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Morphological comparison between *in vitro* and *ex vivo* models to evaluate the direct keratinocyte responses to a psoriatic proinflammatory milieu

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Considering the psycho-socioeconomic impact of psoriasis, affecting 2-3% of the whole population, the need to understand the pathogenetic early epidermal events is still acute. The development of experimental models easy to use, but also able to reproduce the main psoriatic features is a compelling challenge. In this study, we compared the keratinocyte response after 24 hours to a proinflammatory milieu composed of TNF-alpha, interleukin (IL)-17A, IL-22, and IL-23 (MIX) in i) a 2D model of *in vitro* keratinocytes (HaCaT cells) induced to differentiate with $CaCl_2$ 1.8 mM for 4 days and ii) in an *ex vivo* 3D model of healthy human skin obtained after aesthetic surgery (n=5). Based on the hyperproliferative feature and the impairment of terminal differentiation reported in the psoriatic plaque, we evaluated cell proliferation analyzed as 5-bromo-2'-deoxyuridine incorporation and keratinocyte differentiation by indirect immunofluorescence in both models. Keratin (K) 10/K14 were considered as cytoskeletal markers of suprabasal and basal layers, respectively, and claudin 1/zonula occludens (ZO)-1 components of tight junctions (TJs). In HaCaT cells, both cell proliferation and K14 immunostaining increased as early as 24 hours, while in bioptic fragments keratinocyte proliferation was decreased at the same time point. MIX treatment always reduced K10 distribution. Claudin 1 fluorescence intensity was reduced after MIX incubation in the uppermost differentiated epidermal layers and HaCaT cells, while ZO-1 immunoreactivity was redistributed in the epidermal compartment and resulted fainter in MIX-incubated HaCaT cells. The modulation of TJ composition and the impairment of terminal differentiation are psoriatic events occurring earlier than the proliferation impairment, the latter representing a "response to injury" when the epidermis faces an inflammatory milieu. In conclusion, our observations are relevant not only as it applies to general skincare, but also to clinics.

Keywords: HaCaT cells; psoriasis; cytokines; tight junctions