Monitoring macrophage activation by soluble CD163 in plasma

The macrophage is a key cell type in the pathogenesis of several infectious, autoimmune and malignant diseases. The haemoglobin receptor – CD163 – is a specific monocyte-macrophage protein, predominantly expressed on anti-inflammatory cell-types. CD163 is also present in plasma because of regulated shedding of the receptor. The shed soluble CD163 (sCD163) is a promising marker for the monitoring of macrophage activation and is especially useful for the detection of immune-paresis during severe inflammation.

by Dr HJ Møller and Dr SK Moestrup

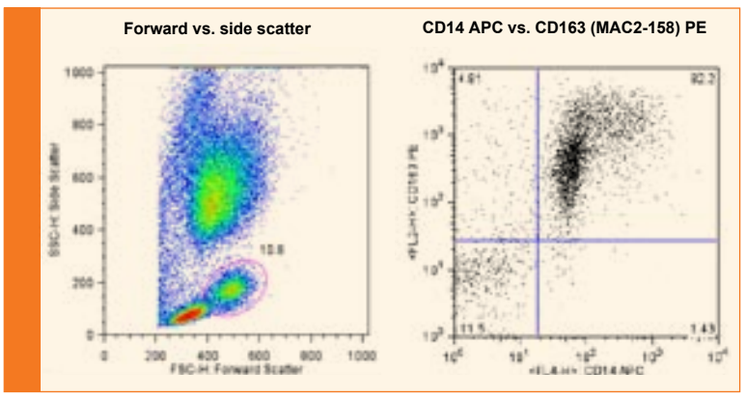

Figure 2. Identification of CD163-expressing monocytes in full blood by flow cytometry using the monoclonal antibody Mac2-158 (IQ Products /Trillium Diagnostics).

CD163 was recognised in 2001 as the receptor scavenging haptoglobin-haemoglobin (Hp/Hb) complexes from plasma [1]. This important physiological function explains the use of declining plasma-haptoglobin as a marker of haemolysis. The Hp/Hb complex binds specifically to the extracellular part of CD163 [Figure 1], and the complex is subsequently endocytosed and degraded in the lysosomes. The removal of "free" Hb from the circulation is essential since free Hb gives rise to toxic symptoms (hypertension, pain, clot formation) due to scavenging of the vasodilator NO and oxidative stress caused by haem. Furthermore, the catabolism of haem by the inducible haem-oxygenase-1 counteracts pro-inflammatory conditions by the production of CO, bilirubin and ferritin, which are reported to have anti-inflammatory effects.

Importantly, CD163 is specifically present only on cells of monocytic origin (i.e. monocytes, macrophages and some dendritic cells) [Figure 2], and is not expressed on other immune, endothelial or connective tissue cells. This feature, combined with the very high expression on macrophages, has made CD163 an important specific immunological marker molecule in medical pathology for diagnostic and research purposes.

Pro- and anti-inflammatory macrophages

It has become clear that macrophages are heterogeneous and are modified by stimuli from the cellular environment. Different subpopulations exist with regard to e.g. surface molecule expression and secretion of cytokines. Although the characterisation of the distinct phenotypes is complex, two main populations can be described: the pro-inflammatory classically activated macrophage (M1) (the well known effector cell of the innate immune defence, producing reactive metabolites for intracellular killing of microbes, and for the activation of the adaptive immune defence) and the alternatively activated macrophage (M2) with reparative functions such as anti-inflammation, scavenging of toxic substances, phagocytosis of apoptotic cells, matrix remodelling and neovascularisation. In view of these diverse roles of M1 and M2 macrophages it is not surprising that CD163 is highly expressed on macrophages with an anti-inflammatory phenotype, which dominate late-phase inflammatory conditions. Accordingly, CD163 is upregulated by IL-6, glucocorticoid and IL-10.

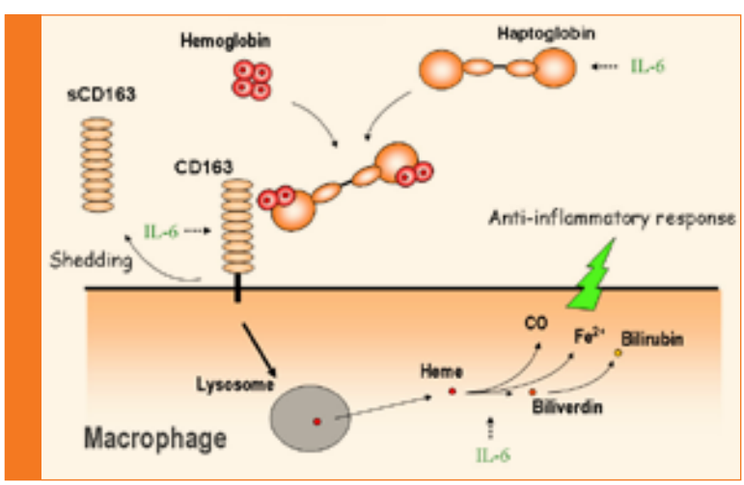

Figure 1. Schematic illustration of the removal of plasma-haemoglobin. Haemoglobin (Hb) binds strongly to haptoglobin (Hp), and the complex subsequently binds to CD163 on the macrophage surface.

Shedding of soluble CD163

The extracellular domain of CD163 is apparently shed to the extracellular environment in a constitutive fashion, and around 1 µg/mL soluble CD163 (sCD163) is present in normal plasma. Upon Toll-like receptor stimulation of macrophages (e.g. by LPS) a steep increase in the plasma concentration is observed due to increased shedding in an acute-phase manner [2]. However, sCD163 does not reflect the general (liver-generated) acute-phase response, but seems to rely specifically on proliferation and activation of macrophages. This differentiates sCD163 from classical acute-phase markers such as C-reactive protein (CRP), and accordingly, clinical studies performed so far have disclosed no or only very weak correlations between the levels of sCD163 and CRP or ESR.

The function of the shed molecule is not known. The high concentrations present (µg/mL) may indicate some functional role in the immune response, but no definite functions have yet been established. It is possible that sCD163 may aid removing Hp-Hb complexes, and inhibitory effects on lymphocytes have been described.

Besides its presence in serum, sCD163 is also present in other body fluids like cerebrospinal fluid (CSF), synovial fluid and ascites fluid. The levels are much higher in synovial fluid than in plasma in patients with arthritis, indicating a local production in the affected joint. Also a relatively high concentration in CSF in healthy subjects indicates a local production by monocyte-derived cells in the central nervous system.

Measuring sCD163

sCD163 is a very stable protein in plasma and serum and can readily

be measured by ELISA, even after repeated thawing and freezing of samples [3]. We have performed a number of methodological and clinical studies using an in-house sandwich assay employing a polyclonal rabbit antibody and a monoclonal antibody for detection. This assay principle has now been adopted in a new commercial ELISA (Macro163, IQ Products/Trillium Diagnostics, based on a monoclonal antibody and patent rights from Dartmouth College). The two assays correlate well (r^2=0.97), which should facilitate transferability of results from different research groups.

A new primary CD163 calibrator has been employed in the Macro163 ELISA. Using this calibration, where the concentration was determined by hydrolysis and amino-acid determination of purified CD163, the level in healthy subjects ranges from 0.5-2 µg/mL. In the individual healthy person the variation over time is quite small (intra-individual variation is 9 % CV). Some apparently healthy elderly individuals may have moderately increased levels. There is no gender difference. The level in children is still poorly defined, although some unpublished data indicate a higher level in children less than two years old.

Soluble CD163 reflects macrophage proliferation and activation: lessons from Gaucher disease and haemophagocytic syndrome

Initial studies in clinical conditions with overt macrophage proliferation and activation have underscored the potential use of sCD163 as a specific serum-marker for macrophage activity. Patients with Gaucher disease (an inherited lysosomal storage disease due to lack of the enzyme glucocerebrosidase) accumulate high numbers of CD163-positive macrophages in bone marrow, spleen and other tissues. These patients have highly elevated sCD163 that correlates with disease severity. Accordingly, sCD163 decreases during enzyme replacement therapy.

Even more pronounced elevations of sCD163 are seen in patients with haemophagocytic syndrome, a sepsis-like condition caused by severe hypercytokinaemia as the consequence of a highly stimulated but ineffective immune response. The syndrome is characterised by haemophagocytosis by activated, morphologically benign macrophages. In its serious form, haemophagocytosis may be lethal due to sustained neutropenia leading to bacterial and fungal infections. A special form of haemophagocytic syndrome occurring in patients with autoimmune disease (estimated to affect 7 % of patients with systemic onset juvenile rheumatoid arthritis) is termed "macrophage activation syndrome".

Awareness of the symptoms of haemophagocytic syndrome (cytopenias, hepatosplenomegaly, prolonged fever) is important in order to start prompt life-saving immunosuppressive therapy. However, the non-specific symptoms often delay treatment. sCD163 is highly elevated in these patients (10-80 µg/mL) and has been proposed as a primary marker for identification of the condition and for the monitoring of the disease cause, which may be characterised by multiple relapses during treatment [4].

	sCD163 (µg/ml)*		Putative clinical use		
	mean	range	diagnosis	prognosis	monitoring
Normal subjects	1	0.5-2			
Gaucher disease	4.5	2-12	++	?	++
AML M4/M5	5	0.5-33		?	+
Hemophagocytic syndr.	19	10-80	+++	+++	+++
Bacteremia/sepsis	3.5	0.5-16		+++	?
Acute liver failure	11	2-38		+++	?
Tuberculosis	1.5	0.5-15		+++	?
Rheumatoid arthritis	1.5	0.5-5		?	(+)
Coeliac disease	Increased			?	+++

*levels are adjusted to the standardisation of the Macro-163 ELISA (IQ-Products/Trillium-Diagnostics)

Table 1. Examples of soluble CD163 in clinical conditions.

sCD163 predicts mortality in sepsis patients: unrecognised haemophagocytosis ?

In local infectious disease or mild sepsis, sCD163 is only slightly elevated, however, in some patients with bacteraemia/severe sepsis, sCD163 reaches levels comparable with those seen in patients with haemophagocytic syndrome. Importantly, patients with bacteraemia and sCD163 plasma levels >5 µg/mL have a 10-times higher risk of mortality from the condition than sepsis patients with lower values of sCD163, and sCD163 may be important in defining patients for intensified treatment [5].

The reason for the increased mortality may be linked to an unfavourable immunological balance in these patients, with an anti-inflammatory predominance ("immune paresis"). In fact, post-mortem studies on bone marrow samples have shown haemophagocytosis (not recognised clinically) in a large number of sepsis patients combined with high CD163 and HO-1 expression in the macrophages. Haemophagopcytosis (and Hb scavenging by CD163) fuels HO-1, producing anti-inflammatory bilirubin and CO, and furthermore stimulates the cell to produce anti-inflammatory cytokines like IL-10 and TGF-ß [Figure 3]. It therefore seems likely that the elevated sCD163 in high-risk sepsis patients may reflect unrecognised haemophagocytosis and immune

paresis, which may have wide implications for individualised therapy [6].

High levels of soluble CD163 in liver disease

Elevated levels of sCD163 are also seen in liver disease (hepatitis, cirrhosis), and Kupffer cells of the liver heavily express CD163. Since CD163-positive macrophages may be directly involved in the fibrosis process of cirrhosis (e.g. by TGF-ß production), sCD163 should be evaluated for the identification of patients at risk (e.g. alcoholic liver disease and non-alcoholic fatty liver disease). In acute liver failure, a sepsis-like state of hyper-inflammation is present in which sCD163 reaches very high levels, comparable to those of haemophagocytic syndrome. Again, high levels of sCD163 correlate to increased risk of fatal outcome, and sCD163 may add important information when making the clinically difficult decision of whether or not to perform a liver transplant [7].

sCD163 in chronic inflammatory conditions

CD163 was originally described as a marker of macrophages in late (chronic) states of inflammation. For example, CD163 is strongly expressed in inflamed synovium in rheumatoid arthritis and in perivascular macrophages plus microglia of multiple sclerosis patients. In coeliac disease, sCD163 is highly increased in untreated cases, and the levels of sCD163 follow the disease course (as judged by histological evaluation of intestinal biopsies) better than tissue-transglutaminase antibody tests [8]. Further research is needed for the evaluation of sCD163 as a prognostic and/or disease-marker in autoimmune diseases like sarcoidosis, rheumatoid arthritis and Crohn's disease.

Is sCD163 a risk marker for cardiovascular disease in low-grade inflammation/ metabolic syndrome?

Marginally increased serum levels of sCD163 are seen in diabetes and in patients with atherosclerosis. For example, a highly significant correlation between sCD163 and the number of atherosclerotic vessels in blood samples collected during coronary angiography has been found. Adipose tissue macrophages are considered to be the source of inflammatory cytokines in the metabolic syndrome, and sCD163 has been shown to correlate to truncal fat mass, which hypothetically may reflect sCD163 production in low-grade inflammatory states related to the metabolic syndrome. It is therefore tempting to speculate that marginally elevated levels of sCD163 may by a risk factor for the development of atherosclerosis and other conditions associated with the metabolic syndrome. This is currently under investigation in a large cohort from the Danish general population.

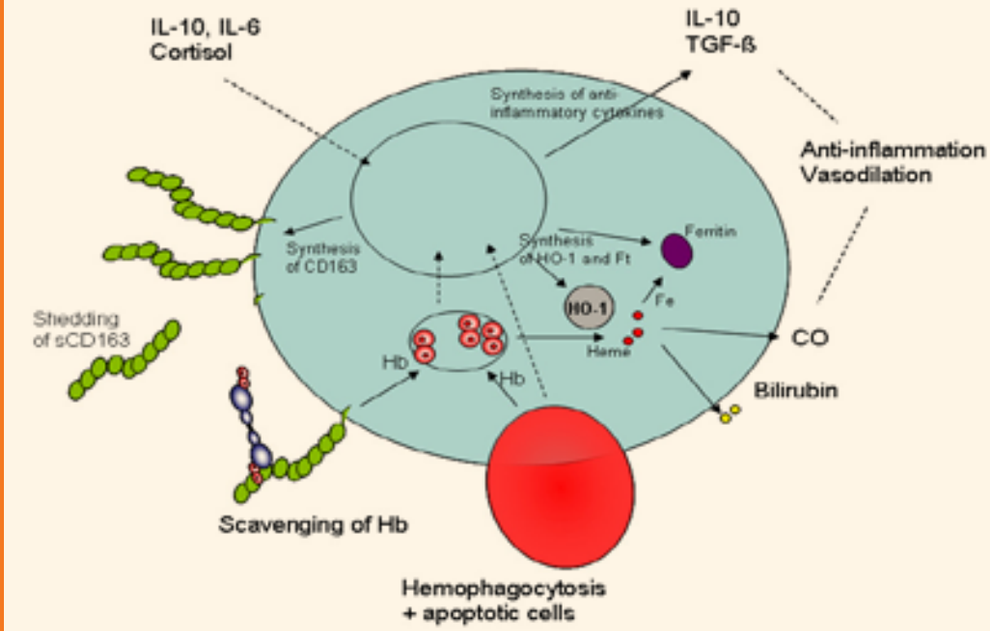

Figure 3. Illustration of some of the functions characterising the alternatively activated (anti-inflammatory) M2-macrophage.

Future studies

A number of reports indicate that sCD163 may be useful in many other clinical situations (e.g. cancer, tuberculosis, HIV, asthma and pregnancy), but more extended studies are needed in order to determine applications for the serum marker. For example, no study has yet thoroughly investigated sCD163 levels in haemolytic conditions (e.g. malaria, sickle cell disease), which obviously may influence expression, internalisation and shedding of CD163. sCD163 should also be carefully investigated for use in monitoring autoimmune diseases (e.g. sarcoidosis) for the evaluation of prognosis in various cancers, and for monitoring organ transplant rejection. Finally, studies are now evaluating the use of sCD163 in multimarker assays (e.g. Luminex assays).

Conclusion

Soluble CD163 is a newly identified specific marker of macrophage activity detectable in plasma and other human body fluids. The levels in plasma reflect general proliferation and activation of macrophages and seem valuable for the management of several autoimmune, infectious and malignant diseases. sCD163 has already proven to be an important prognostic marker in severe sepsis, liver failure and haemophagocytic syndrome.

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A complete bibliography relevant to this article is available from the authors.

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