CROSSROAD BETWEEN INFLAMMATION, IRON AND LIPIDS IN ATHEROGENESIS

Liliana Marques[1, 2,3,4,5*], Lorenne Robert[3,4,5], Anne Auriac[3,4,5], François Canonne-Hergaux[3,4,5] and Luciana Costa[1,2]

[1] Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; [2] Center for Biodiversity, Functional and Integrative Genomics, Lisboa, Portugal; [3]INSERM U1043-CPTP, Toulouse, France; [4]CNRS, U5282, Toulouse, France; [5]Université de Toulouse, UPS, Centre de Physiopathologie de Toulouse Purpan (CPTP), Toulouse, France.

Phone: +351 21 7508128 E-mail: lcosta@insa.min-saude.pt

INTRODUCTION

Atherosclerosis (ATH) is recognized as a chronic inflammatory condition and it is the leading cause of cardiovascular disease. The process of atherogenesis 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.

MATERIAL & METHODS

Mouse bone marrow-derived macrophages (BMDM) 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 for complete differentiation into macrophages before experiments. BMDM were then treated with LPS (10ng/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, ferroxidase), ceruloplasmin (Cp, ferroxidase) were analyzed by western blot of subcellular fractions (cytosol, membrane extracts and lipid raft fractions DRM 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 polyclonal goat anti-human Cp (1/100, KOMA BIOTECH INC.)

ANALYSIS OF RESULTS

APP is expressed in mouse BMDM and is upregulated by iron

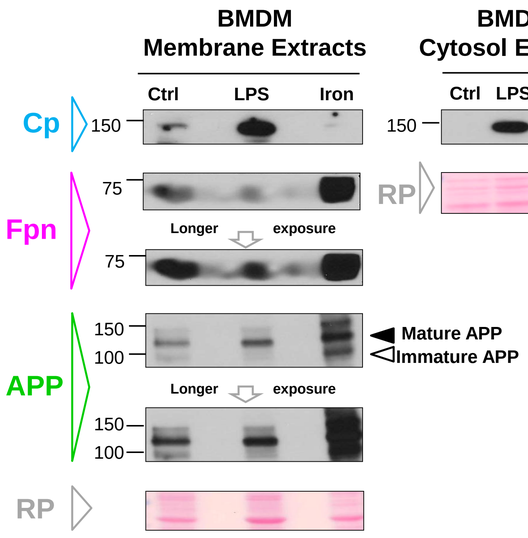

Fig.1 – Analysis of protein expression in mouse BMDM by Western Blot. Immunoblotting analysis showed that Cp is upregulated by LPS in both membrane and cytosol extracts whereas Fpn is downregulated by LPS and upregulated by iron overload as previously reported [4;6]. APP is only slightly upregulated by LPS compared to Cp, but it is strongly upregulated by iron overload like Fpn.

APP and Fpn are localized in lipid raft in iron-treated BMDM

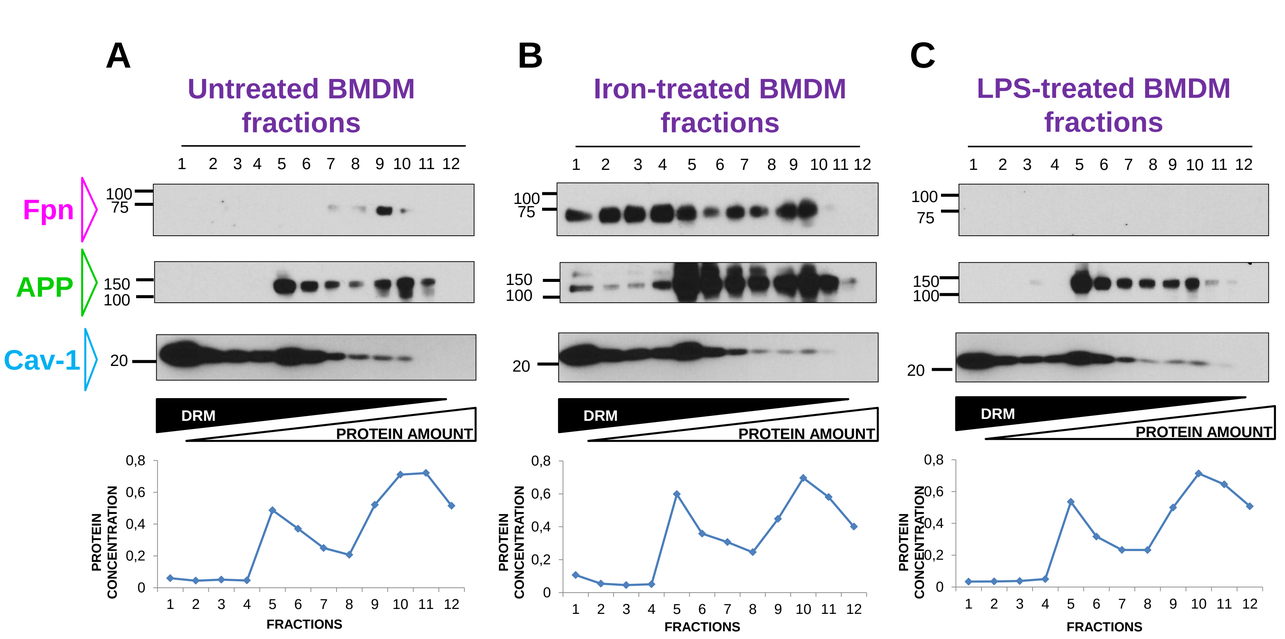

Fig.2 – Expression of Fpn, APP and Caveolin-1 (Cav-1) in detergent resistant membranes (DRM : lipid raft) from BMDM after iron (Fe-NTA) or LPS treatment. Immunoblotting analysis of Cav-1 (raft marker) in BMDM iodixanol fractions showed that fractions 1-4 are enriched in DRM proteins, with fractions 5 and 6 being enriched in Cav-1 due to higher protein concentration in these fractions (protein retention in interface 20%-30% of iodixanol layers). In untreated BMDM (A), APP is mostly expressed in fractions 5-11 while Fpn is more expressed in fractions 7-10. In iron-treated BMDM (B), recruitment to lipid rafts/DRM fractions of both APP and Fpn is observed. In LPS-treated BMDM (C), Fpn expression was downregulated whereas APP expression was slightly increased when compared to untreated cells. The protein concentration in the different iodixanol gradient fractions was calculated by Bradford quantification method and is represented in the graphics at the bottom of each gradient. The pattern of protein distribution showed the protein retention in fractions 5-6 and high concentration of proteins in the fractions 9-12 corresponding to Non-Detergent Resistant Membranes and cytosolic proteins. Since the protein content in DRM fractions 1-4 is low, detection of APP and Fpn in these fractions suggest a strong enrichment of these proteins in DRM.

BMDM differentiation into foam cell is enhanced by iron overload

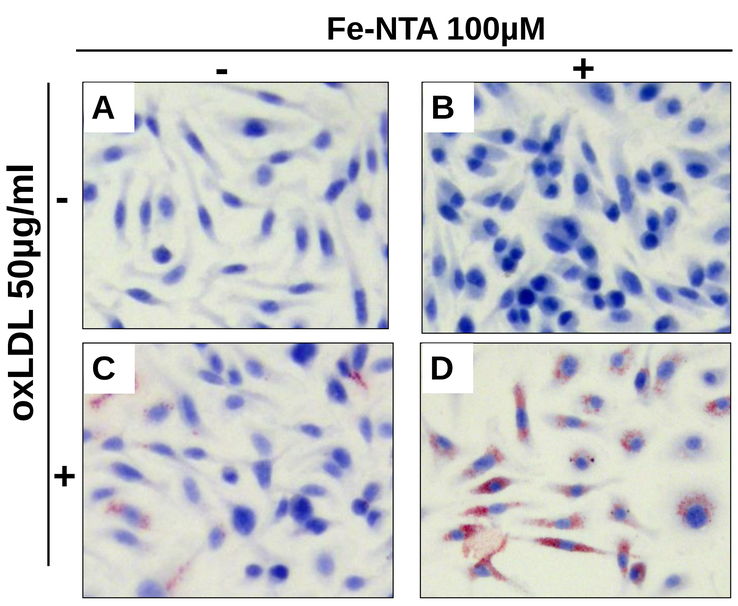

Fig.3 – Effect of iron overload on BMDM differentiation into foam cells. BMDM were treated for 24h with no stimuli (A) or with Fe-NTA (B, 100 µM) or oxLDL (C, 50 µg/ml) or both Fe-NTA and oxLDL (D). Foam cells differentiation (red staining) was only observed in the presence of oxLDL and was enhanced in the presence of iron (Fe-NTA), suggesting an atherogenic role of iron overload in foam cell differentiation.

CONCLUSIONS

Cp and APP, both ferroxidases 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 ferroxidases 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 BMDM 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.

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This work was supported by National Institute of Health Dr Ricardo Jorge, I.P, INSERM (Institut National de la Santé et de la Recherche Médicale), CNRS (Centre National de la Recherche Scientifique), ANR (Agence Nationale de la Recherche, France; ANR- 08-GENO-000) , Fundação para a Ciência e Tecnologia (Grant SFRH/BD/48671/2008) and BioFIG (Center for Biodiversity, Functional and Integrative Genomics).