic fibroblasts (PFs) in some conditioned medium. When PFs were cultured in psoriatic keratinocyte-conditioned medium (PK-CM), IL-6 level was significantly high compared with cultured PFs in normal keratinocyte-conditioned medium (NK-CM). *$P<0.05$.

IL-6 compared with that in NK-CM. On the other hand, when NKs or PKs were cultured in PF-conditioned medium, no significant increase of IL-6 was observed (data not shown).

Discussion

In these experiments, the cell growth of PKs on the collagen membrane was more slowly than that of NKs at the initial phase of culture, demonstrating the similar results to the previous description by Liu et al.[11], in which PKs were cultured on the 3T3 feeder cells. However, our result does not correspond to that of Saiag et al.[12], in which psoriatic epidermis on the dermal equivalent made up with normal fibroblasts grew quickly in contrast to normal epidermis. The reason of the discrepancy between our result and that of Saiag et al. may be attributable to the different culture method. In the experiments of Liu et al.[11] and ours, keratinocytes and fibroblasts were cultured as the individual and single cells. On the other hand, Saiag et al.[12] directly cultured the skin tissues from the biopsy specimen. Growth of epidermal and dermal sheets might be influenced by the lymphocytes and neutrophils infiltrating in the psoriatic skin lesion. In our method, it was possible to observe the pure interaction between keratinocytes and fibroblasts, in which no other cell components were included.

IL-6 and TGF-α hyperexpressed in the

lesion of psoriatic skin act as proliferators to keratinocytes[4, 13, 14]. Our study is the first report that psoriatic keratinocytes and fibroblasts were co-cultured in high Ca^{2+} condition. When NKs were cultured together with PFs, the level of IL-6 increased in time-dependent manner, suggesting the existence of an interaction between keratinocytes and psoriatic fibroblasts. This interaction was further activated by the culture of PKs in place of NKs. Medium transfer experiments indicated that IL-6 was mainly secreted from fibroblasts by the stimulation of humoral factors derived from keratinocytes. These factors from keratinocytes are assumed to be IL-1α[13] or IL-1β[14]. Our study clearly showed that PKs highly enhanced IL-6 secretion from PFs in contrast to NKs.

As Miyauchi et al.[8] have reported previously, the level of TGF-α secretion from PKs was much higher than that from NKs. We found that TGF-α secretion from NKs was suppressed by PFs, which did not affect TGF-α secretion from PKs.

In conclusion, our results indicate that the keratinocyte-fibroblast interactions in psoriasis were altered in demonstrated cytokine secretion, which might take part in the pathogenesis of psoriasis, especially in abnormal keratinocyte proliferation and differentiation. Further studies are needed to elucidate precise mechanisms of these abnormal interactions of cytokine-networks in psoriasis.

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