IMMUNOCOMPETENT CELLS INFILTRATION INTO RECONSTRUCTED HUMAN CORNEAL EPITHELIUM: APPLICATION TO DRY EYE SYNDROME

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INTRODUCTION

Inflammatory mechanisms play a pivotal role in regulating the ocular surface environment. It was demonstrated that anti-inflammatory factors such as the expression of vascular endothelial growth factor receptor 3 (VEGFR-3) in corneal cells, increase corneal resident antigen-presenting cells and regulatory T cells play an active role in protecting the ocular surface. In particular, inflammation plays a crucial role for the chronicity of Dry eye disease (DED), its symptoms, and its clinical signs. Dry eye symptoms range from mild, transient irritation to persistent dryness, burning, redness, stinging, sensation of foreign body, and visual disturbance; furthermore, molecular modifications related to dry eye symptoms are modifications of the structural components of the corneal surface (corneal trehalose network). Corneal epithelium is an avascular tissue but keratocytes, and hematopoietic cells respond to inflammation by changing phenotype and Antigen presenting cell (APC) could be trafficking from the eye. Furthermore, it is well known that intraocular lens and DRY EYE are often associated to the use of preserved eye drops. Moreover, in the case of glaucoma treatment, compliance may be further compromised by the development of ocular surface disease caused, not by the active component of treatment, but by the preservatives used to protect from bacterial contamination.

AIM OF THE STUDY

In order to better recapitulate the corneal response to inflammatory diseases like DED and preservatives toxicity mechanism taking into account a possible contribution of antigen presenting cells (APC) in an in-vitro immunocompetent model of 3D reconstructed human corneal epithelium (HCE-CMM) has been developed by a co-culture with THP-1 cells allowing the infiltration of APCs into the corneal tissue as a consequence of different toxic and inflammatory stimuli.

This research project is still under development in order to demonstrate the following rationale treatment with chemicals known as irritant for the eye or different strategies by activating the corneal epithelial inflammatory response could stimulate APCs to be trafficking from the eye thus involving the whole ocular response. The first step of the project being to set up the experimental conditions of the co-culture and to have evidences at molecular and morphological level of THP-1 cells recruitment and infiltration to the corneal tissue suffering their differentiating position and immunological role. The final goal being to increase in reproducible and reliable experimental conditions a 3D corneal immune-competent system.

THP-1 CELL LINE

THP-1 cells, Acute Monoctytic Leukemia, are supplied by Interlab Cell Line Collection (ICLC, code 983 - B0015)

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In the Fig. 1 are reported the major inflammatory immunosignals on normal ocular surface and their disruption in dry eye disease.

Materials and Methods

The infiltration and migration process of THP-1 in the HCE-CMM model has been monitored after 4h by Immunofluorescence technique trough expression and localization of CD68 and CD14 in THP-1 cells and at transcriptional level -mRNA gene expression of TNF-α, CD14, Cytokines and 8-18 by qPCR.

- Immunofluorescence: performed on FFPE sections using anti-human rabbi polyclonal CD14 (HPA002137) and CD68 (HPA014519) from Sigma-Aldrich. All the images have been captured by using Leica Instruments (DinoEye microscope or SP2 confocal microscope) and LASX software.

- Gene expression: total RNA has been extracted from duplicate HCE samples (RNAqueous kit, Life-technologies) and retro transcribed in cDNA (QUIAqplex kit, Life-technologies). RTqPCR analysis by ΔΔCt methods to quantify expression levels of target genes and GAPDH (as reference gene) from primary human corneal epithelium (HCE-CMM) has been exposed to different conditions: BAC (0,01%).

CONCLUSION

The 3D corneal surface immune-competent system is still under development and it seems to be a promising and innovative tool for research study focusing immunomodulatory therapies to effectively treat inflammatory based-ocular surface diseases.

The treatment with BAC has modified the inflammatory response directly activating THP-1 cell differentiation into mature dendritic cells under following BAC exposure. On the right it is very well visible the structure of the differentiated cell with its dendrites (arrow).

ACKNOWLEDGMENTS

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It is interesting to discover that presence of THP1 cells infiltrated and not infiltrated visible on the tissue filter (yellow arrows) induces a signal that upregulated the CD14 expression of in the corneal tissue and THP1 cells undifferentiated and not yet infiltrated into the tissue (white arrow) are visible on HCE filter.

HCE-CMM : 3D RECONSTRUCTED HUMAN CORNEAL EPITHELIUM

The EPISCREEN Reconstructed Human Corneal epithelium of 0,5 cm² is produced by Episkin (Lyophil-Epipak) Ethical human immortalized cells (HCEC) are deposited on a polycarbonate filter and cultured in a chemically-defined medium in order to form a structured epithelium. The HCE model (standard inserts) has been validated by EURL-EUVIM (EPISKIN HCE Epithelial Irritation Test) as alternative method to identify chemicals not requiring classification and labelling for eye irritation or serious eye damage (OECD 492, Revised July 2016).

The density and the diameter of the pores of the membrane of the HCE standard model allows medium diffusion for cell nutrition but are too small for cells circulation.

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