ImmunoTools *special* Award 2018

Charlotte Moulin, PhD-student

Supervisor: Prof. Charles Pilette

IUCL-IREC-PNEU, Pôle Pneumologie – ORL – Dermatologie, Tour Bernard +5, Avenue Hippocrate 54 Bte B1.54.04, 1200 Bruxelles, Belgique

Lung IgA-producing B-cells characterization and regulation in asthma

Asthma is the most prevalent chronic respiratory disease, affecting 5-10% of the World's population. Asthma is associated with, at least, a local lung-IgE increased production (*Takhar P. et al., 2007, Froidure A. et al., 2016)* but also a serum IgE increased rate in atopic patients (2/3). In a healthy context, the most abundant Ig class in mucosal secretions is the IgA, produced by plasma cells (last stage of B-cells differentiation). Several lines of evidence support there being a protective role for IgA in allergy and asthma (*Schaffer FM. et al, 1991, Urm SH. et al, 2013, Jutel M. et al, 2005, Sletten GB. et al, 2007, Konstantinou GN et al, 2014*).

Ig-producing plasma cells originate from B-cells, where class switch recombination (CSR) from IgM (expressed on naïve mature B-cells) to IgG, IgA or IgE occurs under the influence of micro-environmental factors (e.g. IL-4, IL-10, IL-21, IL-13, BAFF). CSR can only occur following the segment order in the constant heavy chain locus (IgM - IgD - IgG3 - IgG1 - IgA1 - IgG2 - IgG4 - IgE - IgA2, up- to downstream). In the human heavy chain locus, Cα2 is the only C_H gene located downstream of Cε, meaning that the only possible CSR for IgE^+ B-cells is to IgA2. We hypothesise that the patterns of local CSR differ between asthmatic and healthy subjects favouring IgE over IgA and that this underlies the impaired synthesis of IgA from lung B-cells in patients with asthma. We suggest that an IgE-to-IgA2 switch could be induced and would represent a potential way to recover the mucosal tolerance for asthma patient.

To carry out our research, the ImmunoTools antibodies and growth factor will be used in three major part of the project.

<u>Objective 1:</u> to establish the nature of lung IgA producing B-cells

To characterize the b-cells from lung, we developed a lung-tissue digestion protocol to isolate mononuclear cells and analyses them by flow cytometer. We developed a 15-colours analysis panel (including CD38-FITC, CD45-PerCP, CD27-APC, CD3-PE) to identify naïve, memory IgM/IgG/IgA1/IgE/IgA2 ($CD27^+$ and $^-$), plasma cells/blasts, B1, B reg. We will also characterize the Ig-producing B-cells subtype for their micro-environmental factors receptors such as CD40 (with APC-CD40).

In a second phase, to confirm the identification of our Breg cells, we will sort this population and assess their IL-10 production *in vitro* by ELISA.

Objective 2: to study the IgA1/IgA2 synthesis in lung B-cells

To study the IgA1/IgA2 production regulation by lung b-cells. We will sort, with flow cytometer, naïve and non-IgA memory B-cells from alveolar lavage of asthma patient versus control to study the impact of some known CSR inducible factors on the IgA1/2 production. We will use *in vitro* human recombinant cytokines such as rhBAFF, hIL-4, IL-10, IL-13, IL-21, TGFb3 and the soluble rh sCD40L and assess the IgA1/IgA2 production by ELISA in diverse culture condition. These results will be also compare to b-cells from blood of the corresponding patient.

Objective 3: to proof that IgA has a protective role against asthma with an in vivo model

We are developing a new IgA-depleted mice model to study the impact of IgA level following HDM-intratracheal sensitization. During this part of the project, lung B-cells from control and IgA depleted mice model will be also deeply characterized by flow cytometer.

ImmunoTools *special* AWARD for **Charlotte Moulin** includes 19 reagents

APC – conjugated anti-human CD27, CD40
FITC - conjugated anti-human CD38, mouse Control IgG1
PE - conjugated anti-human CD3
PerCP - conjugated anti-human CD45

APC – conjugated anti-mouse CD19
FITC - conjugated anti-mouse CD45R

Human ELISA-set (for one 96 plate): human IL-10

recombinant human cytokines: rh BAFF/sCD257, rh sCD40L/CD154,
rh IL-4, rh IL-10, rh IL-13, rh IL-21, rh TGF-beta3

DETAILS more AWARDS