REVIEW ARTICLE

SCAVENGER RECEPTOR CD163 AND ITS BIOLOGICAL FUNCTIONS

Gabriela Onofre, Martina Koláčková, Karolina Jankovičová, Jan Krejsek

Charles University in Prague, Faculty of Medicine and University Hospital Hradec Králové, Czech Republic: Department of Clinical Immunology and Allergology

Summary: CD163 is a member of scavenger receptor super family class B of the first subgroup. It is mapped to the region p13 on chromosome 12. Five different isoforms of CD163 have been described, which differ in the structure of their cytoplasmic domains and putative phosporylation sites. This scavenger receptor is selectively expressed on cells of monocytes and macrophages lineage exclusively. CD163 immunological function is essentially homeostatic. It also has other functions because participates in adhesion to endothelial cells, in tolerance induction and tissues regeneration. Other very important function of CD163 is the clearance of hemoglobin in its cell-free form and participation in anti-inflammation in its soluble form, exhibiting cytokine-like functions. We review the biological functions of CD163 which have been discovered until now. It seems apparent from this review that CD163 scavenger receptor can be used as biomarker in different diseases and as a valuable diagnostic parameter for prognosis of many diseases especially inflammatory disorders and sepsis.

Key words: *CD163; Scavenger receptor; Hemoglobin; Haptoglobin; Functions*

Introduction

In the last years it has been studied a lot about members of SRCR – SF (scavenger receptor cysteine-rich super family), among them CD163, but the exact function of many molecules of this family still remains unclear.

The aim of this review is to summarize the biological functions of CD163 which have been discovered until now. CD163 can be used as biomarker in different diseases such as inflammation and as a valuable diagnostic parameter for monitoring macrophages activation.

This receptor was first identified in 1987 (21). CD163 antigen is a member of the scavenger receptor cysteine-rich (SRCR) super family class B (4, 26). CD163 was previously called M130 and P155 before it finally received its CD designation. Now it has several names, which are used very promiscuiously: CD163 antigen, hemoglobin scavenger receptor (HbSR), hemoglobin/haptoglobin complex receptor, macrophage-associated antigen, RM3/1 antigen, M130 antigen precursor, MM130, Ki-M8, Ber-MAC3, SM4, and GHI/61, respectively (2, 26).

Several investigations on CD163 have been conducted in humans and marmosets, but also in porcine and rodents. The most important studies were done in rats and humans (3, 4, 14, 24, 37).

Homologous gene to human CD163 has been identified in the porcine, which is encoding as 2A10 antigen. The monoclonal antibody 2A10 recognizes 120-kDa protein with sequence homology to the human CD163 and whose expression is restricted to the cells of the porcine monocyte/macrophage lineage (25).

In rats, CD163 has been identified and described as ED2 antigen. Recently it has been demonstrated by purification and peptide sequencing that the rat ED2 antigen represents the ortholog of CD163. It is located on a distal rat chromosome 6 (29). The monoclonal antibody ED2 is used to define mature tissue macrophages in the rats. In contrast to humans, it is not present on monocytes and alveolar macrophages (4). CD163 is a cell surface glycoprotein receptor with a molecular weight 130 kDa (26, 32).

Molecular characterization

The SRCR – SF (scavenger receptor cysteine-rich super family) members are structurally divided in two groups. Group A contains SRCR domains with six cysteine residues, which are encoded by two exons. Group B usually contains six to eight cysteines and are encoded by a single exon. Structurally, group B is also subdivided into two subgroups, according to the presence or absence of extracellular domains other than the SRCR (26).

It is recognized that CD163 is structurally a member of group B SRCR-SF. It belongs to the first subgroup because it is a protein that exclusively comprises of SRCR domain in its extra cellular region. The extracellular domain of CD163 is composed of nine SRCR domains.

The basic transcript encodes for a protein of 1076 amino acids. The extracellular part contains 1003 amino acids. The transmembrane single segment comprises 24 amino acids; it is encoded by 72 nucleotides of exon 14. Short cytoplasmic domains consists of 49 amino acids (17, 26).

Five different isoforms of CD163 have been described so far. They differ in the structure of their cytoplasmic domains and putative phosporylation sites. Three of these isoforms display different splicing forms of the cytoplasmic domain, which vary from 49 to 84 or 89 amino acids, respectively. The first 42 amino acids after the membrane spanning segment are common for all three isoforms (7). Two possible alternatives splice sites are observed in an extracellular part of the molecule, one generating a stop codon resulting in a truncated form of protein only consisting of the first three SRCR domains, the other introducing additional 33 amino acids between SRCR5 and 6 domains (26, 34).

The genomic molecule sequence of CD163 gene consists of 17 exons and 16 introns and spans at least 35 kD (17, 34).

The exon 1 contains the start codon and encodes the N – terminal part of the signal peptide. The C-terminal part of the signal sequence is encoded by exon 2 and two nucleotides of exon 3. Each of the nine SRCR – domains of the CD163 protein is encoded by a separate exon. The length of these exons varies between 309 and 324 nucleotides (17). It is very important to mention that these exons are separated by phase I introns.

The chromosomal location of the human CD163 gene was mapped to region p13 on chromosome 12. Rearrangements of the short arm of chromosome 12 is one of the most common cytogenetic abnormalities found in a broad range of hematological malignancies (24). These include translocations, insertions, inversions and deletions, among them the most frequent involve band 12p13 (14, 17).

Expression of CD163 scavenger receptor

In general, this scavenger receptor is selectively expressed by cells of monocytes macrophages lineage. It is described that monocytes are positive from 5 % to 30 %. This is a low to moderate expression; but it has been claimed that all monocytes and almost all tissue macrophages are positive for CD163 (10, 26, 32).

CD163 is expressed at high levels by mature tissue macrophages, which are abundant in spleen, placenta and liver (Kupffer cells), lymph nodes, thymus, bone marrow, brain, lung and peritoneum, respectively (4).

The expression of CD163 is influenced by several factors, such as: cyclosporine A, phorbol ester (PMA). Treatment with phorbol ester, cyclosporine A, decreases expression of CD163 on the cell surface (11, 31, 36). CD163 is regulated by pro and anti-inflammatory mediators. For example: TNF-α (tumor necrosis factor alfa) and interferon gama, LPS, transforming growth factor B, lead to decreased expression of this receptor. On the other hand the glucocorticoids, IL-6, IL-10, steroids, induce the increase of CD163 expression (1, 9, 11, 19, 28, 32, 34, 35).

Functions of CD163 scavenger receptor

The specific functions of CD163 have been identified a few years ago, CD163 has been known as macrophages specific membrane protein for which it was suggested the immunological function and the best characterized one is the homeostatic one, essentially.

It has been demonstrated that CD163 is involved in the adhesion of monocytes to endothelial cells. It was reported that this marker is a novel surface molecule that contributes to the inhibition of the adhesion of glucocorticoid treated (dexamethasone-induced) monocytes to activated endothelial cells, indicating a function of CD163 in the adhesion of monocytes to activated endothelium (2, 34). These authors suggest that in the anti-inflammatory phase, CD163-expressing monocytes are recruited from the blood and adhere to the endothelium, whereas during an early steps of inflammation, CD163 on monocytes adhere via different adhesion proteins. The ligand for CD163, on endothelial cells is still unknown (3, 36).

This receptor was identified on macrophages as an adhesion receptor for erytroblasts in erytroblastic islands. It suggests a possible regulatory role for this molecule in erythropoiesis (33).

Furthermore, it has been revealed that CD163 is a mediator against systemic inflammation and is taking part in the resolution of inflammation in the late down regulatory phase both acute and chronic inflammation (1, 19, 23, 38). It is also found in a high number in inflamed tissue, and its function is to down modulate the process of inflammatory macrophages response. We can see that CD163 expression is elevated on circulating monocytes of patients with systemic inflammation. It is important to know that CD163 expression on monocytes is also regulated during the early innate immune response (35). Recently it has been studied that CD163 participates in tolerance induction and tissue regeneration (6, 19). Also some reports show that CD163 soluble molecule may inhibit human T lymphocytes activation and proliferation *in vitro* (12, 22), thereby facilitate suppression of the inflammatory response (5, 22). Frigs and co-workers indicated that the soluble form of CD163 might have a direct suppressive action on CD4+ T helper cells (5).

The best known function of CD163 is the clearance of hemoglobin in its cell-free form and participation in anti-inflammation as a soluble factor, exhibiting cytokine-driven functions.

Free hemoglobin is released into the blood by two mechanisms: the extra and intravascular haemolysis, which is also known as erythrolysis.

Extra vascular haemolysis is the mechanism of red cells phagocytosis by macrophages in the spleen and bone marrow. At the same time alternatively to the process of pha-

gocytosis the erythrocytes can suffer intravascular haemolysis in a few per cent (10 % to 20 %) too. In this mechanism hemoglobin is released from the ruptured red blood cells and dissociates (dimerizes) in two dimers αβ. Subsequently they are captured by plasma protein haptoglobin, thus they form the complex haptoglobin/hemoglobin (7, 19).

Haptoglobin is a plasma protein for which there are two common alleles in humans, denoted 1 and 2. It is a hemoglobin binding protein expressed due to genetic polymorphism as three major phenotypes: 1-1 (homozygous for the 1st allele), 2-1 (heterozygous) and 2-2 (homozygous for the 2nd allele) (8, 16, 18). CD163 reveals higher affinity for the complexes haptoglobin/hemoglobin-2 subtype 2-2 complexes compared to complexes haptoglobin/hemoglobin-1 (27). Both differ in the cytokines response, which they induce via this binding. CD163 binding of the haptoglobin-1 and forming the haptoglobin/hemogobin complexes-1 rapidly stimulates production of anti-inflammatory cytokines such as IL-10. On the other hand, CD163 binding to the haptoglobin-2 and formed haptoglobin/hemoglobin complexes-2, does not stimulate anti-inflammatory cytokines (8, 30). Binding the complex haptoglobin/hemoglobin to CD163 requires the presence of calcium (13, 19). It is very necessary for maintaining the proper tertiary structure of CD163.

Recently it has been reported that the stimulation of anti-inflammatory cytokines by the haptoglobin hemoglobin complex 1 is critically dependent on casein kinase II (CK II) signalling. The studies show that haptoglobin/hemoglobin 1 complexes signal transduction was mediated by changing the specific activity of CK II associated with CD163 (30). It is known that cross-linking of CD163 by antibodies induces a transmembrane signal casein kinase II and protein kinase C dependent intracellular calcium mobilization. It includes phosporylation of the cytoplasmic tail, induction of inositol triphosphate synthesis, resulting in macrophages activation and secretion of cytokines, such as IL-6, IL-1B and GM-CFS (27, 33). The increased activity of CK II appears to be mediated by its phosphorylation.

CD163 expressed on macrophages internalizes the complex hemoglobin/haptoglobin by endocytosis. Subsequently, the heme subunit of Hb is degraded by the rate-limiting heme oxygenase (HO) in lyzosomes. HO is a potent anti-inflammatory and anti-oxidative enzyme. Two main isoforms of HO have been characterized, HO-2 being constitutively present under physiological conditions and HO-1 being inducible by anti-inflammatory stimuli (4, 21). The latter one has been suggested as a potent cytoprotective enzyme. The heme molecule is converted by this enzyme in anti-inflammatory metabolites, as well as biliverdin, carbon monoxide molecule (CO), which has also cytoprotective effects, and free iron (19, 21). Furthermore, Il-6 is known as a regulator of the degradation pathway of the free hemoglobin by stimulating the synthesis/expression of haptoglobin, CD163 and heme oxygenase-1. The induction of intracellular downstream signaling pathways ultimate to increased HO-1 activity and secretion of anti-inflammatory cytokines, such as Il-10. Finally, the reduction of biliverdin to bilirubin by biliverdin reductase occurs in the reticulo-endotelial system macrophages. The bilirubin is then transported into the blood bound to the albumin or hemopexin. It is conjugated in the liver and excreted into the bile. In the intestine the bilirubin is reduced to the urobilinogens. Normally, urobilinogen is excreted almost completely in the feces, a little or no urobilinogen is excreted in the urine (Fig. 1).

The elimination of the complexes haptoglobin/hemoglobin is necessary to remove free hemoglobin from plasma. This remove overcomes oxidative damage, prevents glomerular filtration of hemoglobin and heme intoxication of the kidney by accumulation of free hem and iron because it has oxidative and toxic properties. This mechanism also avoids the oxidative stress, the reactive oxygen species (ROS), and cells injury (13, 19).

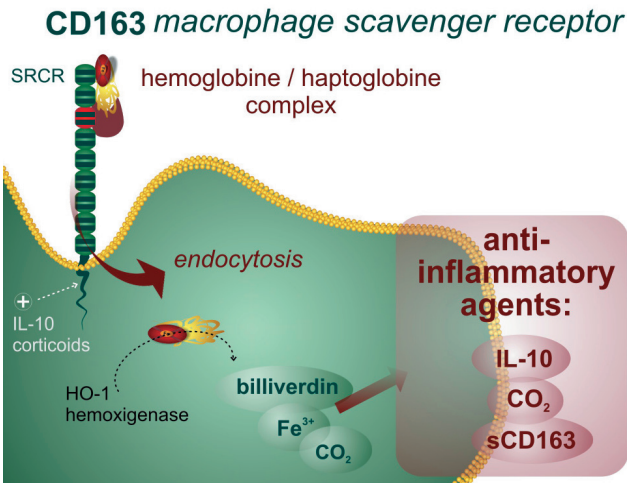

Fig. 1: Schematic representation of free hemoglobin elimination by macrophages via CD163 scavenger receptor.

Soluble form of CD163 (sCD163)

The CD163 soluble form represents a novel, abundant natural protein in human plasma, which is present in other fluids of organism apart from plasma too. Fluids like cerebrospinal, synovial, or ascitic fluid, respectively, contain sCD163. Detectable levels of sCD163 in plasma of healthy subjects range from 0.73 to 4.69 mg/L, with a median value of 1.87 mg/L (2, 4, 7, 31).

The soluble form of CD163 is a biomarker associated with several clinical conditions. It represents a new class of macrophage specific biomarkers relevant to the detection of coronary artery disease, transplantation, atherosclerosis, rheumatoid arthritis, lysosomal Gaucher storage diseases and cancer, cardiac surgery patient respectively (4, 15).

It also plays an important role in the detection of inflammation, systemic inflammatory response syndrome (SIRS), sepsis, bacteriemia, mononucleosis, leishmaniasis,

Crohn's disease, celiac sprue, spondylarthropathy, synovitis, sclerosis, hepatitis and fulminant hepatic failure (9, 19, 22).

It is found in inmunohematological diseases as: lymphoma, reactive haemophagocytic syndrome, histiocystic neoplasm, myeloproliferative diseases, myelo-monocytic leukemia. It has also been documented in other inflammatory conditions characterized by monocytic infiltration. The most interesting part is that there has been an increased level of this receptor and it has been correlated with resolution of these diseases (1, 7).

In many of this diseases, the patients are extensively exposed to a large amounts of free heme/hemoproteins due to intravascular haemolysis and tissues damage; CD163 represents a highly efficient system to remove potentially toxic an pro inflammatory hemoglobin from the circulation and local sites of inflammation; for this reason is considered to be a prognostic marker in different diseases; mainly in inflammation (15).

As highly sensitive and standardized comercial diagnostic kit produced by IQ Products, Gronigen, NL is available, we can detect sCD163 by ELISA (enzyme-linked immunosorbent assay) technique in serum, plasma and other body fluids. We can also use other methods as: flow cytometry, immunohistochemistry, immunoprecipitation and western blotting, to detect the expression of CD163.

Conclusions

As a conclusion it could be summarized that CD163 is a member of scavenger receptor class B with a majority of functions that still remain unclear, however it has several applications in clinical medicine where its soluble form has a biological significance as an interesting candidate marker of inflammation, sepsis and imunohematological diseases. It is considered as a prognostic marker, which can be used for monitoring these diseases. In comparison with other markers of inflammation, this marker is exclusively expressed in the monocytes/macrophages cells. It has been confirmed that its expression is not correlated with other parameters of inflammation like C reactive protein (7).

The up-regulation of CD163 by cytokines and glucocorticoids contributes to anti-inflammatory response attenuating inflammation by two mechanisms. The first one shows CD163 as a soluble factor, exhibiting cytokine-driven functions. The second reveals that CD163 acts like a scavenger of haptoglobin/hemoglobin complexes preventing the hemoglobin intoxication and toxic effects of free hem. This mechanism also avoids the oxidative stress and cells injury.

In addition CD163 as an endocytic receptor selectively expressed in machropages can be used for macrophage targeting by several substances, for instance DNA and drugs chemically linked to haptoglobin/hemoglobin complexes.

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Corresponding author:

Prof. RNDr. Jan Krejsek, CSc., Department of Clinical Immunology and Allergology,
University Hospital in Hradec Králové, Sokolska 581, Hradec Králové 500 05, Czech Republic; e-mail: krejsek@fnhk.cz