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ESTABLISHMENT OF A CELL CULTURE OF CANINE ORAL KERATINOCYTES: ALPHA6 AS A MARKER FOR POSITIVE SELECTION

Hypothesis of the study

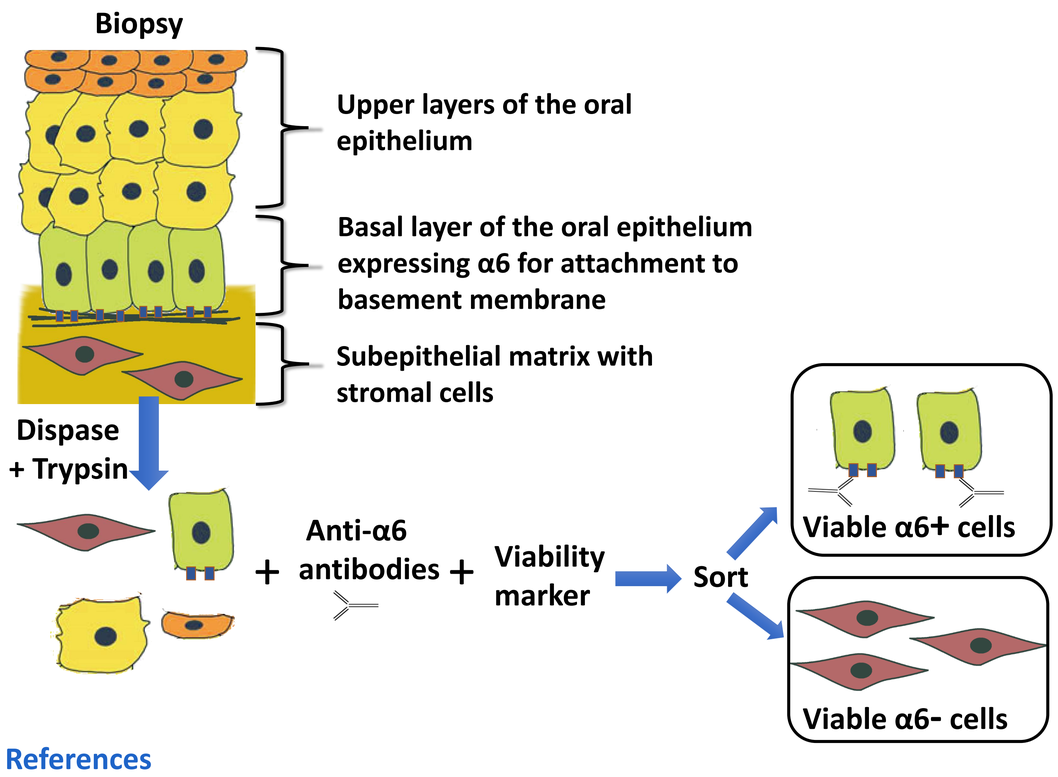
The surface marker α6 (CD49f) can be used to select canine primary oral epithelial cells derived from biopsies in order to establish a cell culture of purified oral keratinocytes.

Background

Apart from forming an important physical barrier, the oral epithelium is an immunologically active first contact zone to a variety of non-self antigens. For skin keratinocytes such an immunologic response has been shown to influence subepithelial cells, such as antigen-presenting cells, with a potential impact on the development of antigen-specific diseases like allergies.[1] To objectively study these immune responses in oral keratinocytes, a homogenous cell culture should be generated. Human oral keratinocyte stem cells can be selected using the integrin subunit α6,[2] a surface marker which is also expressed by canine epidermal keratinocytes.[3] In this study we evaluated whether α6 can be used to characterize and positively select basal keratinocytes in a cell population derived from a biopsy of the canine oral mucosa.

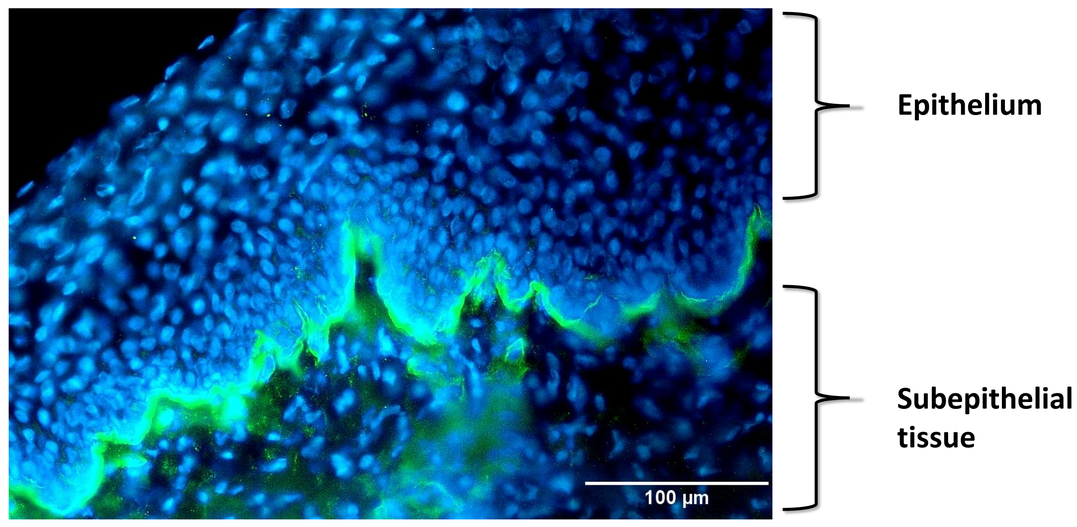
Methods

Oral biopsies were collected from dogs that were euthanized at the faculty clinic for causes independent of this study. One half of each biopsy was snap-frozen in liquid nitrogen and cryosections were stained for α6. The other half was used to generate a purified population of epithelial cells using the following procedure:

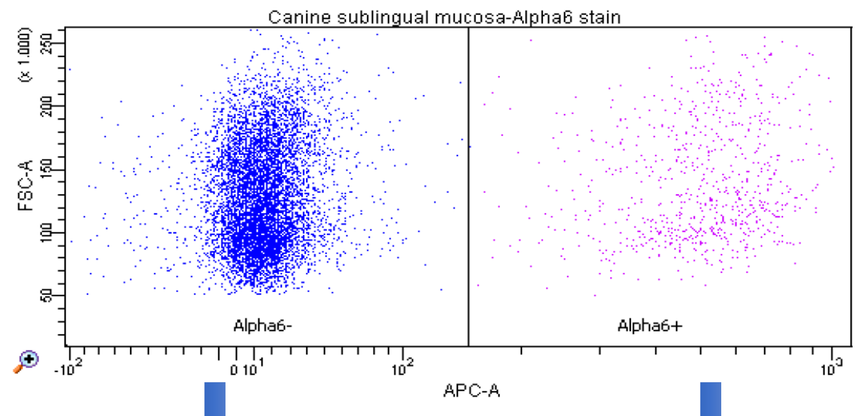


Results

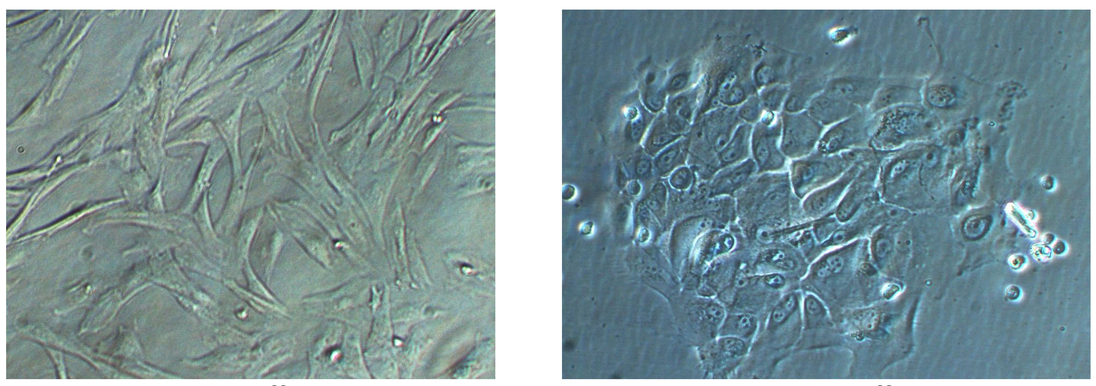
Cryosection of the canine sublingual mucosa stained for α6



Sorting of α6- and α6+ canine oral mucosal cells



96h in culture



α6- cells | α6+ cells

Conclusion:

The integrin subunit α6 can be used to positively select canine oral keratinocytes. The establishment of a purified cell culture can facilitate our understanding of the immunologic signalling mechanisms generated by oral keratinocytes. It will allow to map the cell mediators that are produced by these cells after exposure to a variety of immunologically active molecules such as toll-like receptor ligands and allergens. Knowledge of the repertoire of mediators produced by these cells will provide further opportunities to study how to modulate this immune response for *in vivo* applications such as desensitization strategies for allergies or the oral administration of vaccines.

References

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