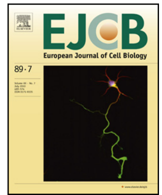
European Journal of Cell Biology 94 (2015) 71–77

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb



Tumor necrosis factor-alpha and interleukin-17 differently affects Langerhans cell distribution and activation in an innovative three-dimensional model of normal human skin

Francesca Prignano[a], Francesca Arnaboldi[b], Laura Cornaghi[b], Federica Landoni[b], Lara Tripo[a], Franz William Baruffaldi Preis[c], Elena Donetti[b,*]

[a] *Department of Surgery and Translational Medicine, Section of Clinical Preventive and Oncology Dermatology, Università di Firenze, 50125 Florence, Italy*
[b] *Department of Biomedical Sciences for Health, Lab of Structural and Ultrastructural Morphology, Università degli Studi di Milano, 20133 Milan, Italy*
[c] *I.R.C.C.S. Istituto Ortopedico Galeazzi, 20161 Milan, Italy*

ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 1 December 2014
Accepted 8 December 2014

Keywords:
Psoriasis
Dendritic cells
Pro-inflammatory cytokines
Immunofluorescence
Transmission electron microscopy

ABSTRACT

Among the several cytokines involved in the psoriasis pathogenesis, tumor necrosis factor (TNF)-alpha and interleukin (IL)-17 play a central role. Many biomolecular steps remain unknown due to difficulty to obtain psoriatic models. To investigate the effect of TNF-alpha and IL-17 on the ultrastructure, immunophenotype, and number of epidermal Langerhans cells (LCs), human skin explants ($n=7$) were cultured air–liquid interface in a Transwell system. Four different conditions were used: medium alone (control), medium added with 100 ng/ml TNF-alpha or 50 ng/ml IL-17 or a combination of both cytokines. Samples were harvested 24 and 48 h after cytokine addition and were frozen. Samples harvested at 24 h were also processed for transmission electron microscopy (TEM). By immunofluorescence analysis with anti-human Langerin antibody (three experiments/sample) we calculated the percentage of LCs/mm^2 of living epidermis after 24 and 48 h of incubation (considering control as 100%). At 24 h LC number was significantly higher in samples treated with both cytokines (216.71 + 15.10%; $p < 0.001$) and in TNF-alpha (125.74 + 26.24%; $p < 0.05$). No differences were observed in IL-17-treated samples (100.14 + 38.42%). After 48 h, the number of epidermal Langerin-positive cells in IL-17- and TNF-alpha treated samples slightly decreased (94.99 + 36.79% and 101.37 + 23% vs. their controls, respectively). With the combination of both cytokines epidermal LCs strongly decreased (120 + 13.36%).

By TEM, upon TNF-alpha stimulus LCs appeared with few organelles, mostly mitochondria, lysosomes, and scattered peripherical BGs. Upon IL-17 stimulus, LCs showed a cytoplasm with many mitochondria and numerous BGs 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 affect LC ultrastructure.

Our study showed that IL-17 induced significant changes in LC ultrastructure, while the combination of both cytokines seems to have a strong chemo-attractant effect on epidermal LCs, supporting the relevance of investigating the interplay between LCs and pro-inflammatory cytokines in the ongoing of the disease.

© 2014 Elsevier GmbH. All rights reserved.

Introduction

Psoriasis is a chronic inflammatory skin disease affecting almost 3% of the world's population with a high negative impact on the quality of life, especially if localized to sensitive body areas. Typical clinical features of psoriasis consist in plaques with clear-cut borders, silvery scales, and erythema. During the pathogenesis of psoriasis a specific and complex interplay occurs among different cytotypes, i.e. T-lymphocytes, dendritic cells, and fibroblasts together with soluble factors as cytokines. For a long time T-lymphocytes have been identified as the main actors of the disease (Davison et al., 2001), but recent advances provided strong evidences that dendritic cells (DCs) actively participate in the psoriatic inflammatory process (Johnson-Huang et al., 2009). DCs are bone-marrow derived cells and act as central players in the potentially self-sustaining type-1 inflammatory network. They are considered

* Corresponding author at: Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Via Mangiagalli, 31, 20133 Milan, Italy.
Tel.: +39 0250315400; fax: +39 0250315387.
E-mail address: elena.donetti@unimi.it (E. Donetti).

http://dx.doi.org/10.1016/j.ejcb.2014.12.003
0171-9335/© 2014 Elsevier GmbH. All rights reserved.

the only cells equipped for working as an “immunogenic bridge” between innate and acquired immunity and, for this reason, have a unique position in the immune system as antigen-presenting cells. Three resident subsets of DCs have been identified in human skin, i.e. epidermal Langerhans cells (LCs), dermal myeloid DCs (mDCs), and plasmacytoid DCs (pDCs) (Zaba et al., 2009; Collin et al., 2013). LCs possess a unique intracytoplasmic organelle which represents their characteristic ultrastructural feature: the Birbeck granule (BG). It is well established that BG is associated with the internalized Langerin (Valladeau et al., 2000). Each subset expresses definite markers and is specifically located in different skin compartments. In the immune mechanism of psoriasis DCs may be important drivers thanks to their capacity to stimulate resting T cells. In psoriatic plaques a high number of immature mDCs and pDCs has been observed (Prignano et al., 2009) while in the unaffected skin of psoriatic patients only pDCs are increased, suggesting a precise role of this subset in the progression of the disease (Nestle et al., 2005).

DCs produce several pro-inflammatory cytokines such as interleukin-17 (IL-17), interleukin-22 (IL-22), interleukin-23 (IL-23), and tumor necrosis factor (TNF)-alpha (Zaba et al., 2009). The central role of TNF-alpha in psoriasis was clearly confirmed by the therapeutic efficacy of TNF-alpha-targeting agents (etanercept, infliximab, and adalimumab) (Marble et al., 2007), but the exact molecular and cellular mechanisms in which IL-17 is involved are not fully understood. IL-17 is produced by a T helper subfamily, i.e. Th17, which have been detected in dermal infiltrates of psoriatic lesions as well as in synovial fluid (Lowes et al., 2008). This cytokine specifically acts on keratinocytes increasing the expression of other chemokines, which recruit most of the cells participating to the plaque formation, i.e. mDCs, Th17 cells, and neutrophils (Gaffen, 2009; Lin et al., 2011). The key regulator cytokine for Th17 and IL-17 production is IL-23, mainly synthesized by DCs (Jariwala, 2007). In the last few years, also anti-IL-17 agents showed their effectiveness in reducing psoriasic plaques in clinical trials (Girolomoni et al., 2012).

However, up to now, experimental and clinical evidences concerning epidermal LCs in psoriasis are puzzling. Prignano et al. (2009) and other Authors thereafter (Alshenawy and Hasby, 2011; Fujita et al., 2011) showed an increased number of Langerin-positive DCs in the epidermis of psoriatic patients, while some previous studies showed a reduction of LCs within psoriatic plaques (Jones et al., 1994). These contrasting results are partially due to the lack of in vitro/in vivo models for studying the cellular processes of psoriasis, in particular the direct effect(s) of pro-inflammatory cytokines on human epidermal cells.

We have recently demonstrated that IL-17 and TNF-alpha strongly inhibited keratinocyte proliferation in a three-dimensional model of normal human skin culture mimicking a psoriatic microenvironment (Donetti et al., 2014). In the present study we considered the effects of these two cytokines in the same experimental setting on the morphology, immunophenotype, and number of epidermal LCs by immunofluorescence and transmission electron microscopy analysis.

Materials and methods

Organotypic cultures and cytokine exposure

Normal human skin explants were obtained from plastic surgery of healthy 20–40-year-old women ($n = 7$) after informed consent. The bioptic fragments were placed epidermis upwards at air–liquid interface in a Transwell system (Costar, Corning, NY, USA) and maintained at 37 °C and 5% CO_2 overnight in order to reduce the acute effects of surgery as previously described (Bedoni et al., 2007). No hydrocortisone was added to avoid an anti-inflammatory activity on epidermal keratinocytes as previously described (Donetti et al., 2014). Specimens were further divided in three different groups before adding either 100 ng/ml TNF-alpha (TNF-alpha group) or 50 ng/ml IL-17 (IL-17 group) or a combination of both cytokines (IL-17 + TNF-alpha group). Samples were harvested 24 and 48 h after cytokine addition and were processed for light microscopy analysis. Samples harvested at 24 h were also processed for transmission electron microscopy. For each patient, represented in all experimental groups, a control sample was cultured without any cytokine (control group).

Morphological analysis

Three specimens (5 mm × 5 mm) from each patient were frozen using the embedding medium for cryostat (Killik, Bio Optica), placed at −80 °C, and cut with a cryostat (HM 500, Microm, Zeiss).

Further bioptic fragments (2 mm × 2 mm) were immersion-fixed in 3% glutaraldehyde diluted in 0.1 M Sorensen phosphate buffer (pH 7.4) overnight at 4 °C. Each sample was then repeatedly washed with Sorensen phosphate buffer (3 washes of 30 min), post-fixed in 1% osmium tetroxide, dehydrated through an ascending series of ethanols, and embedded in Durcupan (Durcupan, Fluka, Milan, Italy). Semithin sections (2 μm) were stained with toluidine blue. Ultrathin sections were obtained with an Ultracut ultramicrotome (Reichert Ultracut R-Ultramicrotome, Leika, Wien, Austria) and stained with uranyl acetate/lead citrate before examination by a Jeol CX100 transmission electron microscope (Jeol, Tokyo, Japan). At least 30 cells were observed for each experimental group.

Quantitative analysis of epidermal LCs

For immunodetection of LCs on frozen skin sections, samples were placed for 10 min at room temperature (RT) and then immersion-fixed in cold acetone for 10 min at 4 °C. Sections were then air-dried, washed with phosphate buffer saline (PBS) 0.1 M (pH 7.4) three times for 5 min each, and then incubated with PBS–bovine serum albumine (BSA) 2% for 1 h to block non-specific binding sites. As primary monoclonal antibody a mouse anti-human Langerin was used (Immunotech, France) at a dilution of 1:100 in PBS–BSA 0.5% for 2 h at RT, washed with the same buffer three times for 5 min each, and then incubated with the secondary antibody goat anti-mouse TRITC (Jackson Immunoresearch, Baltimora, USA) 1:100 in PBS–BSA 0.5% for 1 h at RT. After 3 washes in PBS–BSA 0.5%, sections were incubated with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) 1:50,000 in PBS 0.1 M (pH 7.4) for 5 min at RT for a nuclear counterstaining and then washed with H_2O for 10 min. Sections were mounted with Mowiol 4-88 (Calbiochem; La Jolla, CA). Negative technical controls were treated as described above, but the primary antibody was omitted and substituted with PBS–BSA 0.5%.

For the quantitative analysis of LCs at least three immunofluorescence experiments were carried out in samples harvested at 24 and 48 h, with two slides per sample and no less than two sections on each slide. Two independent double-blinded investigators counted the Langerin-positive bodies of LCs localized in the epidermis using a 40× objective. Results were expressed as percentage of LCs per mm^2 of living epidermis + 1 standard deviation, considering control as 100%. Epidermal area was calculated on adjacent hematoxylin and eosin-stained sections, excluding the stratum corneum, to normalize the immunofluorescence counts. For the area measurement the software Image Pro-Plus (version 4.5.019; Media Cybernetics, Inc., Silver Spring, MD, USA) has been used following a previously standardized procedure (Donetti et al., 2005). All histological and immunofluorescence analysis were

Download English Version:

https://daneshyari.com/en/article/2178415

Download Persian Version:

https://daneshyari.com/article/2178415

Daneshyari.com