Monocyte-derived macrophages and dermal fibroblasts-derived ECM making small talk

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Introduction: 3D in vitro skin models allow studying molecular pathways that otherwise are only possible to explore using animal models. The incorporation of immune cells, such as macrophages, has been implemented to create immunocompetent analogues. Yet, the human biology representation in those models is limited, among other aspects, by the minimal extracellular matrix (ECM) mimicry[1]. In order to tackle this limitation, we have established a protocol to extract fibroblasts-own ECM. In this work, we hypothesised that those ECM extracts were not able to promote macrophage activation, which could compromise the physiologic phenotype of the model.

Methods: Structural (strECM) and soluble (sECM) ECM components were extracted from fibroblasts cultures using an in-house established approach. Blood monocytes were differentiated into macrophages (MDMs) with M-CSF (50ng/mL) and exposed to a range of ECM concentrations (5, 25 and 50 µg/mL - sECM or 250, 500 and 1000 µg/mL - strECM). The response to the ECM was also assessed in the presence of TNFα (50ng/mL and 100ng/mL), to understand its influence on a pro-inflammatory signal. Pro-inflammatory control cells were attained with LPS/IFNγ (10ng/mL,20ng/mL). MDMs response was monitored after 24h of exposure by RT-PCR (NF-kB, STAT1, PPARG and JMJD3), flow cytometry (CD14, CD86, CD197, CD319, CD206,) and immunofluorescence (CD86, CD206) analyses, and multiplexing of cells' supernatants (IL-6, CXCL10, TNF-α, CCL18, CCL22).

Results: Exposure to the ECM extracts led to downregulation of transcription factors associated with pro-inflammatory pathways (NF-kB, STAT1) and unchanged mRNA expression of anti-inflammatory (PPARG and JMJD3) genes. Moreover, lower/unchanged levels of pro-inflammatory surface markers (CD86, CD197, CD319) were detected. All cytokine's levels were similar to the unstimulated MDMs condition, with the exception of CXCL10 that was increased in the presence of the lowest sECM concentration. When MDMs were simultaneously exposed to ECM and TNFα, only the expression of the anti-inflammatory molecule CD206 was observed.

Discussion & Conclusions: Fibroblast-derived ECM support MDMs basal or anti-inflammatory phenotype, even if obtained from different donors. Moreover, the presence of ECM seems to attenuate the response to a pro-inflammatory stimulus. Overall, our study shows that cells-own ECM do not activate macrophages, warranting the representation of the physiologic skin phenotype although might have a role in an inflammatory microenvironment.

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References:1. Chau, D.Y.S., et al., *The development of a 3D immunocompetent model of human skin.* Biofabrication, 2013. **5**(3): p. 035011.