KERATITIS

Background

Microbial keratitis is a common ocular infection caused by bacteria, fungi, viruses or parasites. Breakdown in ocular immune privilege by infections resulting in inflammation of the cornea that can lead permanent eye damage in untreated infection. Keratitis is the second most significant cause of monocular blindness, particularly in certain developing countries.

Pathology Model

Our in vitro models are based on the activation of Toll-like receptors (TLRs) on Human corneal epithelial cell (HCEC), upon recognition of the specific ligands, in order to reproduce a pathogen-associated molecular pattern and to induce innate immune response. Human corneal epithelial cell are the first line of defence to protect cornea from harmful pathogens. TLRs are innate sensors that detect pathogen associated molecular patterns from a variety of pathogens and active rapid host innate immune response to microbial. Recently the expression of functional TLRs was been demonstrated in the eyes.

HCEC line will be cultured in the presence of specific TLR ligand/s and their functional parameters will be evaluated. The choice of the specific TLR ligand will be done on the basis of the different (antimicrobial) compound analyzed:

- TLR2 agonist: heat-killed bacteria Gram-positive, Gram-negative;
- TLR3 ligand: a synthetic analog of dsRNA (polyinosinic-polycytidylic acid: poly(I:C)), associated to viral infection;

TLR4 ligand: LPS, the major structural component of the wall of all Gram-negative bacteria. Recently TLR4 was recognized as a receptor for protozoan (such as *Acanthamoeba*)

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Readouts

Cells will be quantitatively assayed for the following functional readouts:

- **Cell viability, toxicity and proliferation:** quantitative evaluation of OCP fibroblast proliferation and modulation by selected Client's compounds.
- **Apoptosis:** qualitative evaluation of the apoptotic caspases-3 and -7 in order to evaluate the modulation of programmed cell death by Client's compounds.
- **Chemokines/Cytokines profile:** the expression profiles of pro- and anti- inflammatory factors and chemokines will be characterized (i.e. IL1, IL6, TNF□, IL24, CXCL1, CCL20) using real-time PCR and multiplex bead-based immunoassay.
- **Antimicrobial peptides expression:** the expression of peptides with known antimicrobial activity, such as: beta-defensines (hBD), Cathelicidin LL-37 will be evaluated by real-time PCR and immunoenzymatic assay in cells treated with TLR ligand or proinflammatory cytokines IL1b and TNFa.