Identification of equine blood dendritic cell subsets and evaluation of their significance for allergic diseases and immunotherapy in horses

Aim
The aim of our project is to identify and characterise dendritic cell subsets in the blood of horses, with special focus on plasmacytoid dendritic cells.

Background
Horses can be affected by various allergic diseases, such as Insect Bite Hypersensitivity or Recurrent Airway Obstruction. Dendritic cells (DC), as professional antigen-presenting cells, can initiate and modulate the allergic immune response. Plasmacytoid DC (pDC) in particular have been shown to play a crucial role in inducing tolerance to inhaled allergens by exerting an inhibitory effect on the development of Th2 responses through the secretion of IFN-α. They could therefore be potential key players in the efforts to improve allergen-specific immunotherapy for equine allergic diseases. However, while DC subsets are well characterised in humans and mice and research is ongoing in several veterinary species, very little is yet known about DC subsets in horses.

Project Description
Peripheral blood mononuclear cells (PBMC) from healthy horses will be used for phenotypical identification of putative DC subsets by flow cytometry. For the immunophenotyping a wide range of fluorescently-labelled cell surface markers will be employed. The list includes, but is not limited to, monoclonal antibodies against CD4, CD11b, CD11c, CD13, CD14, CD16, CD172a, MHC class II and CADM-1 that are either equine-specific or cross-react with equine cells, as well as recombinant bovine Flt3 ligand and recombinant equine IL-3.

First results show that IL-3 receptor expression, detected by binding of fluorescently-labelled IL-3, appears to be a suitable tool to identify pDC. Further, we could show a strong production of IFN-α in a cell population containing IL-3R⁺ cells, upon stimulation with a TLR-9 agonist and equine herpesvirus-1. Meanwhile, no IFN-α production could be detected in IL-3R⁻ that had undergone the same stimulation conditions.

These results indicate that the IL-3R⁺ cells are in fact equine pDC. In a next step, we are planning to sort the IL-3R⁺ cells and perform further functional assays for firm identification of the pDC. Also, we aim to assess the effect of pDC and their IFN-α on
the allergic T-cell response. For this purpose, equine monocyte-derived dendritic cells (MoDC) will be generated using recombinant equine IL-4 and GM-CSF and phenotypically analysed using antibodies against CD14, CD206, CD80, CD86 and MHC class II. These MoDC will then be exposed to various equine allergens and matured using a number of pro-inflammatory cytokines, such as IL-1, IL-6, TNF-α and IFN-γ. The mature MoDC will be co-incubated with autologous T-cells from healthy or allergic horses in the presence or absence of IFN-α secreted by pDC or, if cross-reacting, recombinant human IFN-a. The effect on T-cell proliferation and polarization will be evaluated by Thymidine proliferation assay and intracellular cytokine staining.

**Significance**
In order to improve allergen immunotherapy for horses, a more detailed knowledge about equine dendritic cells and their role in the induction and modulation of the allergic immune response is crucial. By characterizing equine DC subsets and proposing a classification in line with what has been found in other species, this study will contribute to this knowledge. Furthermore, we will hopefully identify possible applications for plasmacytoid dendritic cells in equine immunotherapy.

**References**
(1) Deifl et al. Differential activation of dendritic cells by toll-like receptors causes diverse differentiation of naïve CD4+ T cells from allergic patients. *Allergy* 69, 1602-7
(3) Summerfield et al. Comparative Dendritic Cell Biology of Veterinary Mammals *Annu Rev Anim Biosci* 3, 533-57. Review

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recombinant human cytokines: rh IFN-a

human IL-6 ELISA-set for 96 wells, (3 reagents)

recombinant equine cytokines: req IL-1a, req IL-1RA, req IL-3, req IL-4, req IL-6, req IL10, req TNF-a

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