ImmunoTools *multiplex* Award 2014

Ilse Van Brussel, PhD

Laboratory of Physiopharmacology
University of Antwerp
Universiteitsplein 1, B-2610 Antwerp, Belgium

Unraveling the dynamic network of inflammatory cell subsets in human atherosclerotic plaques.

Atherosclerosis is a chronic immune-mediated inflammatory disease of the large and medium-sized arteries. Treatment of atherosclerosis is currently based on reducing risk factors, e.g. by use of statins and beta-blockers. Yet, many patients remain at risk for acute coronary events, and as a consequence the disease remains the number one cause of death in the Western World. Thus, new therapeutic strategies are needed.

Recent studies have highlighted the important role of inflammation in the development and progression of human atherosclerosis. However, so far, studies that aimed to investigate inflammatory subsets in human atherogenesis were focused on *systemic* immune activation. Here, we want to compare systemic inflammation with *local* immune cell subsets present in atherosclerotic plaques within the same patient. Better knowledge of the specific immunological and inflammatory mechanisms that occur in plaques can lead to: (1) novel immunomodulating therapeutic strategies, in addition to the current treatment, and (2) a better identification of patients at risk for acute cardiovascular events, including stroke and myocardial infarction.

Peripheral blood samples and atherosclerotic plaque samples are currently collected from patients that are eligible for endarterectomy at the carotid, aorto-iliac and femoro-popliteal level at a rate of 3-4 patients/week, from the Antwerp University Hospital and the Antwerp hospital network. We already succeeded in isolating immune cell subsets from human atherosclerotic plaques, which is very challenging, given the high amount of (extra)cellular debris in human samples. We have shown that treatment of samples with collagenase IV and DNase I for 2h at 37°C is very efficient as enzymatic digestion mixture with a large cell surface marker preservation. Peripheral blood samples of age and sex matched healthy controls (recruited from the same sites) are collected as well. The control group has no known pre-existing cardiovascular or other inflammatory or immune diseases and is symptom-free.

To compare the systemic inflammation with the local inflammation within the same patient (hereby using healthy controls as a reference), we have developed a flow cytometric cell sorting strategy for the phenotyping of multiple immune cell subsets, including conventional and plasmacytoid dendritic cells, natural killer cells, T cells,

monocytes and macrophages. However, the complex 11-parameter flow cytometrix analyses can be overcome by the use of the **multiplex array** for human cytokines and different CD markers. This array would be of great value to investigate even more inflammatory cell subsets at the same time (including $CD4^+$ and $CD8^+$ T cell subsets). It can reveal which immune cell subtypes are altered in patients compared to controls, and in plaques compared to blood from the same patient. Moreover, it will give information about possible alterations in cytokine levels as well, indicating immune cell functionality.

The new insights in the underlying inflammatory mechanisms in atherosclerosis might lead to the identification of novel potential targets for immunomodulating therapies.

ImmunoTools *multiplex* AWARD for **Ilse Van Brussel**

includes free analysis of samples on several antibody arrays with large range of antibodies against human CDs, human cytokines, and others ...