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 (PBMMN) 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 etiopathology 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 ferrooxodative activity [5], which is expressed in human PBL [6] and PBMMN [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).

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 HCl, pH 7.4) for 10min at 37°C, followed by washes in PBS before culture.

Immunomodulation of Cp expression

PBMC were incubated in RPMI 1640 at 37°C in a 5% CO2 atmosphere with/without the following stimuli: 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, resuspended in Staining Buffer and subsequently analyzed by Flow Cytometry.

Immunophenotyping and Flow Cytometry analysis

Cells were plated in 96-well round-bottom microtiter plates at 5×10^3 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 PBMMN populations under study. Flow cytometry analysis was performed in a FACSScalibur (BD Biosciences) and protein expression levels were analyzed using Weasel v.2.7.4 software.

**RESULTS**

PBMMN show higher surface Cp expression compared to PBL.

Both activated PBMMN and PBL express higher surface Cp than their non-activated counterparts.

T cells show decreased surface Cp expression compared to non-T cells.

**CONCLUSIONS**

The results obtained during this study showed:

- A consistently higher cell surface Cp expression in untreated PBMMN vs PBL, activated mononuclear cells and non-T cells;
- Cp expression at surface of PBMMN 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.

**REFERENCES**

7. The work was supported by National Institute of Health Dr Ricardo Jorge, I.P. and by Fundação para a Ciência e Tecnologia (Grant SFRH/BPD/48671/2008).

**ACKNOWLEDGEMENTS**

This work was supported by National Institute of Health Dr Ricardo Jorge, I.P. and by Fundação para a Ciência e Tecnologia (Grant SFRH/BPD/48671/2008).