Assessing the effect of allergens, Toll-like receptor ligands and calcitriol on immune responses in an in vitro model of canine primary sublingual epithelial cells

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Contact person

Eric Cox

Organisation

Name of the organisation Ghent University (UGent)

Department Department of Virology, Parasitology and Immunology

Country Belgium

Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo

Species from which cells/tissues/organs are derived	Dogs
Type of cells/tissues/organs	Primary sublingual epithelial cells

DESCRIPTION

Method keywords

epithelial cells

Dermatophagoides farinae

Toll-like receptor

Calcitriol

Cxcl8

Dog

Scientific area keywords

Sublingual immunotherapy

Allergen-specific

Desensitization

Allergic disease

Method description

The response of sublingual epithelial cells to house dust mite allergen and potential tolerance-promoting adjuvants such as Toll-like receptor (TLR) ligands and calcitriol was investigated using primary sublingual epithelial cells isolated from dogs and cultured *in vitro*. After 24-h incubation with a Dermatophagoides farinae extract, a Dermatophagoides pteronyssinus extract, TLR2 ligands (FSL-1, heat-killed Listeria monocytogenes, Pam3CSK4), a TLR3 ligand (poly I:C), a TLR4 ligand [lipopolysaccharide (LPS)], and calcitriol (1,25-dihydroxyvitamin D3), viability of the cells was analyzed using an MTT test, and their secretion of interleukin 6 (IL-6), IL-10, CXCL8, and transforming growth

factor ?1 (TGF-?1) was measured by enzyme-linked immunosorbent assay. Additionally, to evaluate its potential effect as an adjuvant, sublingual epithelial cells were incubated with calcitriol in combination with a D. farinae extract followed by measurement of CXCL8 secretion. Furthermore, the effect of D. farinae and calcitriol on the transcriptome was assessed by RNA sequencing. The viability of the sublingual epithelial cells was significantly decreased by poly I:C, but not by the other stimuli. CXCL8 secretion was significantly increased by D. farinae extract and all TLR ligands apart from LPS. Calcitriol significantly decreased CXCL8 secretion, and co-administration with D. farinae extract reduced CXCL8 concentrations to levels seen in unstimulated sublingual epithelial cells. Although detectable, TGF-?1 secretion could not be modulated by any of the stimuli. IL-6 and IL-10 could not be detected at the protein or at the mRNA level. It can be concluded that a D. farinae extract and TLR ligands augment the secretion of the proinflammatory chemokine CXCL8, which might interfere with sublingual desensitization. On the other hand, CXCL8 secretion was reduced by coapplication of calcitriol and a D. farinae extract. Calcitriol therefore seems to be a suitable candidate to be used as adjuvant during sublingual immunotherapy.

Lab equipment

- Cell culture equipment;
- Fluorescence microscope;
- Flow Cytometer.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

Fast screening of inflammatory or tolerogenic responses induced by molecules of micro-organims, plants and proteins animal origin. Also fragments, extracts or suspensions can be screened.

Challenges

The method uses an epithelial cell line. There is currently no co-culture with fibroblast and/or cells of the immune system (antigen-presenting cells and/or lymphocytes). The interaction is static, whereas *in vivo* mucosa are exposed to potential allergens in a dynamic context.

Modifications

The development of more complex 3D cultures in which immune cells will be incorporated will be a next step.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Pelst MP, Höbart C, Wallaeys C, De Rooster H, Gansemans Y, Van Nieuwerburgh F, Devriendt B and Cox E (2020) Adjuvanting Allergen Extracts for Sublingual Immunotherapy: Calcitriol Downregulates CXCL8 Production in Primary Sublingual Epithelial Cells. Front. Immunol. 11:1033. doi: 10.3389/fimmu.2020.01033

Links

Laboratory of Immunology, Department of Virology, Immunology and Parasitology, …

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