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Soluble macrophage-derived CD163: A homogenous ectodomain protein with a dissociable haptoglobin–hemoglobin binding ☆

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Abstract

Background: The soluble form of the haptoglobin–hemoglobin (Hp–Hb) scavenger receptor (sCD163) is a specific plasma/serum marker for macrophage activity. Here, we have characterized molecular forms in serum and investigated a role of sCD163 as a binder of Hp–Hb complexes.

Methods: The sCD163 species in serum (from 50 healthy subjects and 29 patients) were measured with domain-specific ELISAs, purified from serum (from 6 individuals) by affinity chromatography and identified by western blotting and MALDI-TOF/TOF mass spectrometry. Binding to Hp–Hb complexes was investigated by gel-chromatography, surface plasmon resonance (SPR) analyses, and inhibition of Hp–Hb endocytosis in CD163-transfected Chinese hamster ovary (CHO) cells.

Results: By using C- and N-terminal-specific ELISAs, no sCD163 concentration differences in plasma were seen, thus indicating a homogenous sCD163 species. Affinity-purified sCD163 from serum migrated as a single band of 130 kDa, and spanned at least 945 amino acids (94%) of the total extra-cellular part of CD163. In solution sCD163 only weakly competed for Hp–Hb uptake in CD163-expressing cells, and Hp–Hb saturation of sCD163 in serum was only seen with large excess of Hp–Hb complexes. However, upon immobilisation, recombinant sCD163 bound Hp–Hb with high affinity. This suggests that Hp–Hb is less dissociable when bound to the membrane form of CD163, presumably because of the di- or multivalent nature of Hp–Hp complexes in terms of CD163 binding.

Conclusions: Serum sCD163 is a homogenous protein covering more than 94% of the CD163 ectodomain including the Hp–Hb-binding region. However, CD163 is a poor competitor of Hp–Hb uptake, probably because of its soluble nature, where Hp–Hb cannot take advantage of receptor cross-linkage.

Keywords: Hemoglobin scavenger receptor; HbSR; Macrophage

Introduction

CD163 is the 130 kDa endocytic receptor for haptoglobin–hemoglobin (Hp–Hb) complexes and it is specifically expressed by macrophages and monocytes (Kristiansen et al. 2001).

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It consists of a large ectodomain of nine scavenger receptor cysteine-rich (SRCR) domains, a transmembrane segment and a short cytoplasmic tail with signals for endocytosis. The expression of CD163 is, like its ligand, highly regulated by inflammatory factors. A soluble form of CD163 (sCD163) present in serum (Sulahian et al. 2001; Moller et al. 2002b) was some years ago identified and shown to be a marker of macrophage activity. Recent clinical studies have further revealed that sCD163 is a promising marker-molecule for macrophage activation in diseases such as hemophagocytic syndrome, sepsis, autoimmune disorders, and liver disease (Baeten et al. 2004; Schaer et al. 2005; Moller et al. 2007, 2006; Daly et al. 2006; Hiraoka et al. 2005).

A fraction of sCD163 is known to originate from shedding from the cell surface upon inflammatory toll-like receptor activation (Droste et al. 1999; Weaver et al. 2006), but the origin and nature of the entire amount of circulating sCD163 are hitherto unknown. A small and putatively soluble CD163 variant has been described at the mRNA level (Law et al. 1993), and furthermore, a region in the CD163 domain 3 has been shown to be very sensitive to proteolytic degradation *in vitro* (Madsen et al. 2004).

Whereas the function of membrane CD163 as a high-affinity Hp–Hb receptor is well established, the functional roles of sCD163 are unclear. Shedding of sCD163 may reduce cellular iron uptake and thereby inhibit growth of intracellular pathogens (Weaver et al. 2006). It has been proposed that sCD163 may be directly involved in iron sequestration by binding of Hp–Hb complexes in the circulation (Weaver et al. 2006; Madsen et al. 2004). The binding of sCD163 to Hp–Hb complexes, however, has not been described in detail.

In order to provide a better basis to understand the biological role of sCD163 in Hp–Hb clearance and to provide more knowledge on what is measured by different immunological assays for sCD163 (Matsushita et al. 2002; Hintz et al. 2002), we have further characterized sCD163 and its ligand-binding properties.

Materials and methods

Materials and patient samples

Full-length CD163 used for calibration of ELISA assays was purified from solubilized human spleen membranes by Hp–Hb affinity chromatography as previously described (Kristiansen et al. 2001). The CD163 monoclonal antibodies GHI/61 and Mac2-158 were from BD PharMingen and Trillium Diagnostics, respectively. A polyclonal rabbit CD163 antibody has previously been described (Kristiansen et al. 2001). Plasma samples from 50 blood donors and 29 hematological patients were collected and stored at −80 °C until analyses. Approval from the local ethics committee was obtained.

Production of recombinant human CD163 proteins

CD163 cDNA fragments were amplified by polymerase chain reaction, purified with the QIAEX II gel extraction kit (Qiagen) and subcloned into the expression vector (pcDNA5/FRT or pSecTag2B from Invitrogen) as described (Madsen et al. 2004). Chinese hamster ovary (CHO) K1 cells (BioWhittaker) were transfected and stable-transfected clones were established by limited dilution and selection with Hygromycin B (Invitrogen) or ZeocinTM (Invitrogen). Clones were grown in serum-free medium for CHO cells and were lysed in a buffer containing Triton X-100 and phenylmethylsulfonyl fluoride as described (Madsen et al. 2004). The expression products were detected by Western blotting of growth medium and cell lysate using a rabbit polyclonal anti-CD163 antibody (Kristiansen et al. 2001).

The following 5 constructs designated according to their SRCR domain composition were subcloned and expressed: r-CD163SRCR1-5 (aa 1-574); r-CD163SRCR1-6; r-CD163SRCR1-7 (aa 1-815); r-CD163SRCR1-9 (aa 1-1025); and r-CD163SRCR5-9 (aa 470-1025).

Enzyme-linked immunosorbent assays specific for SRCR domain-1 and domain-7 of CD163 (Mac2-158 ELISA and GHI/61 ELISA)

A sandwich ELISA for CD163 has previously been described, which uses polyclonal (rabbit) anti-CD163 for coating, and the domain-7-specific MAb GHI/61 for detection (Moller et al. 2002a). A variant of this assay was established using the clone Mac2-158 (0.5 mg/l) in replacement for GHI/61. We have previously shown that Mac2-158 binds to the SRCR domain-1 of CD163, and that the polyclonal coating antibody binds to all CD163 SRCR domains (Madsen et al. 2004). The specificity of the two assays was tested by reactivity to recombinant human CD163 proteins. As expected r-CD163SRCR1-5, r-CD163SRCR1-6, r-CD163SRCR1-7, and r-CD163SRCR1-9 reacted strongly in the Mac2-158 ELISA whereas r-CD163SRCR1-7, r-CD163SRCR1-9, and r-CD163SRCR5-9 reacted strongly in the GHI/61 ELISA.

Gel filtration

Sera from 6 individuals were diluted 1:2 with 0.05 M Tris, 0.3 M NaCl, 0.1% human serum albumin, 0.02% sodium azide, pH 7.2, containing 5 mM EDTA. Diluted