INFLAMMATORY STIMULI MODULATES THE EXPRESSION OF CERULOPLASMIN AT SURFACE OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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INTRODUCTION

Atherosclerosis (ATH) is the leading cause of cardiovascular disease [1], and its development is characterized by the passage of LDL through the endothelial layer, and the early infiltration and activation of peripheral blood lymphocytes (PBL) and monocytes (PBMN) that contribute to a pro-inflammatory state in specific locations [2,3]. However, the functional interaction between immune cells and the oxidation of LDL is still not fully understood. One hypothesis for the etiopathogeny of ATH may be associated with an ongoing inflammatory process caused by a pro-oxidant/anti-oxidant imbalance induced by metals such as iron (Fe) or copper (Cu) [4]. Ceruloplasmin (Cp) is an acute-phase protein but also a multicopper oxidase with a relevant role in Fe metabolism mainly due to its ferroxidase activity [5], which is expressed in human PBL [6] and PBMN [7]. Herein, we aim to study the effect of putative pro-atherogenic immune stimuli on the expression of Cp at the surface of peripheral blood mononuclear cells (PBMC).

MATERIAL & METHODS

Isolation of Peripheral Blood Mononuclear Cells

Total peripheral blood from healthy volunteers was collected in citrate CPT™ vacutainer tubes (BD Biosciences) and PBMC were isolated according to the manufacturer's instructions. Remaining red blood cells (RBC) were removed by incubating PBMC with RBC lysis solution (10mM Tris, 16mM NH_4Cl, pH 7.4) for 10min at 37ºC, followed by washes in PBS before culture.

Immunomodulation of Cp expression

PBMC were incubated in RPMI 1640 at 37ºC in a 5% CO_2 atmosphere with/without the following stimulli: PMA (5 ng/mL, 2h), IL-1β (20, 50 ng/mL, overnight (o.n)), IL-6 (10, 50, 100 ng/mL, o.n), IL-8 (14, 73, 143 ng/mL, o.n) or TNF-α (2, 12, 20 ng/mL, o.n). PBMCs were then collected, ressuspended in Staining Buffer and subsequently analyzed by Flow Cytometry.

Immunophenotyping and Flow Cytometry analysis

Cells were plated in 96-well round-bottomed microtiter plates at 5×10^5 cells/well. PBMCs were stained for Cp by indirect staining using anti-human Cp (Koma Biotech) as primary antibody and goat F(ab')2 anti-rabbit FITC-conjugated (Rockland) as secondary antibody. Also, conjugated CD3-PE, CD69-PerCP, CD45-PerCP (BD Biosciences) and CD14-APC (MACS) antibodies were used to define PBL and PBMN populations under study. Flow cytometry analysis was performed in a FACScalibur (BD Biosciences) and protein expression levels were analyzed using Weasel v.2.7.4 software.

ANALYSIS OF RESULTS

PBMN SHOW HIGHER SURFACE CP EXPRESSION COMPARED TO PBL

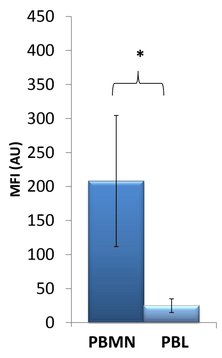

Fig.1 - Flow Cytometry analysis of surface Cp expression in PBMC. Surface Cp expression in untreated PBMN and PBL (n=16, *p < 0.01).

BOTH ACTIVATED PBMN AND PBL EXPRESS HIGHER SURFACE CP THAN THEIR NON-ACTIVATED COUNTERPARTS

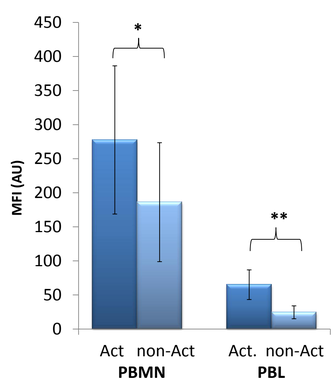

Fig.2 - Flow Cytometry analysis of surface Cp expression in PBMC. Surface Cp expression in untreated PBMN and PBL depending on their activation state (n=12, *p < 0.05, **p < 0.01).

T CELLS SHOW DECREASED SURFACE CP EXPRESSION COMPARED TO NON-T CELLS

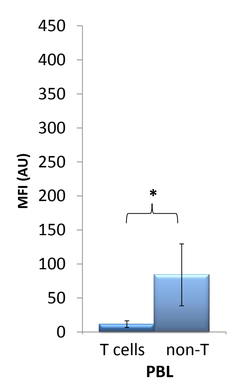

Fig.3 - Flow Cytometry analysis of surface Cp expression in PBL subpopulations. Surface Cp expression by in untreated PBL subpopulations (n=16, *p < 0.05).

CP EXPRESSION AT SURFACE OF PBMN AND PBL IS DIFFERENTLY MODULATED BY PMA AND IL-1β

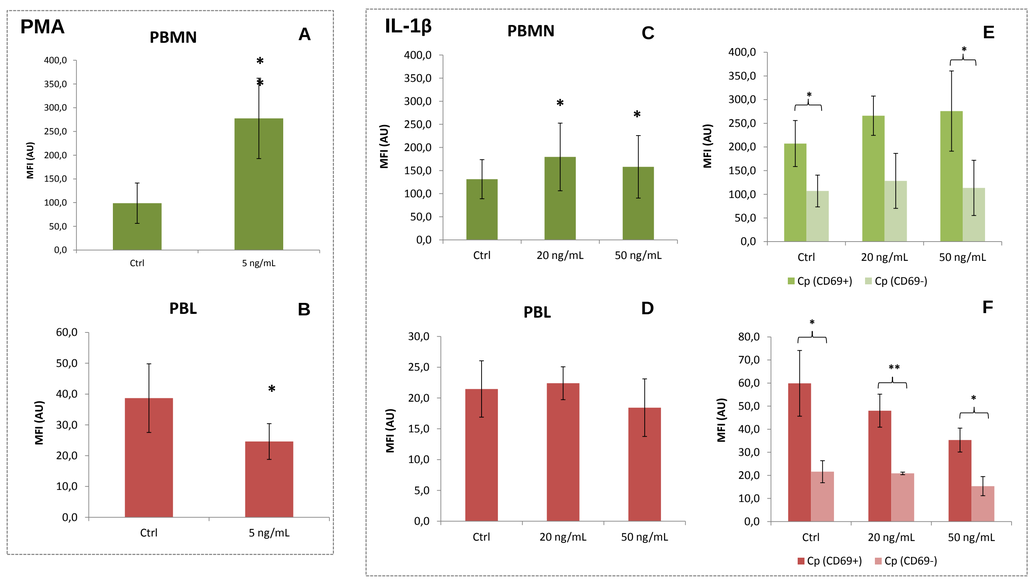

Fig.4 – Effect of PMA and IL-1β on surface Cp expression of PBMC. Results of flow cytometry analysis of surface Cp expression in untreated vs stimulated PBMN and PBL by PMA (A,B) and IL-1β (C-F). Analysis of Cp expression at surface of activated vs non-activated PBMN (E) and PBL (F) incubated with/without IL-1β. All experiments were performed in triplicate, *p < 0.05; **p < 0.01.

EXPRESSION OF CP AT SURFACE OF PBMC IS NOT MODULATED BY: IL-6, IL-8 AND TNF-α

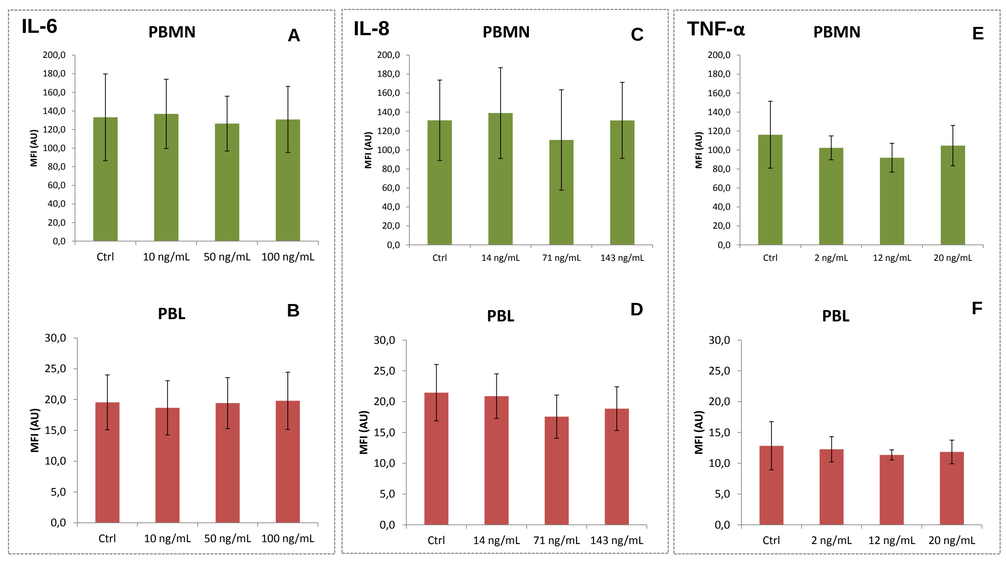

Fig.5 - Effect of IL-6, IL-8 and TNF-α on surface Cp expression of PBMC. Results of flow cytometry analysis of surface Cp expression in untreated versus stimulated PBMN and PBL by IL-6 (A,B), IL-8 (C,D) and TNF-α (E-F)**.** All experiments were performed in triplicate.

CONCLUSIONS

The results obtained during this study showed:

- A consistently higher cell surface Cp expression in untreated PBMN *vs* PBL, activated *vs* non-activated cells and non-T *vs* T cells;
- Cp expression at surface of PBMN and PBL is differently modulated by PMA and IL-1β;
- Expression of Cp at surface of PBMC is not modulated by IL-6, IL-8 and TNF-α.

These results demonstrate that specific inflammatory mediators could be, at least in part, involved in the modulation of Cp expression at surface of PBMC and thus, could have a putative role in atherogenesis.

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