Research Focus 3 – Identification of Individual Target Structures and CO-Factors for the Targeted Modulation of the Allergic Immune Response:

- **Exploration of the immune modulatory properties of the flagellin:allergen fusion protein rFlaA:Betv1**

Adjuvants conjugated to antigens, including fusion proteins incorporating the TLR5- and NLRC4-ligand flagellin, have been repeatedly described to have strong immune modulating properties, making them interesting candidate therapeutics in allergen-specific immunotherapy. To further study the immune modulating properties of such fusion proteins as well as their underlying mechanisms, we generated a recombinant flagellin:antigen fusion protein incorporating the major birch pollen allergen Bet v 1 (rFlaA:Betv1).
rFlaA:Betv1 significantly suppressed allergic sensitization in vivo in mice while also inducing tolerogenic, IL-10-producing myeloid dendritic cells (mDCs) in vitro. Interestingly, the tolerogenic phenotype of these rFlaA:Betv1-stimulated mDCs was shown to depend on a JNK-MAPK-dependent activation of the mTOR pathway. mTOR is a conserved serine/threonine protein kinase that belongs to the phosphatidylinositol 3-kinase-(PI3K) family. mTOR not only integrates various nutritional and environmental stimuli, including levels of growth factors, cellular energy reserves, and stress levels, but is also a master regulator of cellular metabolism that affects innate and adaptive immune responses. mTOR activation in rFlaA:Betv1-stimulated mDCs resulted in alterations of mDC metabolism with increased rates of glycolysis, Warburg metabolism, and reduced mitochondrial respiration. Inhibition of either glycolysis or mTOR activation suppressed rFlaA:Betv1-induced IL-10 production. In addition to glycolysis, fatty acid synthesis also significantly contributed to rFlaA:Betv1-mediated cytokine secretion, the production of antimicrobial molecules, and the modulation of T cell responses.
Our current studies aim to further understand the molecular mechanisms in mDCs and other cell types contributing to the immune modulating properties of flagellin:antigen fusion proteins. These results will provide valuable insights for the future development of adjuvants and allergen therapeutics.

- **Investigation of the immune modulating potential of novel adjuvants in the context of allergen-specific Th2 responses**

Type I hypersensitivity, or so-called type I allergy, is caused by Th2-mediated immune responses directed against otherwise mostly harmless environmental antigens. Currently, allergen-specific immunotherapy (AIT) is the only treatment with disease modifying properties towards induction of allergen-specific immunological tolerance. However, conventional AIT has certain drawbacks, including long treatment durations, the risk of inducing allergic side effects, and the rather low immunogenicity of isolated allergens.
To improve AIT, adjuvants can be a powerful tool not only to increase the immunogenicity of co-applied allergens but also to induce the desired allergen-specific Th1- or regulatory responses. However so far, the number of adjuvants that are established in AIT are limited. Therefore, better understanding the immunological mechanisms underlying the effects of both novel and already clinically used adjuvants may help to us improve AIT.
In our projects, we analyze the immune activating potential of both novel and established adjuvants (the latter present in approved allergen products) on isolated mouse and human immune cells. In addition to investigating molecules and signal transduction events classically associated with the activation of immune cells, we focus on the functional link between immune cell metabolism and the induction and modulation of immune responses. Here, metabolic

parameters (e.g. metabolic flux analysis using Agilent Seahorse technology, analysis of Warburg, glucose, amino acid, and fatty acid metabolism) can be directly linked at the cellular level with immune cell function (e.g. intracellular signaling, immune cell activation, cytokine secretion, or effector function such as T-cell activation or direct antimicrobial activity) and analysis of OMICs (e.g. transcriptomics).