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Morphological analysis of the effects of tumor necrosis factor-alpha and interleukin-17 in a three-dimensional organotipic model of normal human skin

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Psoriasis is an autoimmune chronic inflammatory disease in which epidermal keratinocytes and innate immunity effector cells play a pivotal role in the lesion formation in genetically predisposed subjects (Bonifati et al., 1999). Among the several cytokines involved in psoriasis pathogenesis, tumor necrosis factor (TNF)-alpha and interleukin (IL)-17 play a relevant role. TNF-alpha stimulates the production of many chemokines, induces cell proliferation, and is proapoptotic. IL-17 is involved in the recruitment/activation of neutrophils and induces keratin 17 (K17) expression in psoriatic lesions. The present study is focussed on the early effects of these proinflammatory cytokines on i) the molecular composition of intercellular junctions (desmocollin (DSC)1/desmoglein (DSG)1, E-cadherin, and occludin) ii) on K17 expression iii) on immunophenotype/number of epidermal Langerhans cells (LCs) after cytokines exposure. Ultrastructural analysis was performed in on all samples.

Skin explants obtained from plastic surgery of healthy 20-40 year-old women (n = 7) after informed consent, were cultured overnight in Dulbecco's modified Eagle's medium and divided before adding 100 ng/ml TNF-alpha or 50 ng/ml IL-17 or a combination of both cytokines (Donetti et al., 2014). Samples were harvested 24, 48, and 72 hours after cytokine incubation.

Occludin immunostaining was non homogeneous in cytokine treated samples, starting from 24 hours of culture. Interestingly, K17 expression was induced only in IL-17-treated samples only. No differences were observed in DSC1, DSG1 and E-cadherin expression by immunofluorescence. LC number was significantly higher in samples treated with both cytokines (216.71±15.10%) than in TNF-alpha (125.74±26.24%) or IL-17 (100.14±38.42%) alone.

TEM analysis revealed that spaces were enlarged in the basal and spinous layer, especially upon TNF-alpha treatment, but desmosomes were uniformly distributed. Upon TNF-alpha stimulus LCs appeared with few organelles, mostly mitochondria, lysosomes, and scattered peripherical Birbeck granules. Upon IL-17 stimulus, LCs showed a cytoplasm with many mitochondria and numerous Birbeck granules close to the perinuclear space and Golgi apparatus, but also at the periphery, at the beginning of the dendrites. The addition of both cytokines did not modify LC ultrastructure.

Altogether this study strongly suggests that this model is useful to study the early, direct, and specific effects of specific psoriatic cytokines on the different cell population.

References

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Keywords

Psoriasis, keratinocyte proliferation, cell junctions, Langerhans cells, transmission electron microscopy.