**TLR4 IS INVOLVED IN S1P/S1P1 PATHWAY IN THE LUNG AND CONTRIBUTES TO S1P-INDUCED ALLERGIC INFLAMMATION**

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**Background:** Sphingosine-1-phosphate levels significantly increase in BAL of asthmatic patients following segmental allergen challenge and this increase well correlates with pulmonary inflammation. Allergen challenge promotes S1P signaling in the lung and determines in the mouse a disease closely mimicking the cardinal features of severe asthma in humans.

**Objective:** To understand the role of TLR4 in S1P/S1P1 pathway in the lung and its contribute to S1P-induced allergic inflammation.

**Methods:** BALB/c and C3H/HeJ mice received subcutaneous administration of S1P (10ng), LPS (0.1μg) or S1P +LPS at days 0 and 7. A group of BALB/c mice were pretreated with the purified rabbit anti-TLR4 (10μg).

**Results:** Subcutaneous administration of S1P induces airway hyperreactivity and pulmonary inflammation coupled to an increase in the percentage of dendritic cells and macrophages in the lung of BALB/c mice. In addition a reduction in mediastinic lymph node dendritic cell percentage occurs. Conversely, S1P does not affect lung function and the pulmonary immune microenviroment in C3H/HeJ mice which is a strain with a TLR4 defective. On the other hand, pretreatment with the purified rabbit anti-TLR4 significantly inhibits, while LPS potentiates airway hyperreactivity and pulmonary inflammation of S1P-challenged BALB/c mice. Following S1P subcutaneous administration TLR4 up-regulation occurs in the lung and S1P1 coprecipitates with TLR4 in a S1P dependent manner. TLR4+ cells are further increased when mice were pretreated with LPS as well as increase colocalization of S1P1 and TLR4 in the lung.

**Conclusions and clinical relevance:** Our data confirm the influence of TLR4 on the immune microenvironment in the lung of Th2 biased and suggest a novel receptor cooperation in which functional interaction of two distinct pattern-recognition receptors e.g. S1P1 and TLR-4 results in an enhanced allergic inflammatory response in the lung.