Ocular Diseases

OCULAR CICATRICIAL PEMPHIGOID

Background

Ocular cicatricial pemphigoid (OCP) is a systemic autoimmune disorder characterized by recurrent episodes of inflammation and progressive subepithelial conjunctival fibrosis, as a result of excessive deposition of matrix proteins.

The disease is associated with autoantibodies directed against basement membrane zone proteins, together with recruitment and accumulation of macrophages in the conjunctiva and inflammation. Chronic inflammation and repair lead to excessive deposition of connective tissue elements that remodel and destroy normal tissue architecture.

Pathology Model

1. **Direct challenge** - To reproduce the pathological microenvironment, fibroblast will be cultured in medium containing cytokines over-expressed in the disease (TNFα, IL4 or IL13) and their functional parameters will be evaluated as reported below.

2. **Immune cell mediated challenge** - To better reproduce the pathological microenvironment, fibroblasts will be cultured in microfluidic connection with conditioned medium derived from macrophages, important participants in immunoinflammatory diseases.

In this model, THP-1 monocytic cell line-derived macrophages will be used. THP-1 cells will be induced to differentiate into macrophages with phorbol 12-myristate 13-acetate (PMA) and then cultured 48h to obtain macropaghe-conditioned medium with high levels of pro-inflammatory cytokines, for fibroblasts exposure.

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Readouts

RGC cells will be assayed for the following biological responses:

- **Cell proliferation and vitality:** quantitative evaluation of OCP fibroblasts proliferation (MTT assay) and modulation by selected Client's compounds
- **Immunomodulators expression:** the expression profiles of immune mediators will be examined in OCP fibroblasts using real-time PCR and multiplex bead-based immunoassay. TNFa, IL13, IL4, macrophage colony-stimulating factor (mCSF), TGF-1, conjunctive tissue growth factor (CTGF), MMPs, TIMPs, macrophage migration inhibitory factor (MMIF) will be analyzed.
- **Flow cytometric analysis of surface molecules** involved in crosstalk between fibroblasts and T lymphocytes: it will be evaluated the expression of ICAM, CD80, CD86, CD40, CD40-ligand on the surface of OCP fibroblasts.