MUCOSAL DISEASES (ORAL, ANOGENITAL), EXTERNAL EYE DISEASE

DEVELOPMENT OF IN VITRO RECURRENT APHTOUS STOMATITIS (RAS) IMMUNO-COMPETENT MODEL

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Introduction: Recurrent aphthous stomatitis (RAS) is a common ulcerative disease of the oral mucosa characterized by an increase of the mucosal permeability and disorders in the immune response modulation, in particular with a Th1 polarization, with pro-inflammatory cytokines induction and apoptosis.

Objective: To recapitulate the features of RAS in vitro, the human reconstituted oral mucosa (HOE) produced on special polycarbonate CMM inserts, allowing an efficient infiltration and co-culture with monocytes (THP-1 cells) was used. The model was based on a superficial localized ulcer associated to a pro-inflammatory stimulus that resulted in THP-1 cell migration, allowing an immuno-modulated tissue response, and mirroring the most relevant mechanisms related to aphtous lesions.

Materials & Methods: HOE-CMM subjected to a lesion and challenged with the systemic incubation in 1,5 ng/mL TNF-α, was then co-incubated with THP-1 cultured on the CMM polycarbonate membrane insert for 4h. After that, the treatment with the reference molecule 4mg/mL betamethasone was done topically for 24h. The tissue response was evaluated by TUNEL assay on the histological sections and by qRT-PCR of inflammatory genes (IL-1 β, TLR-2).

Results: The RAS model has been recapitulated in vitro as evidenced by the apoptotic cells detection on the HOE superficial layer, down-regulation of TLR-2 and concomitant IL-1β release upon THP-1 stimulation in induced inflammatory conditions, indicating the establishment of a Th-1 polarization.
The reference molecule, betamethasone, caused a significant anti-inflammatory efficacy and immunosuppressive effect, as expected, without modifications at morphological level, confirming the well-known mechanism of corticoids in delaying tissue re-epithelization.

Conclusions: The lesional inflamed HOE-CMM cell migration model appears as a biologically relevant model mimicking the RAS features, suitable for the efficacy assessment of new potential anti-inflammatory products designed to accelerate the healing process of the aphtous lesion.