Atherosclerosis (ATH) is recognized as a chronic inflammatory condition and it is the leading cause of cardiovascular disease. The process of atherosclerosis is characterized by the accumulation and oxidation of LDL (oxLDL) in the vessel wall and subsequent infiltration and activation of immune cells, particularly monocytes in an earlier stage and, later on, lymphocytes. The infiltrated monocytes differentiate into macrophages which then could differentiate into foam cells as a consequence of oxLDL uptake [1]. The recruitment of immune cells to the site of ATH lesion contributes to a local pro-inflammatory state that will promote the development of the atheroma plaque and progression of the disease. However, the exact mechanisms involved in this process are not fully understood. One hypothesis is the contribution of oxidative stress mediated by metals such as iron [2]. Previous authors have shown high iron content in foam cells and also accumulation of hemoglobin and ferritin in the areas rich in foam cells [3]. Herein, we investigate a possible mechanism for cellular iron accumulation by testing the effect of pro-inflammatory as well as pro-atherogenic stimuli in the expression of proteins involved in iron efflux in macrophages.

**Materials and Methods**

Mouse bone marrow-derived macrophages (BMMDD) were prepared from SWISS mice (7-11 weeks of age) as previously described [4]. Briefly, bone marrow cells were extracted from femurs and cultured in RPMI 1640 medium supplemented with 10% FBS, 10% LCCM and 1% antibiotics for 7 days to complete differentiation into macrophages before experiments. BMMDD were then treated with LPS (10 ng/ml: 20h), iron (Fe-NTA 100 µM) 20h) or/and oxLDL (50 µg/ml: 24h). The expression of ferroportin (Fpn, iron exporter), beta-amyloid precursor protein (APP, ferrooxidase), ceruloplasmin (Cp, ferrooxidase) were analyzed by western blot of subcellular fractions (cytosol, membrane, and lipid-rich 5-9-fractones). BMMDD were also prepared as described previously [5]. Oil Red O staining (lipid specific dye) and hematoxylin counterstain was used to follow foam cell differentiation by oxLDL treatment. Antibodies used: Home made polyclonal rabbit anti-mouse Fpn [6], monoclonal mouse anti-APP (Dilution 1/500, clone 22C11, Millipore); polyclonal rabbit anti- caveolin1 (1/500 to 1/10000, Santa Cruz) and polycyclic goat anti-human Cp (1/100, KOMA BIOTECH INC.)

**Analysis of Results**

APP is expressed in mouse BMDM and is upregulated by iron

Fe-NTA 100µM

BMDM differentiation into foam cell is enhanced by iron overload

**Conclusions**

Cp and APP, both ferrooxidases reported to interact with Fpn, are differentially regulated by LPS and iron in BMDM, with Cp being strongly upregulated by LPS and APP by iron overload. Previously, we have reported that membrane Cp and Fpn were only partially colocalized in BMDM and proposed that other ferrooxidases could be interacting with Fpn for iron export [7]. Considering that Fpn is upregulated by iron, APP is a potential partner for Fpn in BMDM. Supporting this hypothesis, we observe that after iron treatment, APP is recruited in DRM/lipid rafts fractions along with Fpn. Modulation of protein involved in iron export by inflammatory stimuli could also contribute to disruption of iron metabolism in plaque macrophages. In addition, foam cell differentiation of BMMDD by oxLDL was enhanced in the presence of iron overload, supporting the idea that iron overload in atheroma plaques may constitute a pro-atherogenic factor. Moreover, the effect of oxLDL on the expression and localization of iron-related proteins on lipid raft microdomains may constitute an important pathway for iron efflux disruption in the plaque environment and is currently being investigated.