Effects of Fibroblast-Keratinocyte Interactions on the Secretion of Interleukin-8

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Using two models for preparing skin equivalent, the effects of interactions between various types of fibroblasts and keratinocytes on their secretory activity in the production of interleukin-8 are studied. The secretion of an antiinflammatory cytokine is found to depend on the type and function of fibroblasts, the level of their proliferative activity, and the extent of formation of the dermal layer. Keratinocytes are capable of recognizing fibroblast function and stimulate or inhibit the secretion of interleukin-8 depending on the functional status. A new concept of hyperkeratosis and parakeratosis in psoriasis is proposed, which is based on a defect in the autoregulation of mediators, specifically, interleukin-8.

Key Words: *proliferation; differentiation; cytokines; fibroblasts; keratinocytes*

The discovery of mechanisms of cell-to-cell cooperation realized in the course of the immune response is crucial to solving problems of both experimental and clinical immunology. This applies above all to the immunodeficiency states caused by disorders of cell-to-cell cooperation, among which are autoimmune and some inflammatory and malignant diseases.

It is now clear that the key role in the realization of the interactions between immunocompetent cells and in the processes of proliferation regulation, differentiation, and migration of somatic cells is played by interleukins, interleukin-8 (IL-8) being one of the most important [2,3].

IL-8 is known as one of the principal cytokines of the skin, which is largely responsible for the interactions between immunocompetent cells of the skin, chemotaxis of neutrophils, and the processes of inflammation and formation of skin infiltrates [4]. The role of this agent in the interplay between somatic cells and disease development, specifically in psoriasis pathology, is not clear. Published reports describe the active participation of IL-8 in immune reactions of the skin in healthy subjects and in patients with psoriasis [1,6].

This study was aimed at measuring the secretion of IL-8 during interactions between one of the principal skin cells, dermal fibroblasts (FB), and keratinocytes (KC).

MATERIALS AND METHODS

Human dermal FB and KC were used in the study. FB were isolated from the skin of adult donors and patients with psoriasis. FB from apparently healthy skin sites and from plaques and lyzed skin areas of psoriasis patients were used. KC were isolated from the umbilical skin of newborns and from the skin of adult donors.

A 4-6-mm skin biopsy was carried out in patients and donors. Trypsin treatment [5] was used, consisting in a 12-hour incubation of skin fragments in trypsin in the cold, after which the skin was separated into derma and epidermis.

Two methods were employed for isolating FB: using slides and collagenase [4]. For the first method the derma was sliced into 0.5-1.0-mm fragments, put in 3-cm petri dishes, covered with slides, and incubated until FB began to migrate from the sliced frag-

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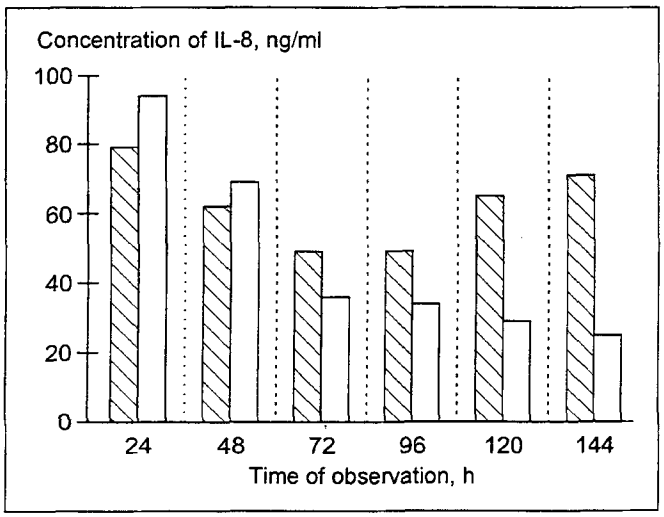

Fig. 1. Time dependence of the kinetics of interleukin-8 (IL-8) secretion by SE consisting of fibroblasts (light bars) and keratinocytes (dark bars) of a newborn.

ments to the bottom of the dish. After 50% fusion was attained by trypsinization, FB were transplanted into flasks. For the second method a separated dermal component of the skin was placed in a culture flask with collagenase for a 1-2-hour incubation. The cells were then repeatedly resuspended, washed with DMEM solution (containing 10% bovine serum), and centrifuged at 600 g for 7 min. Thereupon the cells were transferred to special plastic 75 ml flasks, DMEM culture growth medium (with 10% bovine serum) was added, and incubation was carried out at 37°C with 5% CO_2 until the cells covered 80 to 100% of the bottom of the flask. The cells were passaged by trypsinization.

KC were isolated from skin biopsy specimens using trypsin. A washed biopsy specimen was put in

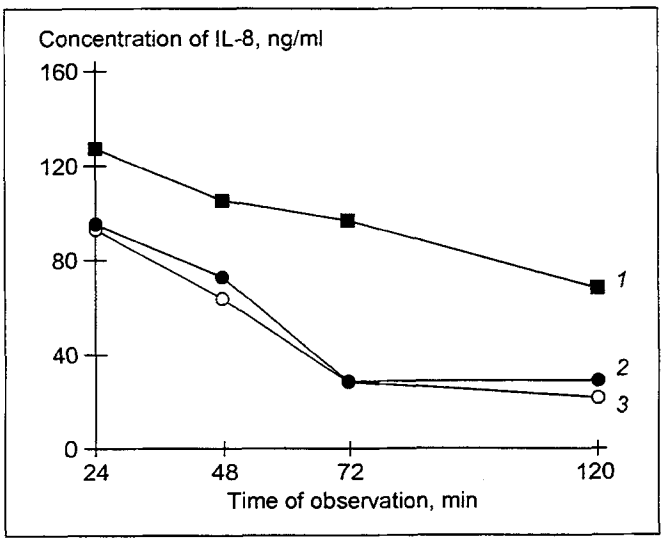

Fig. 2. Expression of interleukin-8 (IL-8) by SE containing similar types of newborn keratinocytes and dermal fibroblasts. *1*) from apparently intact skin sites of a psoriasis patient; *2*) from skin plaques of a psoriasis patient; *3*) from a healthy donor.

5% phosphate buffer saline with 2.5 mg/ml trypsin and kept in the cold for 18 h. Separated epidermis was resuspended and neutralized with DMEM solution containing 10% bovine serum, centrifuged for 5 min at 600 g, and the supernatant discarded. Cells resuspended to a solitary state were placed in special culture flasks with KGM (Keratinocyte Growth Media, Clonetic) medium with complement. The cells were incubated at 37°C at a constant humidity in a 5% CO_2 incubator. The culture medium was replaced every third day. After the formation of a monolayer, phosphate buffer saline with 0.2% EDTA and 5% trypsin was added. Then the cells (the first or second passage of KC) were transplanted into a new flask.

To elucidate the effect of KC-FB interactions on the secretion of IL-8, we used a skin equivalent (SE) model, which makes it possible to assess the effects of different types of FB on the secretion of IL-8 during their interactions with normal KC of newborns and adults.

The insert method was used to create SE. This method represents our modification of the mesh method developed at Madeleine Duvic's laboratory at the University of Texas. First, an FB equivalent was prepared. A collagen solution containing 10 ml of type 1 collagen (Biocoat), 0.1 ml of 10-fold DMEM, 0.011 ml of penicillin-streptomycin sulfate, and 0.01 ml of sodium bicarbonate was placed on ice. In parallel with this, a suspension of dermal FB in a concentration of 1×10^6 cells/ml of phosphate buffer saline was prepared. Resuspended cells were mixed with the collagen solution, and 1 ml of the suspension was embedded per insert so that the final concentration of FB collagen in the collagen was equal to 200,000 cells/matrix. After collagen polymerization with FB, 1 ml of FAD (Clonetic) was added per matrix for FB feeding. The plate with the cells was incubated for 24 h at 37°C in 10% CO_2. The culture medium was not changed over the entire period of formation of FB equivalent. For the formation of SE, 50,000 KC were layered onto each FB component. Medium was added to the lower part of the culture dish, 1 ml every 24 h.

RESULTS

Figure 1 presents the time dependence of IL-8 secretion by the SE created from adult donor FB and adult and newborn KC. We see that the time dependence of IL-8 for the studies of SE are similar, the secretion of IL-8 by these SE consistently diminishing right up to day 4 of incubation. With an incubation of more than 4 days the secretion of IL-8 by an SE containing neonatal KC appreciably surpasses the secretion by SE containing adult KC.

In addition, the secretion of IL-8 in an SE containing normal adult donor FB and normal newborn

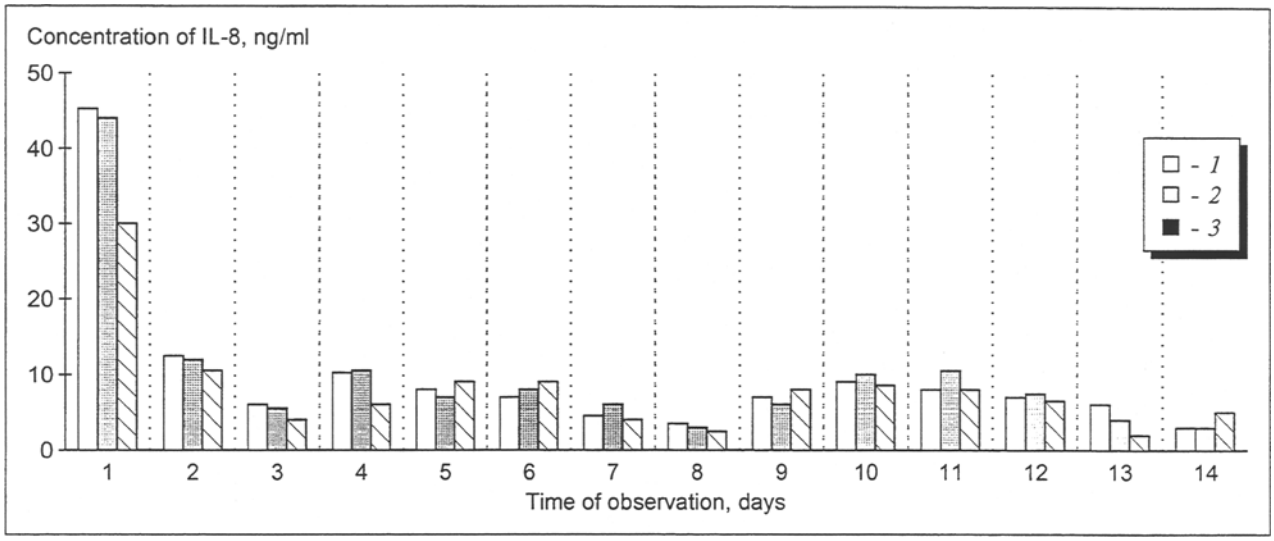

Fig. 3. Time dependence of the secretion of interleukin-8 (IL-8) by SE containing psoriatic fibroblasts (from plaques) and newborn keratinocytes during a long follow-up period (24 days). Fibroblasts isolated from one (*1*, *2*) and from another (*3*) patient (similar skin sites were used).

or adult KC surpassed the secretion of IL-8 by individual normal donor FB or neonatal KC in culture 20 to 60 times. The level of secreted IL-8 decreased as the incubation went on, but the minimal level of secretion of IL-8 by KC was still elevated by day 4 of incubation, at least 20 times as high as the secretion of IL-8 by individual cells. By day 6 of incubation the level of IL-8 secretion in the SE containing normal newborn KC increased again.

If we consider the SE composed of normal donor FB and neonatal KC as the donor SE, then the SE containing KC and FB from apparently intact skin sites of psoriasis patients suppresses the higher level of IL-8 1.5-3.5-fold, depending on the time of incubation (Fig. 2).

An appreciable difference in the secretion of IL-8 in the SE is observed for FB isolated from apparently intact skin sites and from plaques of psoriasis patients. It is noteworthy that SE containing FB from apparently intact areas is a more active producer of IL-8. Similar relationships of IL-8 secretion were observed for other SE consisting of other FB strains isolated from apparently intact skin sites and from plaques of psoriasis patients.

Figure 3 shows secretion of IL-8 in a SE containing FB from plaques upon 24-day incubation of the SE. Three peaks of IL-8 secretion by this SE were observed. The first occurred between the 20th and 24th hours, the second on days 6-7, and the third on days 10-12 of SE incubation, depending on the FB strain.

Similar time relationships were obtained for SE containing fibroblasts from normal donors and from apparently intact skin sites of psoriasis patients, but the periods of increased IL-8 secretion were somewhat shifted.

Previously we demonstrated that KC and FB of healthy subjects and patients are capable of spontaneous (without the addition of other agents, such as IL-8, tumor necrosis factor, etc.) production of IL-8 in culture. This means that the secretion of agents such as IL-8 is a normal physiological process for these cells, but the level of IL-8 secretion is low in such cases. The secretion of IL-8 by FB starts not directly after their inoculation in a culture, but after some 6 to 12 h, the maximal secretion occurring between the 12th and 24th hours of culturing. The higher the initial density of FB inoculation, the earlier the start of IL-8 secretion. These facts indicate that the functional activity of both FB and KC depends in great measure on the degree of differentiation of these cells and their cell-to-cell cooperation. These results are in line with data on murine FB, for which the functional and phenotypic status of a cell was shown to depend on the density of FB inoculation in the test system [4,5].

The observed drop of IL-8 secretion after a 24-hour incubation for all studied skin cells may be due to the fact that FB proliferation leads to the secretion of collagen by these cells, which, in turn, interferes with cell-to-cell cooperation and the cooperative effect. In addition, a change in the expression of surface markers of FB and KC is possible because of the involvement of mechanisms inhibiting the proliferative process. The slight peak (Fig. 3) by days 6-7 of incubation of the SE coincides in time with the formation of the basal membrane and basal layer. According to our findings, starting from this time the epidermis becomes the principal source of IL-8. During the further formation of the epidermis, IL-8 is secreted not only in the basal layer, but in the prickle-cell and granular layers as well. This corresponds to days 10-12 of SE incu-

bation in our case. Immunohistochemical analysis showed IL-8 even in the horny layer on days 21-27 of incubation for SE containing FB isolated from apparently healthy skin sites and from psoriatic plaques.

Our data permit us to conclude that the secretion of the antiinflammatory cytokine IL-8, which plays a large part in the chemotaxis of neutrophils, depends greatly on the type and functional status of FB, the level of their proliferative activity, and the degree of formation of the dermal layer. KC are capable of recognizing the functional status of FB and, depending on this, stimulate or inhibit the secretion of IL-8. Not only IL-8, but other agents as well are secreted in the course of cell-to-cell interaction, which can stimulate or inhibit the secretion of IL-8, depending on the maturity of the SE and the activity of KC and FB in it.

Hence, IL-8 is a key player in the regulatory processes of the major somatic skin cells, which in health stimulates (in the case of a deficit) or inhibits (in the case of hyperactivity) the KC. Psoriasis may involve a defect of autoregulation, leading to hyperproduction of IL-8, which results in hyperproliferation of KC and the formation of plaques. The detected high level of IL-8 in the horny layer of the skin (scales) is additional evidence in favor of this concept. However, the mechanisms and causes of this shift are still to be investigated.

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