Decoding Equine IBH: Single-Cell Insights into Allergen-Specific Immune Dysregulation

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Equine insect bite hypersensitivity (IBH), an IgE-mediated allergic dermatitis caused by *Culicoides* midge bites, severely impacts horse welfare. Despite its prevalence, its systemic immunopathogenesis remains partly uninvestigated. Allergen immunotherapy (AIT) using recombinant *Culicoides* allergens has emerged as potential treatment option. Icelandic horses, originally allergen-naïve in Iceland, develop IBH at a >50% prevalence within two years post export to *Culicoides*-endemic regions.

This study utilizes single-cell RNA sequencing (scRNA-seq) for high-resolution immune response analysis within the unique model of Icelandic horses—*Culicoides*-naïve in Iceland with high IBH prevalence post-export—to investigate systemic immune dysregulation and enable the evaluation of preventive allergen immunotherapy (AIT).

For our preventive AIT trial, Icelandic horses were immunized before export to Switzerland and monitored for three years. At study's conclusion, PBMCs from IBH-affected and healthy horses, alongside independent Swiss-born non-immunized controls, were restimulated with *Culicoides* allergens and cultured in medium alone. Cultivated PBMCs were sequenced using 10x Chromium scRNA-seq technology. Data was processed with Seurat toolkit, followed by manual annotation of different immune cell types.

We identified twenty-seven distinct immune cell populations, including previously undescribed Th1 and Th2 cells, M2 macrophages, and transitional dendritic cells (tDCs) in horse PBMCs. Differential gene expression analysis in Swiss-born IBH-affected and healthy horses revealed allergen-specific activation in IBH. Specifically, basophils from IBH exhibited increased FCER1A expression, protein synthesis, redox regulation, and a shift in lipid and glucose metabolism, suggesting heightened IgE-mediated responses and oxidative stress. Dendritic cells showed Th2 polarization, upregulated antigen processing, and lipid metabolism, contributing to allergic inflammation. Notably, no transcriptional differences were observed in unstimulated cells, highlighting the allergen-driven immune dysregulation in IBH.

Our findings define a molecular basis for IBH pathogenesis, pinpointing basophils and dendritic cells as key early drivers of allergy and provide a framework to assess the immune-modulatory effects of preventive AIT in Icelandic horses.

#SingleCellRNAseq #Allergy #EquineResearch