

Ultrastructural study of the effect of proinflammatory cytokines in a three-dimensional model of normal human skin

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Psoriasis is an autoimmune disease in which epidermal keratinocytes and innate immunity effector cells play a pivotal role in the lesion formation. Tumor necrosis factor (TNF)-alpha and interleukin (IL)-17 are known to play a relevant role in the immunological activation typical of psoriasis, but the mechanisms leading to disease are still poorly known.

Several experimental models are available, but all of them have intrinsic limitations. A three dimensional model of organotypic human skin culture is a valuable approach for exposing the whole skin to TNF-alpha and IL-17 as specific proinflammatory stimuli mimicking a psoriatic microenvironment. To gain insight on the action of these cytokines on human skin, the present study was carried out in such a model, standardized in our laboratory, looking at the direct and immediate effects of proinflammatory cytokines on the ultrastructure of normal human epidermis. Normal human skin explants were obtained from plastic surgery of healthy 20-40 year-old women (n = 7) after informed consent. Bioptic fragments were cultured overnight in Dulbecco's modified minimum Eagle's medium and further divided before adding either 100 ng/ml TNF-alpha or 50 ng/ml IL-17 or a combination of both cytokines. Samples were harvested 24 hours after cytokine incubation and processed for transmission electron microscopy. Each patient was represented in all experimental groups. At light microscopy observation of semithin sections, the skin three-dimensional architecture appeared unaffected by cytokine treatment. By electron microscopy, the basal compartment of cytokine-treated samples presented a monolayer of cylindrical keratinocytes accompanied by melanocytes, as in normal skin, but occasional cells showed signs of ongoing apoptosis. Desmosomes were uniformly distributed throughout the epidermis. Langerhans cells were present and rich in Birbeck granules. A few dermal cells near the epidermis were well preserved, while more deeply located cells appeared apoptotic or necrotic. After cytokine treatment the intercellular spaces were enlarged in the basal and spinous layer, especially upon TNF-alpha, but desmosomes were still present; Langerhans cells appeared as in controls.

These results suggest that in these experimental conditions neither TNF-alpha nor IL-17 affect the epidermal architecture and cell ultrastructure, but the intercellular oedema suggests that the two proinflammatory cytokines can exert early and direct effects on normal human epidermal keratinocytes.