

A proinflammatory microenvironment induces NFkB activation and beta-defensin expression through specific Toll Like Receptors in a 3D human skin model

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Psoriasis is an autoimmune skin disease characterized by the formation and the progression of silvery plaques on the extensory surfaces of our body. Proinflammatory cytokines as Tumor Necrosis Factor (TNF)-alpha, interleukin (IL)-17, IL-22 and IL-23 represent for the normal skin a psoriatic microenvironment. In the 3D human skin model standardized in our lab in the last decade, we were able to dissect the events in which each cytokine exerts a specific effect, e.g. keratinocyte proliferation, Langerhans cell activation, cytoskeleton arrangement, and, more recently, the epidermal expression of Toll like Receptors (TLRs) 2, 7, 9. Several experimental studies reported that in psoriasis TLRs are expressed and their activation triggers i) NFkB translocation from the cytoplasm to the nucleus and ii) the release of beta defensins (HBDs). The present study was aimed at investigating the intracellular NFkB activation and HBD1 and HBD2 expression induced by a cytokine mix (TNF-alpha, IL-17, IL-22, IL-23) by indirect immunofluorescence. Bioptic samples of normal human skin were obtained after aesthetic surgery of young healthy informed women (n=7). After overnight incubation to reduce mechanical and termical stress, skin fragments were incubated in a Transwell system for 5 (T5), 24 (T24), and 48 (T48) hours with the cytokine mix. Parallel control samples were carried out and each patient was represented at all time points. In controls at all time-points NFkB was localized only in the cytoplasm, while, starting from T5, scattered basal nuclei were observed in the cytokine-incubated samples. At later time points, in the upper spinous and granular layers, NFkB nuclear immunostaining was evident. HBD2 expression was affected after cytokine mix exposure, while HBD1 distribution was similar to controls.

Thanks to this simple but effective model, a deep knowledge of the early events occurring in the normal epidermis exposed to cytokines can be achieved, excluding the contribution of the blood and lymphatic vessels herein absent. This basic research can thus represent an important tool for targeting and counteracting single phenomenon leading to the formation/progression with the most innovative biological drugs.

Keywords

Proinflammatory cytokines, psoriasis, immunofluorescence