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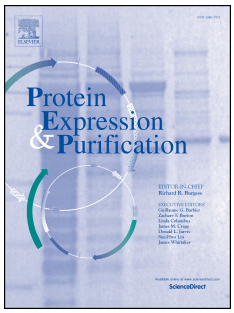

High yield production of human invariant chain CD74 constructs fused to solubility-enhancing peptides and characterization of their MIF-binding capacities

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High yield production of human invariant chain CD74 constructs fused to solubility-enhancing peptides and characterization of their MIF-binding capacities

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Abstract

The HLA class II histocompatibility antigen gamma chain, also known as HLA-DR antigen-associated invariant chain or CD74, has been shown to be involved in many biological processes amongst which antigen loading and transport of MHC class II molecules from the endoplasmic reticulum to the Golgi complex. It is also part of a receptor complex for Macrophage Migration Inhibitory Factor (MIF), and participates in inflammatory signaling. The inhibition of MIF-CD74 complex formation is regarded as a potentially attractive therapeutic target in inflammation, cancer and immune diseases. In order to be able to produce large quantities of the extracellular moiety of human CD74, which has been reported to be unstable and protease-sensitive, different constructs were made as fusions with two solubility enhancers: the well-known maltose-binding domain and Fh8, a small protein secreted by the parasite *Fasciola hepatica*. The fusion proteins could be purified with high yields from *Escherichia coli* and were demonstrated to be active in binding to MIF. Moreover, our results strongly suggest that the MIF binding site is located in the sequence between the transmembrane and the membrane-distal trimerisation domain of CD74, and comprises at least amino acids 113-125 of CD74.

Key words: CD74, MIF, fusion proteins, solubility enhancers.

Running head: **Production of CD74 fused to solubility enhancing peptides**