ImmunoTools *special* Award 2015

Pauline Lehebel, PhD-student

Supervisor: DrSc Olivier Denis

Allergology Program, Communicable and Infectious Diseases
Rue Engelandstraat, 642, 1180 Brussels, Belgium

Analysis of the inflammatory and allergic properties of moulds

Allergic asthma is a multi-factorial chronic lung disease characterized by a deregulation of immunity that can be triggered by inhaled allergens such as house dust mite, pollens, animal dander's and moulds. In general, infectious fungi, and therefore moulds spores, trigger inflammatory protective responses through the generation of Th1/Th17 responses and the production of cytokines such as TNF-α, IL-17 and IFN-γ (1). But mould spores can also activate Th2 responses in the lungs leading to the development of an allergic inflammation (2). The aim of this project is to investigate the inflammatory and allergic activating properties of common indoor moulds.

First, mice were chronically stimulated intra-nasally with fresh mould spores of *Alternaria alternata*, *Aspergillus fumigatus, C. cladosporioides* and *Penicillium chrysogenum*. After five weeks, the Bronchoalveolar Lavage (BAL) cellular composition and the Th1 and Th2 cytokine mRNA levels in cell lungs were analysed. Our results show that mice chronically treated with these mould spores developed a typical allergic lung inflammation with numerous eosinophils. Nevertheless, *A. fumigatus* spores led the highest eosinophil recruitment whereas *A. alternaria* spores induced a mixed eosinophilic-neutrophilic lung inflammation.

Then, we analysed the production of pro-inflammatory cytokines (TNF-a, IL-6 , IL1,etc…) by bone marrow derived dendritic cells (BMDCs). These dendritic cells were obtained from bone marrow cells cultured in the presence of GM-CSF from ImmunoTools. BMDCs were cultured in the presence of various mould spores overnight. All mould species tested induced a secretion of TNF-α, IL-6 and IL-1α in the supernatants but these secretions involved quite distinct pathways. The species triggering an allergic lung inflammation used the MALT-1 pathway since the TNF-α production was absent in dendritic cells generated from the bone marrow (BMDC) of MALT-1 deficient mice.

To further characterize the properties of these moulds *in vivo, w*e have developed a model of allergic lung inflammation based on the transfer of BMDCs, pulsed *in vitro* with mould

spores. In short, pulsed-BMDCs were transferred into the lungs of naïve mice. Two weeks later, mice were challenged with mould spores intra-nasally and lung inflammation was analysed three days later. In this model, BMDCs pulsed with *A. fum* spores activated lung Th2 responses and induced a recruitment of eosinophils into the airways.

Alternatively, we also used DCs pulsed *in vivo* with *A. fum* spores. These DCs were obtained from naïve mice instilled with *A. fum* spores. Four hours later, lungs were digested with collagenase and then $CD11c^+$ DCs were isolated using magnetic particles. After transfer into naïve mice, these purified DCs induced similar *in vivo* responses as compared to the BMDCs pulsed *in vitro* with the spores.

In order to follow *in vivo* the migration of *A.fum* spores, these spores were labelled with CFSE. *In vitro,* only $CD11c^+$ BMDCs were able to internalize $CFSE^+$ *A. fum* spores. To determine the phenotype of cells internalizing the spores *in vivo*, naïve mouse were instilled with $CFSE^+$ labelled *A. fum* spores. Four hours later, bronchoalveolar lavages (BALs) and lungs were collected and $CFSE^+$ cells were detected by flow cytometry. In the BALs, CFSE-labelled spores were detected in double positive $CD11c^+$ $CD11b^+$ inflammatory DCs. However, in the lungs, spores were detected in $CD11c^+$ $CD11b^-$ DCs. We will further analyse the migration of these cells to the regional lymph nodes and investigate the effect of signalling pathway and receptors as Myd88, Malt1 and TLR4 deficiencies in this model thanks to ImmunoTools anti-mouse antibodies for flow cytometry.

(1) Templeton SP, Buskirk AD, Law B, Green BJ, Beezhold DH. Role of germination in murine airway CD8+ T-cell responses to Aspergillus conidia. PLoS One. 2011 Apr 13;6(4):e18777. doi: 10.1371/journal.pone.0018777.
(2) Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. Eur Respir J. 2006 Mar;27(3):615-26.