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An innovative three-dimensional model of normal human skin to study the proinflammatory psoriatic effects of tumor necrosis factor-alpha and interleukin-17

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ABSTRACT

Background: Among all cytokines involved in the pathogenesis and in the progression of psoriasis, Tumor Necrosis Factor (TNF)-alpha and interleukin (IL)-17 play a pivotal role.
Objective: The aim of the present study was to mimic a psoriatic microenvironment and to investigate the early effects of TNF-alpha and IL-17 in a three-dimensional model of organotypic normal human skin.
Methods: Human skin explants were obtained from plastic aesthetic surgery of healthy young women 20–40 years old ($n = 7$). The study was approved by the Institutional Review Board and written informed consent was obtained from all subjects. Bioptic fragments were cultured at the air–liquid interface overnight in a Transwell system and further divided before adding either 50 ng/ml IL-17 or 100 ng/ml TNF-alpha or a combination of both cytokines. For each subject, a control sample was cultured without any cytokine. Samples were harvested 24 or 48 h after cytokine incubation. At both time points and for all cytokine treatments a bioptic fragment obtained from each patient was processed. Epidermal proliferation, expressions of terminal differentiation (keratin 10, K10, and 14, K14) and of intercellular adhesion (occludin for tight junctions and E-cadherin for adherens junctions) biomarkers were investigated by indirect immunofluorescence.
Results: IL-17 and TNF-alpha induced an early and statistically significant inhibition of keratinocyte proliferation (more than 80% compared with their respective controls). At 24 h, the combination of both cytokines did not further reduce cell proliferation. Starting from 24 h of incubation, a non-continuous occludin expression in the granular layer was observed after both IL-17 and TNF-alpha exposure. Immunolabelling for E-cadherin in adherens junctions, for K10 in the suprabasal layers, and for K14 in the basal layer was similar in all experimental groups and unaffected after cytokine treatment.
Conclusions: These results suggest that in this experimental model IL-17 and TNF-alpha induced an early alteration of the homeostasis of the inner proliferative layer and of the upper granular layer, as shown by cell proliferation inhibition and occludin expression.

1. Introduction

Psoriasis is an autoimmune chronic inflammatory pathology in which epidermal keratinocytes and innate immunity effector cells play a pivotal role in the lesion formation in genetically predisposed subjects [1]. The precise sequence of cellular events leading to the psoriatic lesion formation is still unclear, but inflammatory processes can be elicited by stimulated keratinocytes. This interplay between keratinocytes and immune cells, in particular T cells, is responsible for the two main peculiar features of psoriasis, i.e. hyperproliferation and inflammation. When epidermal cells are activated, they can (i) promote the recruitment of circulating immune cells thanks to the expression of vascular adhesion molecules and (ii) secrete a huge variety of proinflammatory cytokines.

Among all the different cytokines involved in the pathogenesis of the psoriatic lesion, Tumour Necrosis Factor (TNF)-alpha and interleukin (IL)-17 are known to play a relevant role in the immunological activation characterizing psoriasis. The role of TNF-alpha has been widely studied and most of its properties are well known

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Table 1
Summary of the indirect immunofluorescence protocols used. Microwave and autoclave antigen unmasking were always performed with slides immersed in citrate buffer (pH 6). Primary, secondary antibodies, and goat serum were diluted in PBS/BSA 1%. RT: room temperature; PBS: phosphate buffered saline; BSA: bovine serum albumin.

Primary antibody	Saturation aspecific sites	Antigen unmasking	Dilution/ incubation time	Secondary antibody
Paraffin sections				
Terminal differentiation				Goat anti mouse FITC-conjugated (Jackson Immuno research)1:200/1 h RT
K10 (DE-K10 – Progen)	Goat serum (Vector) 1:10/30′ RT	Microwave (750w) boiling 3 × 4″	1:10/overnight 4 °C	
K14 (LL002 – Santa Cruz)	Goat serum (Vector) 1:10/30′ RT	Pepsin 0.05% 15′ RT	1:200/overnight 4 °C	
Intercellular adhesion				
Occludin (Invitrogen)	Goat Serum (Vector) 1:10/45′ RT	Microwave (750w) boiling 3 × 1′ and pepsin 0.05%25′ 37 °C	1:100/overnight 4 °C	
E-cadherin (36/E-cadherin – BD Bioscience)	Goat serum (Vector) 2%/45′ RT	Autoclave 10′ 120 °C	1:1000/ overnight 4 °C	

in the pathogenesis of psoriasis; this molecule stimulates the production of many chemokines, induces cell proliferation, and is proapoptotic [2]. IL-17, synthesized only by memory T cells and natural killer cells, has pleiotropic effects, mainly in the recruitment and activation of neutrophils [3,4]. IL-17 receptor is widely expressed on epithelial cells, fibroblasts, B and T cells, and monocytic cells. In psoriatic skin lesions, both Th17 cells and their downstream effector molecules, e.g. IL-17 and IL-22, are highly increased [5].

In order to study the early events induced by proinflammatory cytokines in the epidermis during psoriatic lesion formation, several experimental models are available, but all of them have intrinsic limitations. In vitro studies focused on the psoriatic biomarker expression in keratinocytes with an anomalous terminal differentiation [6]. In vivo, transgenic mice [7,8] showed only hyperplastic keratinocytes in hair follicles, but the specific features of psoriatic skin were not reproduced. More recently, dendritic

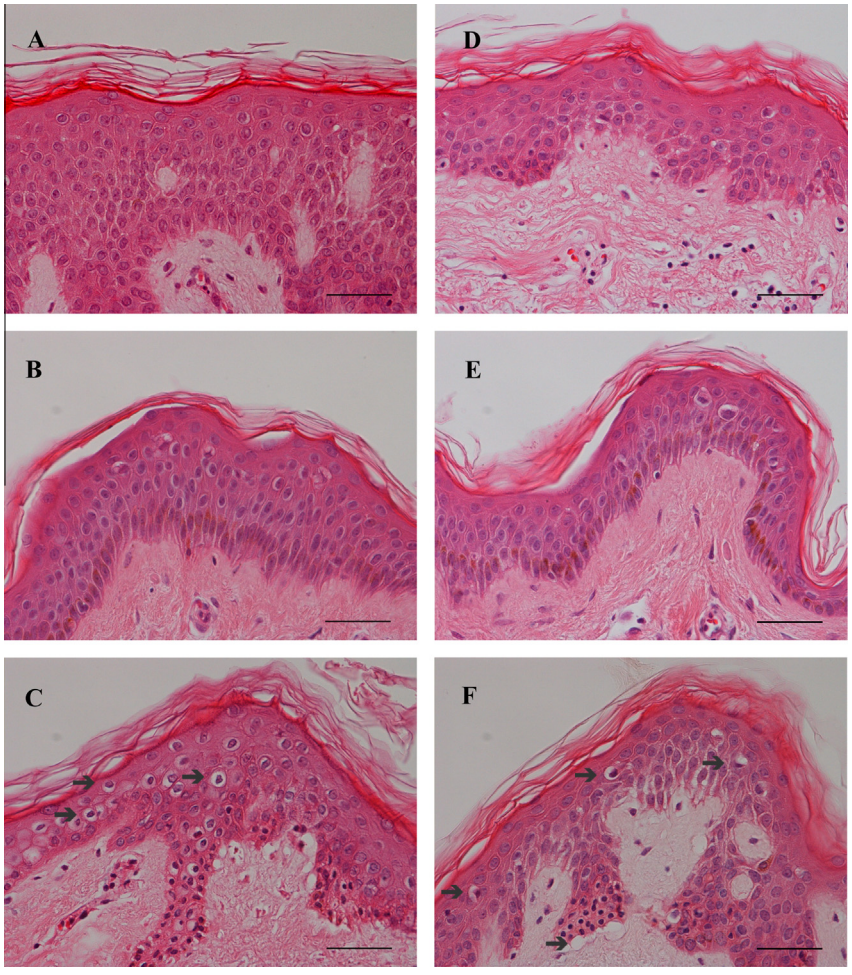

Fig. 1. Haematoxylin and Eosin staining. Representative photomicrographs of human normal skin paraffin sections after haematoxylin and eosin staining. (A–C): Samples harvested after 24 h of culture following the overnight incubation; (D–F): Samples harvested after 48 h of culture following the overnight incubation. (A) and (D): Control samples; (B) and (E): IL-17-treated samples. (C) and (F): TNF-alpha-treated samples. Black arrows indicate keratinocytes in the spinous layer with pale cytoplasm and condensed nuclei. IL-17: interleukin 17; TNF-alpha: Tumor Necrosis Factor-alpha. Bars = 50 μm.