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. For these projects TLR-ligands are either genetically fused or chemically conjugated to the allergen. Modified allergens with a reduced IgE-binding capacity (“hypoallergenic” molecules) will be considered as potential allergen vaccines. We hypothesize that hypoallergenic derivatives with retained T cell epitopes will cause lower rates of adverse side effects, while allowing for the application of higher allergen amounts that will result in improved therapeutic outcomes.
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. In this context immune cell activation by e.g. TLR-ligands triggers a metabolic state that 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, NO, prostaglandins, itaconate).

Specific projects we currently work on in this field are:

(a) The investigation of dual TLR2/7 ligands combining TLR2- and TLR7-ligands into a single molecule.

Dual TLR2/7-ligands such as the commercially available CL531 are an interesting strategy to leverage the beneficial immune modulating properties of both single TLR-ligands by ensuring the simultaneous co-stimulation of the target cell with both components. We showed that CL531 induced IL-10 secretion from myeloid dendritic cells, suppressed allergen-specific TH2 responses, was able to suppress DNP-induced mast cell degranulation and suppress allergen-specific IgE production *in vivo* (Fig.1). Therefore we provide evidence, that dual TLR2/7-ligands have the potential to induce TH1-biased immune modulation *in vivo* and are promising adjuvant candidates to further improve the treatment of allergic diseases.

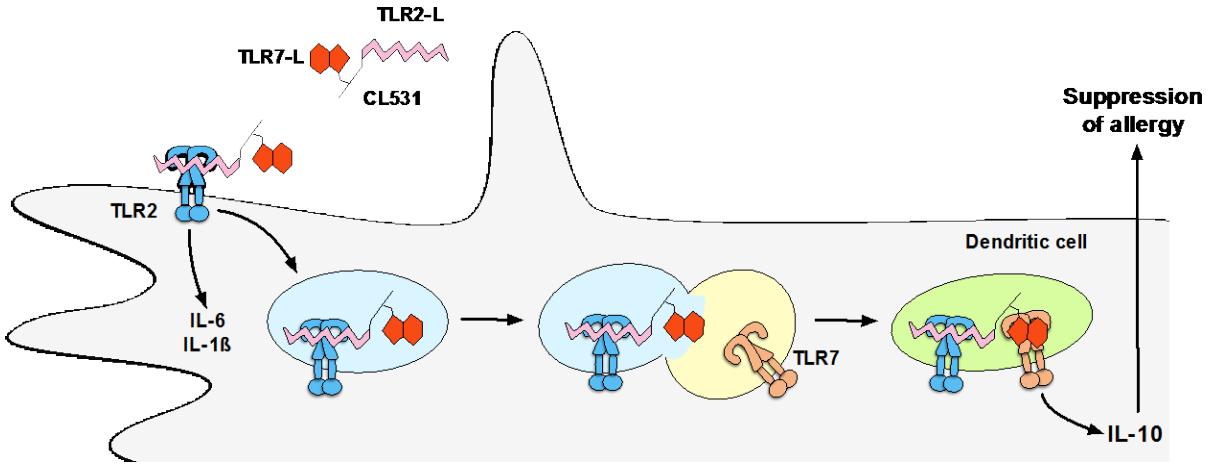

Fig.1. Proposed mechanism of immune modulation by the dual TLR2/7-ligand CL531 in the context of allergy treatment. Source: *PEI*

(b) The generation and investigation of the potency and the immune modulating mechanism of a fusion protein consisting of the TLR5-ligand FlaA from *Listeria monocytogenes* and the major birch pollen allergen Bet v 1.

The fusion protein (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. Mechanistically, we showed that stimulation with rFlaA:Betv1 resulted in an increased metabolic activity of the stimulated mDCs, mediated by an activation of mTOR. 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).

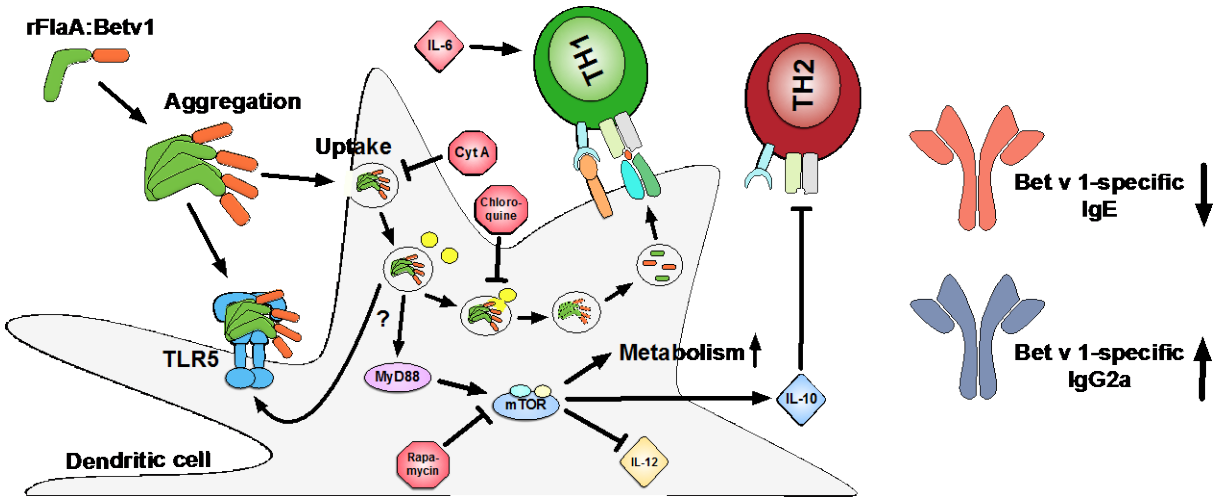

Fig.2: Proposed mechanism of rFlaA:Betv1 induced mDC activation. Source: *PEI*

(c) 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 cytokine secretion are largely unknown. Stimulation of mDCs with MPLA resulted in both a pronounced mDC activation and pro-inflammatory cytokine secretion as well as an activation of glucose metabolism characterized by induction of the Warburg Effect and increased glucose consumption. The MPLA-induced activation of glycolytic metabolism in mouse mDC was shown to depend on a JNK MAPK-mediated activation of mTOR-signaling, while both MAPK- and NFκB-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.

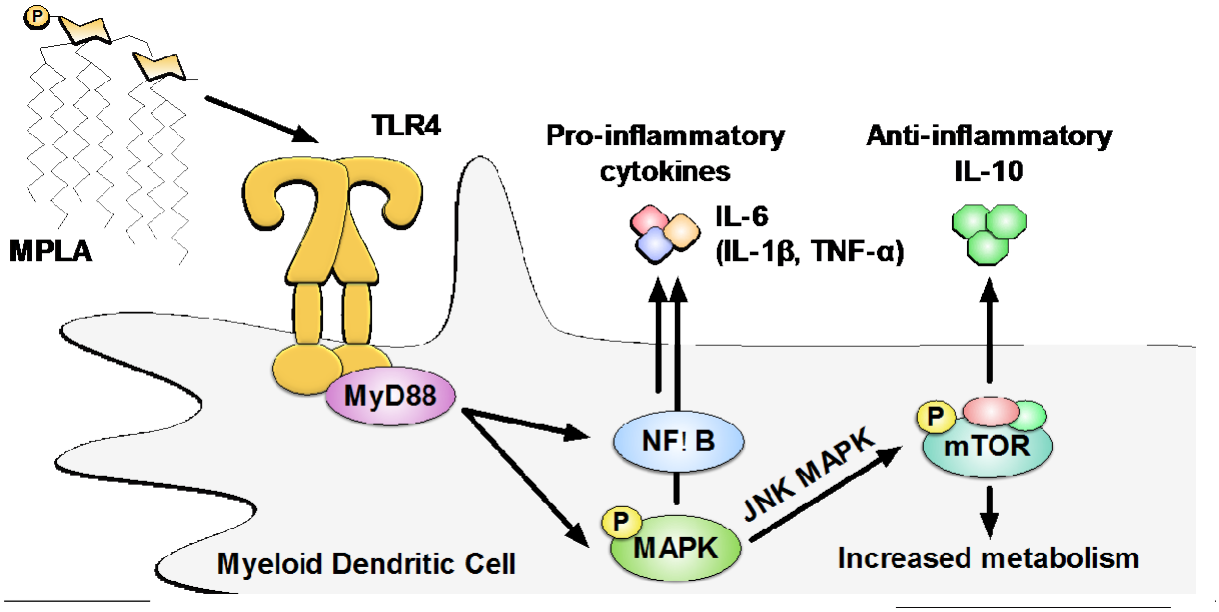

Fig. 3: Effects of the vaccine adjuvant MPLA on dendritic cell activation and metabolism. Source: *PEI*