Preclinical Development of Recombinant Allergen Vaccines

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Currently, conventional allergen immunotherapy (AIT) with allergen extracts is not convenient for patients due to a multi-year treatment regimen. For some allergies, AIT is only partially efficacious and can be hampered by unwanted side effects. To improve AIT, novel vaccine candidates and accompanying adjuvants that increase efficacy while decreasing unwanted adverse-effects are needed.

In this Research Field our Projects Currently Focus on:

1. The evaluation of genetically engineered modular vaccines that act as protein transfer vectors.

As modular vaccines adjuvant:allergen conjugates have several advantages over simple non-conjugated mixtures of both components: (**1**) they target the conjugate to the respective immune cells by binding to specific immune receptors. Upon binding to the target cell they (**2**) deliver the conjugated allergen to the immune cell in the context of the adjuvant-mediated immune cell activation which likely influences allergen uptake, processing, and presentation. Moreover, (**3**) adjuvant and allergen are simultaneously delivered to the same cell in a fixed molecular ratio, thereby preventing potentially detrimental bystander activation. The objective of our studies is to investigate whether the activation of pattern recognition receptors (PRR) such as TLRs expressed on immune cells by TLR-ligands co-administrated with the allergen will be suitable to prevent allergies (Fig. 1). For these projects allergens are either fused or chemically conjugated to TLR-ligands or applied as part of genetically engineered virus-like particles (VLPs) that either display the allergen on their surface or package it inside the particles (Fig. 1).

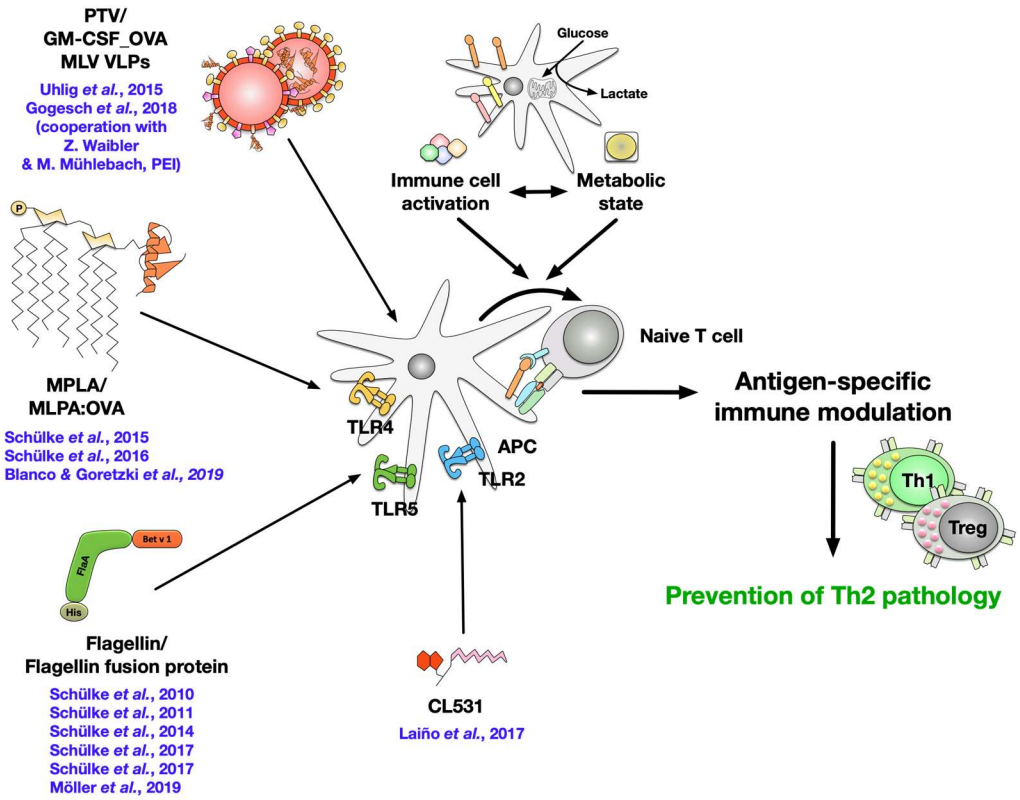

Fig. 1: Overview of projects to modulate antigen-specific immune responses using modular vaccines. Source: PEI

2. The investigation of immune metabolic effects of adjuvants and vaccines.

Over the last years evidence has accumulated suggesting, that classical immune cell activation (intracellular signaling, immune cell activation, and cytokine secretion) and the activation of the respective immune cell's metabolism are closely connected to each other (Fig. 1). To understand the mechanisms underlying these metabolic changes and their functional consequences is the aim of a new research field termed "immune metabolism".

In this context immune cell activation by e.g. TLR-ligands triggers a metabolic state that both fulfills the rapid energy requirements of the activated immune cell. Interestingly, these changes in cell metabolism also both control and contribute to immune effector mechanisms by regulating for example the pattern of the secreted cytokines and providing substrates for the generation of directly anti-microbial effector molecules (e.g. ROS, prostaglandins, or itaconate).

Specific projects we currently work on in this field are:

Generation and investigation of the immune modulating mechanism of flagellin:antigen fusion proteins

The "Toll"-like receptor 5 (TLR5)-ligand flagellin is a bacterial motility protein that forms the main body of the bacterial flagellum. Because of its intrinsic immune activating potential, flagellin was demonstrated to be an effective mucosal adjuvant mediating protective immune responses.

Since flagellin is the only proteinaceous TLR-ligand, its application for the generation of recombinant flagellin:antigen fusion proteins is of special interest in vaccine development. Flagellin-containing fusion proteins have already been shown to be both safe and well-tolerated in clinical trials.

We are currently investigating a fusion protein consisting of the TLR5-ligand FlaA from *Listeria monocytogenes* and the major birch pollen allergen Bet v 1 (rFlaA:Betv1) as a model of how novel types of vaccines can improve the treatment of birch pollen allergies.

In own previous work, rFlaA:Betv1 displayed strong immune modulating properties both *in vivo* and *in vitro*, characterized by a secretion of both pro- and anti-inflammatory cytokines from murine mDCs as well as PBMC from birch allergic patients (Fig. 2). Mechanistically, we showed that stimulation with rFlaA:Betv1 resulted in an increased metabolic activity of the stimulated mDCs, mediated by an activation of mTOR (Fig. 2). Moreover, induction of anti-inflammatory IL-10 secretion by rFlaA:Betv1, but not pro-inflammatory cytokine secretion in mDCs, was inhibited by rapamycin and therefore dependent on mTOR activation showing that immune-modulatory cytokine secretion induced by this vaccine candidate was linked to the activation of mDC metabolism (Fig. 2).

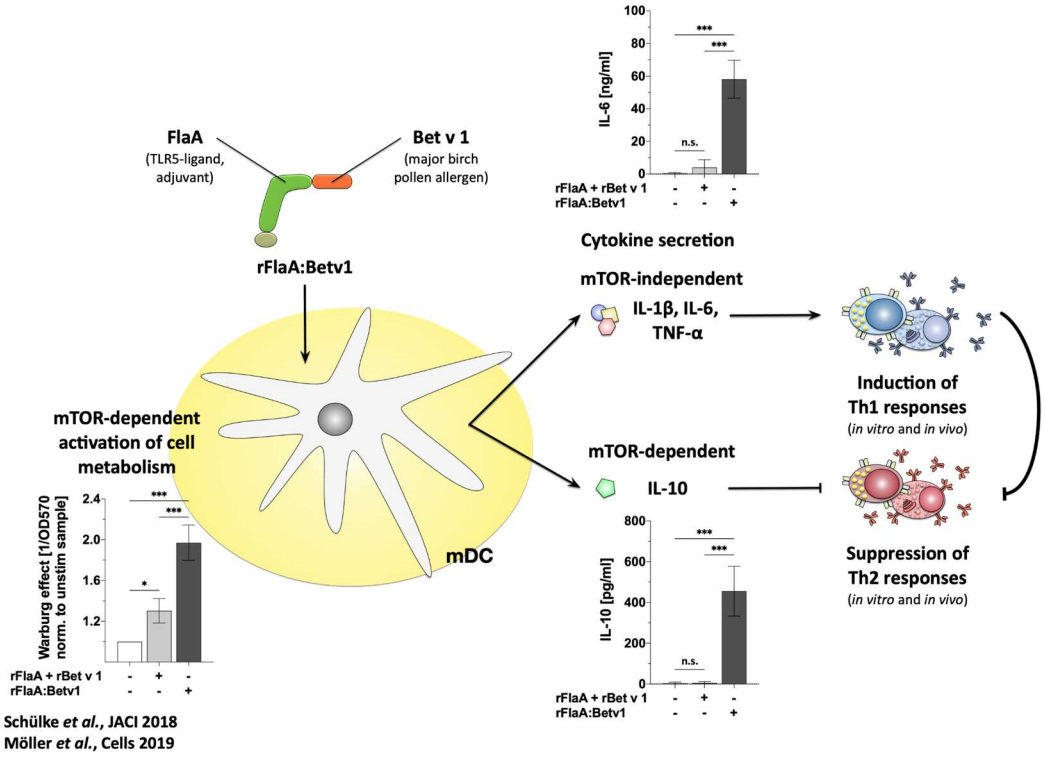

Fig. 2: Proposed mechanism of rFlaA:Betv1-mediated immune modulation. Source: PEI

Activation of immune cell metabolism by the LPS-derivative Monophosphoryl Lipid A (MPLA)

The detoxified TLR4-ligand Monophosphoryl Lipid A (MPLA) is a successfully used adjuvant in clinically approved vaccines. However, its capacity to activate glycolytic metabolism in mDC and the influence of MPLA-induced metabolic changes on immune cell activation are largely unknown. Stimulation of mDCs with MPLA resulted in both pronounced mDC activation and pro-inflammatory cytokine secretion as well as an activation of glucose metabolism. The MPLA-induced activation of glycolytic metabolism in mouse mDCs was shown to depend on JNK MAPK-mediated activation of mTOR-signaling, while both MAPK- and NF$\kappa\kappa$B-signaling contributed to pro-inflammatory cytokine secretion (Fig. 3).

In this context, understanding the mechanisms by which MPLA activates dendritic cells will both improve our understanding of its adjuvant properties and contribute to the future development and safe application of this promising adjuvant.

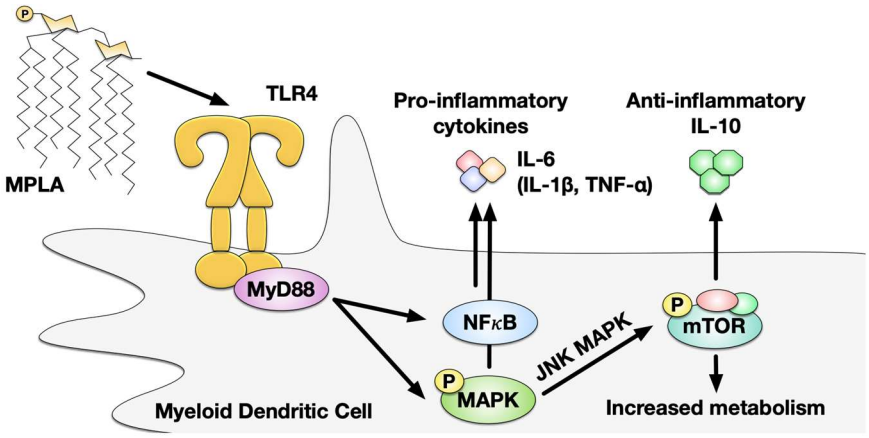

Fig. 3: Effects of the vaccine adjuvant MPLA on dendritic cell activation and metabolism. Source: PEI