ImmunoTools *special* Award 2014

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1,25(OH)2Vitamin D3 as adjuvants in an immunotherapy for allergic asthma

Allergic asthma is characterized by specific IgE and Th2-cell driven eosinophilic inflammation and airway hyperresponsiveness (AHR). So far, allergen vaccination (subcutaneous immunotherapy; SCIT) has been the only disease-modifying treatment providing long-term protection against allergic diseases, asthma included. However, current allergen vaccines are hampered by low efficacy to suppress allergic inflammation. Thus, there is an unmet clinical need to improve the efficacy and treatment regime of allergen vaccination.

Recently, we identified $1,25(OH)_2VitaminD_3$ ($VitD_3$) as a promising adjuvant to improve allergen vaccination. Preliminary data from our clinical study on the adjuvant activity of $VitD_3$ towards grass pollen (GP) vaccination in patients with allergic rhinitis showed a trend for a beneficial effect of $VitD_3$ as adjuvant after one year. The overall aim is the development of a pre-clinical GP-vaccine that uses $VitD_3$ as an adjuvant leading to superior beneficial effects in suppressing asthma manifestations.

Previously, in our animal models of allergic asthma, we optimized doses of GP as well as doses of $VitD_3$. Here, we want to test new formulations of $VitD_3$ supplemented GP-SCIT using a potential carrier, like 1-methyl-4-(cis-9-dioleyl) methyl-pyridinium-chlorid (Saint). Saint has been shown to be very effective in transporting DNA, RNA and proteins to the target destination, both *in vitro* and *in vivo.*

The objective of this research project is to test whether the Saint can be applied as a medical device for the delivery of $VitD_3$-GP-SCIT. To this end, BALB/cByJ mice will be sensitized using alum absorbed GP extract by two intraperitoneal injections. After 14 days, SCIT will be performed by three s.c. injections within one week with PBS, GP, Saint, $VitD_3$ and or a combination of those. Two weeks after SCIT, ear-swelling

responses will be performed to determine immediate allergic reactions to intradermal GP challenge. Hereafter, mice will receive three intranasal GP challenges every other day. Blood serum will be taken before SCIT, before challenge and after challenges on the section day. Asthma manifestations will be determined 48 hours after the last intranasal challenge. We will measure airway resistance in response to metacholine; serum levels of GP-specific IgE, IgG1, IgG2a, and IgA; BALF leukocyte differentials (flow cytometry); Cytokine levels in cell-free BALF and supernatants of re-stimulated cell suspensions of lung cells and LDLNs (IL4, IL5, IL10, IL13, TGFβ, and IFNγ); DCs, Treg and Th2 cell phenotypes in single cell suspensions of lung tissue cells and lung draining lymph nodes (LDLNs) by flow cytometry. To perform all flow cytometry analyses and clearly differentiate between leukocyte populations the following antibodies provided by ImmunoTools would be of great help.

ImmunoTools *special* AWARD for **Laura Hesse** includes 17 reagents

FITC - conjugated anti-mouse CD3e, CD44, CD45R, a/b TCR, g/d TCR, isotype control IgG2b

PE - conjugated anti-mouse CD4, CD8a, CD49d, CD62L, Gr-1, isotype control IgG2b

APC -conjugated anti-mouse CD11b, CD19, CD45, NK-cells, isotype control IgG2b

DETAILS

When successful, a proof of concept emerges and future research can be focused on the improved treatment regime and efficacy of immunotherapy in patients with allergic rhinitis and -asthma.