Ceruloplasmin (Cp) is a multicopper oxidase implicated in iron (Fe) metabolism and protection against free radical–driven cell injury [1]. Through oxidation of Fe(III) to Fe(II), Cp assists the sole identified mammalian iron exporter ferroportin (Fpn) for transporting iron out from the cells [2-3]. Cp can be expressed as a soluble secreted protein (scCp) or as a membrane GPI–anchored protein (GPI–Cp) as a result of alternative splicing [4-5]. scCp is abundant in serum and is known to be mostly expressed by hepatocytes while GPI–Cp has been shown to be mostly expressed in astrocytes, leptomeningeal cells, and sortilin cells [1]. Previously, we reported the mRNA expression of both scCp and GPI–Cp in human lymphocytes and in the hepatocarcinoma cell line HepG2 [6]. Hence, we clarified the protein expression of both Cp isoforms in different immune cells as well as in HepG2 cells.

The subcellular localization of Cp isoforms was analyzed by immunofluorescence and immunoblotting of resting human peripheral blood lymphocytes (PBL) and monocytes (PBMC), mouse bone marrow derived macrophages (BMDM) and HepG2. Cells were treated with PI-PLC, an enzyme that specifically cuts GPI–proteins, followed by analysis of Cp expression at cell surface by immunofluorescence. BMDM and HepG2 were treated with iron (Fe-NTA) and expression of Cp and Fpn was studied by immunoblotting of subcellular fractions (cytosol, membrane and lipid rafts). Colocalization of Cp and Fpn was investigated by immunofluorescence in iron-treated BMDM. Cp antibodies: anti-Cp FITC (Biotrend), anti-human Cp (Koma Biotech) and anti-mouse Cp (BD Bioscience). Fpn antibody: anti-mouse Fpn (Alpha Diagnostic). For identification of lipid rafts/DRM (detergent resistant membrane) fractions, Fitolin-1 (Flotil) and caveolin-1 (Cav) were used as lipid rafts markers.

In this study, we showed that PBL, PBMC, BMDM and HepG2 express both a soluble form of Cp and a membrane-associated form that was shown to be correspond to Gp–Cp. Analysis of immunofluorescence data for Cp in non-permeabilized PBMC, BMDM and HepG2 was suggestive of GPI–Cp localization in lipid rafts microdomains, which was confirmed by immunoblotting analysis of isokinasediagonal fractions of BMDM and HepG2. Also, our results revealed that iron overload conditions upregulate the expression of both Cp isoforms in BMDM but not in HepG2 cells, suggesting that Cp expression is under distinct regulatory mechanisms in these cells. This observation that likely reflects a cell-type specific function of Cp. Interestingly, partial colocalization of GPI–Cp and Fpn was observed in lipid rafts microdomains in iron-treated BMDM, indicating a possible role for GPI–Cp/Fpn interaction in iron metabolism in mouse macrophages.

REFERENCES