ImmunoTools *special* Award 2014

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Macrophage-derived foam cells in Atherosclerosis: Phenotypic and Functional Characterization

Atherosclerosis is a chronic inflammatory vascular disease, having as ultimate outcome the atheromatous plaque, a focal lesion located within the intima of large and medium sized arteries. Subendothelial retention of low density lipoprotein (LDL) and its oxidative modification represent the initial event in atherogenesis, which is followed by infiltration and activation of blood inflammatory cells. Oxidized LDL (OxLDL) in fact activates endothelial cells (ECs) by inducing the expression of several cell surface adhesion molecules which mediate the rolling and adhesion of blood leukocytes (monocytes and T cells); after adhesion to the endothelium, leukocytes migrate into the intima in response to chemokines. Monocytes then differentiate into macrophages that upregulate both toll-like receptors (TLRs), involved in macrophage activation, and scavenger receptors (SRs), that internalize OxLDL, leading to lipid accumulation and foam cell formation. In the later stages of the disease, foam macrophages (FM) undergo apoptosis and secondary necrosis, which leads to the formation of a lipid-rich, procoagulant core of the atherosclerotic lesion. When atherosclerotic lesions rupture, exposure of blood to this procoagulant material causes immediate and massive thrombosis, leading to serious or fatal cardiovascular events including myocardial infarctions and ischemic strokes.

Moreover, in addition to the traditional cardiovascular risk factors, in the last two decades, several infectious agents have been implicated in the pathogenesis of atherosclerosis. *Chlamydia pneumoniae* has been considered as the most plausible additional risk factor for atherosclerosis since it is the sole viable pathogen detected in the atherosclerotic plaque and it is able to multiply and persist within vascular cells and to induce the chronic inflammatory state underlying atherosclerosis.

Foam cell formation from monocyte-derived macrophages is a hallmark of atherosclerosis but up to date no data concerning phenotypic and functional characterization of FM are available. We already developed a simply and good defined experimental *in vitro* model of FM generation. In details we take whole

blood from healthy donors and isolate monocytes that are induced to differentiate to M1 or M2 macrophages in the presence of recombinant human GM-CSF or M-CSF respectively. Then the monocyte-derived macrophages are exposed to OxLDL (50 μg/ml) for a week and then verify foam cell formation by red O staining.

Now we plan to phenotypically characterize the differentiated M1 and M2 FM by flow cytometry using fluorescent monoclonal antibodies specific for differentiation surface molecule CD14, for presenting molecules (MHC class-I and MHC class-II), co-stimulatory molecules (CD80, CD86, CD40), surface receptors (CD16, CD71, CD11b, CD11c, CD18) and migratory marker (CD62L) on live cells (Annexin V). Therefore the fluorescent monoclonal antibodies from ImmunoTools would advance our project and could be very enlightening.

Subsequently we plan to investigate the capacity of FM to secrete inflammatory cytokines upon TLR-stimulation and in details we will measure the release of IL-8, IL-6, human TNF-α by ELISA kits. Now we aim to round off our functional studies with the help of ImmunoTools cytokine ELISAs.

Finally we are interested to analyse the antigen presenting capacity of FM. For this purpose we will use two different strategies: in the first one we will set up mixed leukocyte reactions. With the second strategy we are interested to investigate the capacity of FM to present *C. pneumoniae* and consists in the co-culture among *C. pneumoniae*-infected FM and autologous $CD3^+$ responder T lymphocytes. For both the strategies we will analyse the T cell activation by checking with flow cytometer the expression of surface activation markers (CD25, CD69, CD44) and the release of IFN-γ and IL-2 cytokines on the $CD3^+CD4^+$ T cells or $CD3^+CD8^+$ T cells. The fluorescent antibodies from ImmunoTools would be very helpful to complement our project.

ImmunoTools *special* AWARD for **Sabrina Mariotti** includes 25 reagents

FITC - conjugated anti-human CD11b, CD16, CD18, CD45RA, CD69, CD71, CD86, HLA-ABC, HLA-DR, Annexin V,

PE - conjugated anti-human CD8, CD14, CD25, CD44, CD80,

PerCP - conjugated anti-human CD3,

APC - conjugated anti-human CD4, CD11c, CD40, CD62L,

human IL-6 ELISA-set for 96 wells, human IL-8 ELISA-set for 96 wells, human TNFa ELISA-set for 96 wells (each 3 reagents),

recombinant human cytokines: rh GM-CSF, rh M-CSF DETAILS more AWARDS