Molecular characterization of the haeptoglobin-haemoglobin complex and its receptor

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ABSTRACT

The PhD dissertation "Molecular characterization of the haptoglobin-haemoglobin complex and its receptor" is based on experimental work performed at the Department of Medical Biochemistry, University of Aarhus. The work was conducted to gain further insight into structural and functional aspects of CD163 and haptoglobin (Hp) in scavenging of haemoglobin. The main results of this PhD study are presented in three separate scientific reports and are based on functional analysis of CD163 splice variants and Hp mutant variants expressed by stable transfection in mammalian cells.

CD163 is the receptor responsible for mediating endocytosis of the complex formed between the abundant plasma protein Hp and haemoglobin that has been released into plasma during intravascular haemolysis. CD163-mediated clearance of Hp-haemoglobin prevents toxic effects of the oxidative heme prosthetic group of haemoglobin. Due to alternative splicing the cytoplasmic tail of human CD163 exists in three variants, a short tail variant and two long tail variants.

The first report shows that all three tail variants of CD163 may actively contribute to endocytosis of Hp-haemoglobin, albeit with different efficacy, by means of a common endocytic signal. The CD163 short tail variant displays a high cell surface expression level and efficiently internalizes Hp-haemoglobin complexes. In contrast, the long tail variants 1 and 2 are predominantly intracellular proteins located to the Golgi/endosomes and accordingly show a lower rate of Hp-haemoglobin internalization. Due to the higher endocytic efficacy and a demonstrated higher level of mRNA expression in human blood, liver, and spleen, the short tail variant is expected to account for the majority of Hp-haemoglobin uptake from the circulation. The results have been published in Journal of Leukocyte Biology.

The second report describes functional properties of the haptoglobin-related protein which is 91% identical to Hp at the protein level. The haemoglobin- and CD163-binding properties of human haptoglobin and haptoglobin-related protein are compared.

The third study reports the expression and analysis of a large panel of recombinant Hp mutant proteins with the aim of identifying Hp residues involved in the Hp-haemoglobin high affinity binding to CD163. These analyses identify four Hp residues of critical importance for the high affinity CD163 binding of the Hp-haemoglobin complex.

In conclusion, this PhD study provides new information on the mechanism of CD163-mediated clearance of Hp-Hb by analysis of endocytic properties of CD163 cytoplasmic tail variants and identification of a receptor-binding site in Hp.