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CD163 and Inflammation: Biological, Diagnostic, and Therapeutic Aspects

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Abstract

Significance: The hemoglobin (Hb) scavenger receptor, CD163, is a macrophage-specific protein and the upregulated expression of this receptor is one of the major changes in the macrophage switch to alternative activated phenotypes in inflammation. Accordingly, a high CD163 expression in macrophages is a characteristic of tissues responding to inflammation. The scavenging of the oxidative and proinflammatory Hb leading to stimulation of the heme-oxygenase-1 and production of anti-inflammatory heme metabolites indicates that CD163 thereby indirectly contributes to the anti-inflammatory response. ***Recent Advances:*** In addition to this biological role in inflammation, CD163 is a potential inflammation biomarker and a therapeutic target. The biomarker form of CD163 is the soluble plasma CD163 that arises from the increased shedding of CD163 mediated by the tumor necrosis factor-α (TNF-α) cleaving enzyme. This explains that a steadily increasing literature documents that the plasma level of soluble CD163 is increased in a large spectrum of acute and chronic inflammatory disorders. The nonshed membrane form of CD163 in macrophages constitutes a target for drugs to be directed to macrophages in inflammation. This approach has been used in an animal inflammation model to highly increase the apparent therapeutic index of anti-inflammatory glucocorticoid drug that was coupled to an anti-CD163 antibody. Furthermore, other recent animal data, which indirectly involve CD163 in macrophages, demonstrate that injections of haptoglobin attenuate Hb-induced damages after blood transfusion. ***Critical Issues and Future Directions:*** The diagnostic and therapeutic properties of CD163 await further clinical studies and regulatory approval before implementation in the clinic. *Antioxid. Redox Signal*. 18, 2352–2363.

Introduction

CD163 was originally identified in two independent reports (75, 123) using monoclonal antibodies as an unknown monocyte–macrophage-specific antigen associated with the anti-inflammatory process. Several other groups identified the same protein named as the antigen of different monocyte–macrophage-specific antibodies, such as Ki-M8 (95), GHI/61 and SM4 (94), Ber-MAc3 (6), AM-3K (121), and 2A10 (100). The Leukocyte Typing Workshop V defined the common antigen of the different antibodies leading to the CD163 designation (52, 93) The function of the protein was for long unknown, but its expression, regulation, and primary structure (59) suggested an immunological receptor function (52, 75, 123). In 2001, CD163 was identified as the "hemoglobin (Hb) scavenger receptor" HbSR (57) for the uptake of Hb released into the plasma and complexed to haptoglobin (Hp) during intravascular hemolysis. Although this function appeared distinct from a direct immunological role, it was fully in accordance with the structure, macrophage-specific expression, and regulation (69). In recent years, the receptor has also been reported to bind the tumor necrosis factor-α (TNF-α)-like weak inducer of the apoptosis (TWEAK) protein (15), and some pathogenic bacteria (30) and virus (101, 113).

In addition to providing the basic information about CD163 in terms of structure, function, and expression, the present review will describe the many links between CD163 and human inflammation. This includes the regulated expression in macrophages, direct removal of proinflammatory ligands (in particular Hb), fueling of the pathway for generation of anti-inflammatory heme metabolites, inflammation-induced shedding of CD163, the use of soluble CD163 as a biomarker for inflammatory diseases, and the development of CD163-targed anti-inflammatory therapy.

CD163 Structure

CD163 is a 130-kDa membrane protein with a short cytoplasmic tail, a single transmembrane segment, and a large ectodomain consisting of nine scavenger receptor cysteine-

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rich (SRCR) scavenger receptor class B domains (59). Different isoforms of human CD163 have been described, including three variants with different length of the cytoplasmic tail (59), with the short tail form (42 amino acids) being the most abundant. All variants contain common internalization motifs and exhibit endocytic activity (83).

The SRCR domain is a common 100–110 amino acid domain for molecular interactions (65) and with a determined structural fold of six or seven β-sheets cradling an α-helix (34, 45, 98). The SRCR class A and class B domains share a similar fold (34, 98) and differ only by the presence of an additional disulfide bond in the class B domains. Whereas SRCR class A domains largely are present as single domains in different mosaic domain proteins, including the scavenger receptor, AI and MARCO. The class B domains are largely present as tandem repeat membrane proteins that only contain this type structural motif in the ectodomain (65). Figure 1 shows the human SRCR class B family membrane proteins CD163, CD163b, CD5, CD6, and Scart1. CD163b, which has its encoding gene located close (107) to the CD163 gene on chromosome 12 (97) and represents the closest homologue to CD163 (41). It exhibits similarities in terms of tissue distribution, regulation, and endocytic capability although it seems to have another not yet defined ligand repertoire (67).

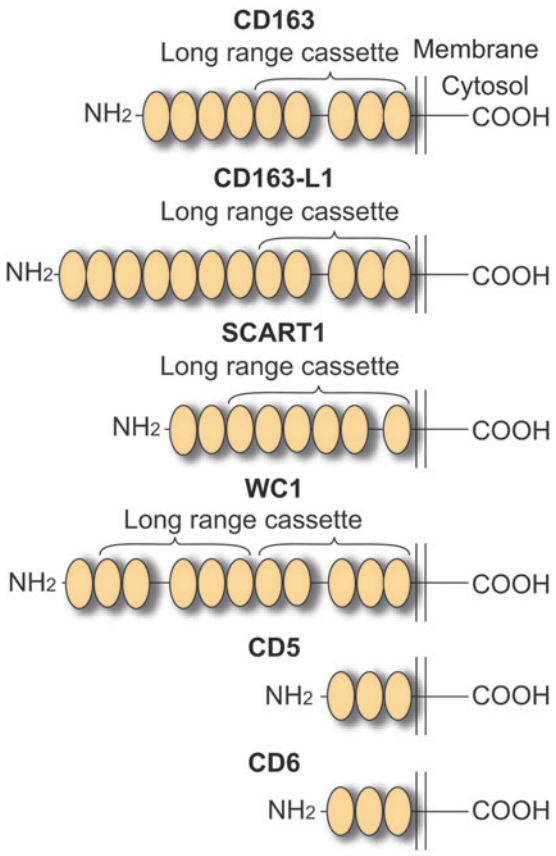

FIG. 1. Scavenger receptor cysteine-rich (SRCR) class B family membrane protein members. All members contain multiple extracellular repeats of the SRCR class B domain. A conserved repeat, known as the long-range cassette, is defined by a cassette of five SRCR domains separated by a 31 amino acid spacer between repeat 2 and 3. All the proteins, except for WC1, which was identified in the pig, are encoded by the human genome. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Some of the CD163 SRCR domains contain consensus sites (88) for calcium binding, and the binding of Hp-Hb complexes, as well as several antibodies (62, 63) exhibit calcium-dependent binding. The calcium binding is pH-sensitive and the calcium-binding structure is therefore suggested to be an essential structural component for the uncoupling of ligand upon internalization (62). Accordingly, the calcium-binding SRCR domain 3 of CD163 has been shown to be essential for the binding of Hb-Hp to CD163 (62).

CD163 Expression and Regulation

Human CD163 expression is restricted to the monocytic–macrophage linage with high expression in, for example, red pulp macrophages, bone marrow macrophages, liver macrophages (Kupffer cells), lung macrophages, and in macrophages of several other tissues (93). A similar expression pattern is observed in the Lewis rat (10, 91). Monocytes have a modest expression of CD163, but the expression level highly increases in culture along with other macrophage characteristics (2). Previous conflicting data on the monocyte expression have been explained by a receptor-based study revealing that the cellular staining in flow cytometry protocols is very sensitive to the antibody used and the incubation conditions, which impacts reproducibility of results (63).

Low or absent CD163 expression is seen in other monocyte-derived cells, such as dendritic cells (64), Langerhans cells (55), and white pulp macrophages in the spleen (93). A number of factors (Table 1) regulate CD163 expression *in vitro*. The most potent stimulators of CD163 expression known are glucocorticoid, interleukin (IL)-6, IL-10, and heme/Hb, whereas IL-4, lipopolysaccharide (LPS), TNF-α and interferon γ, CXC-chemokine ligand 4 (CXCL4), and granulocyte–macrophage colony-stimulating factor downregulate CD163 expression (17, 19, 36, 49, 108, 111). The effect of glucocorticoids *in vitro* (75, 123) has been confirmed *in vivo* by analyzing human monocytes after administration of glucocorticoids to human volunteers (124). The glucocorticoid-mediated regulation of CD163 is further evidenced by the identification of three glucocorticoid receptor-binding sites in the promoter

Table 1. Substances Regulating CD163 Expression in Monocytes/Macrophages *In Vitro*

Compound	*Up- or downregulation*
Glucocorticoid	Up
IL-4	Down
IL-6	Up
IL-10	Up
IFN-γ	Down
LPS	Down
TNFα	Down
CXCL4	Down
GM-CSF	Down
Hb	Up

Refs (16, 19, 36, 49, 75, 108, 111, 123)

IL, Interleukin; IFN γ, interferon γ; LPS, lipopolysaccharide; TNFα, tumor necrosis factor; CXCL4, CXC-chemokine ligand 4; GM-CSF, granulocyte–macrophage colony-stimulating factor; Hb, hemoglobin.

region of the CD163 gene. Furthermore, binding sites for several transcription factors important for myeloid differentiation have been identified. Altogether, the observations on the regulation of CD163 conclude that CD163 is a feature of macrophages that differentiate into the "alternatively activated" macrophages that contrast the classical activated M1-type macrophages (37). Accordingly, CD163-expressing macrophages have been detected in sites of inflammation, such as chronically inflamed arthritis joints (8, 33), atherosclerotic plaques (96), and the vicinity of tumor cells (tumor-associated macrophages) (18).

The CD163-positive macrophage may originate from extravasation of monocytes or may represent macrophage activation switching (92) of already present M1 proinflammatory macrophages. Studies of atherosclerosis (16) and Hb (49) suggest that CD163 and Hb from local microbleedings (plaque hemorrhage) have an atheroprotective role *via* the metabolism of Hb leading to polarization of macrophages. These studies have led to a definition of a new class of CD163-positive atheroprotective and anti-inflammatory macrophages in atherosclerotic lesions (16). These macrophages, now designated Mhem macrophages, are characterized by a high iron load and heme-oxygenase-1 (HO-1) activity in contrast to the low content of those in M1, M2, and Mox macrophages (16). This further underscores the plasticity of macrophages and their multiple and overlapping phenotypes that may be regarded as a pronounced tendency to adapt to the local environment.

Future studies of atherosclerosis and other types of inflammation in CD163 knockout animals should further define the protective role of CD163 in site of acute and chronic inflammation. CD163 knockout animals may better define a recent hypothesis that atherogenesis is reduced in mice with a knock out of the gene encoding the platelet chemokine, CXCL4, might relate to an absent CXCL4-mediated polarization of macrophages with low CD163 expression in these animals (36).

The present literature on CD163 expression is largely based on work on human material and to some extent the rat and pig systems, *in vivo* data are limited. Unfortunately, most of the comprehensive characterization of macrophage differentiation in animal models is based on the mouse system, where a suitable anti-CD163 antibody for tracking CD163 expression until recently has been missing. By implementing CD163 expression in future studies of the many mouse inflammation models, new information on macrophage differentiation and CD163 expression during inflammation will hopefully become available.

CD163- and Hp-Mediated Hb Scavenging

CD163 is a high-affinity receptor of human Hp-Hb complexes (57, 62) that instantly form when Hb is released from erythrocytes during physiological or pathological hemolysis (Fig. 2). Moreover, free Hb can bind to low affinity to CD163 (102) and this may have importance after depletion of Hp during excessive hemolysis. Binding of Hp to Hb is one the strongest protein–protein interactions occurring in plasma (48). The high-resolution structure of the porcine complex is now known (3) and it shows how a previously identified loop region important for CD163 recognition (87) pertrudes from the complex in the proximity of Hb. Surprisingly, studies of mice did not reveal a clearance-promoting effect of Hp (26) suggesting that in this and other species, the Hp role in relation to Hb may be limited to protective functions, such as avoiding peroxidative modification of Hb (14, 20) and impairing filtration of the relatively small Hb molecule by the kidney (31, 51). The binding of the complex to CD163 macrophages leads to a fast degradation of the complex, and in case of an increased intravascular hemolysis as seen in many pathological conditions, such as malaria, hemoglobinopathies (*e.g.*, sickle cell disease), autoimmune hemolyses, and drug-induced hemolysis, Hp may virtually disappear from the human plasma. Hp and the protein moiety of Hb are degraded in lysosomes (57). The fate of Hb is most likely identical to that of Hb present in macrophages upon erythrophagocytosis of outdated red cells. In the macrophage, the heme-oxygenases convert heme to biliverdin, carbon monoxide (CO), and iron. Biliverdin is further converted to bilirubin, which is released and transported by albumin to the liver for conjugation and excretion in the bile. Overall, the removal of Hb and the generation of heme metabolites result in a localized anti-inflammatory response (99).

The anti-inflammatory effects of the heme metabolites CO and biliverdin/bilirubin are the outcome of multiple biological mechanisms not fully elucidated and reviewed in detail elsewhere (40). Among the many effects of CO, where some overlap those of NO, this well-known, but potentially very toxic CO gas, is a potent inhibitor of proinflammatory cytokines, such as IL1 and TNF-α and a stimulator of IL-10 (61). In malaria, where the plasmodium parasites may cause severe hemolysis and heme intoxication, CO is reported to have a specific cytoprotective role because it binds to free Hb and thereby prevents heme release from Hb (32). Biliverdin and bilirubin are antioxidants and their main cytoprotective function is based on the inhibition of lipid and protein peroxidation, but they apparently also exhibit direct anti-inflammatory activity, such as inhibition of the complement cascade, P/E-selectin expression, and attenuation of leukocyte rolling (9, 42, 105). Finally, the release of $Fe2+$ may indirectly lead to protection of cells from oxidative stress even though this atom is indirectly pro-oxidative. The mechanism is that $Fe2+$ stimulates the expression of ferritin, the iron storage protein, which has antioxidative properties (89).

Interestingly, HO-1 may also have cytoprotective effects independent of the effect mediated by the heme metabolites. Elegant studies of cells transfected with catalytic inactive HO-1 have shown that HO-1 itself is involved in cell signaling and different changes in phenotype, including an upregulated expression of catalase, glutathione peroxidase, and GSH (46). However, much seems still to be learned about the HO-1 protein that also may have an adverse affect in the central nervous system, where HO-1 is reported to exacerbate early brain injury after intracerebral bleeding (117).

Altogether, the stimulation of the Hp-CD163-HO-1 metabolic pathway owing to an increased Hb metabolism leads to a counter response of the pro-oxidative effects of Hb. In case of consumption of Hp, the protective effect of the system may be weakened leading to heme-induced damage of tissues, in particular, the kidney. However, CD163 may still play a role because *in vitro* studies have shown a low-affinity binding of Hb to the receptor. Moreover, plasma contains hemopexin, which acts as a kind of back-up protein (110) that binds the heme leading to the uptake of hemopexin–heme complexes by CD91 (47, 85) expressed in macrophages, hepatocytes, and several other cell types (68). The backup by hemopexin has been demonstrated in

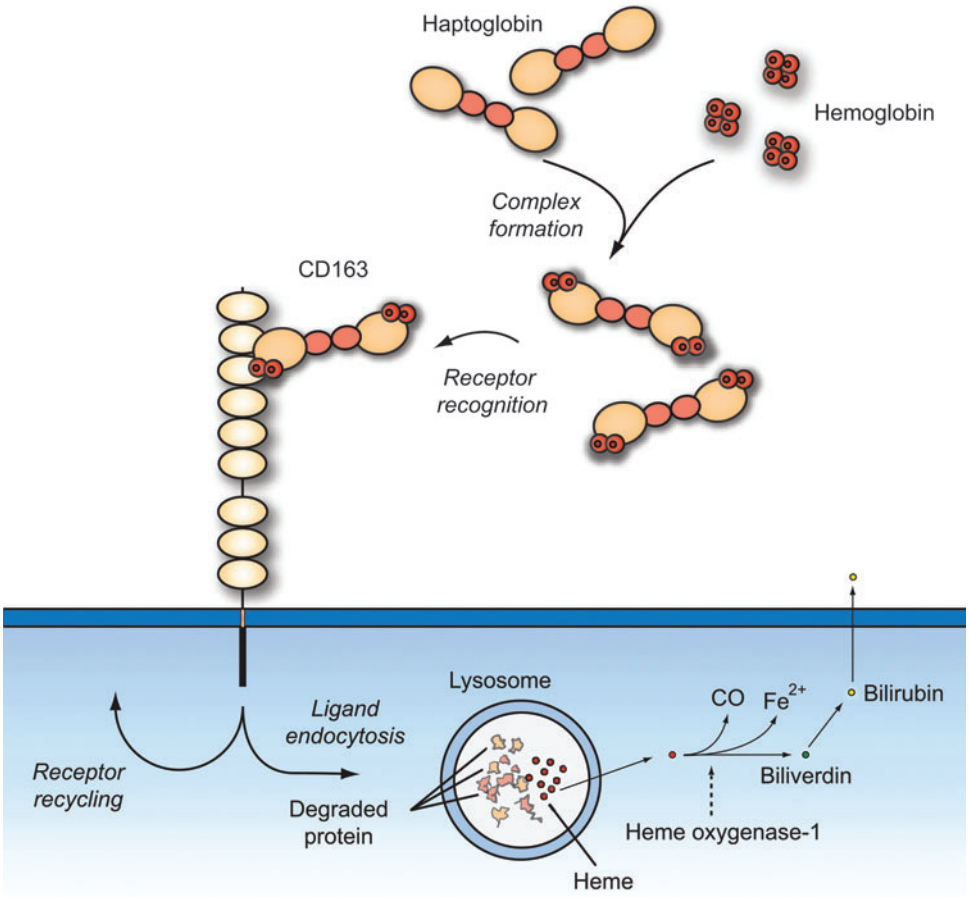

FIG. 2. CD163-mediated scavenging of Hb upon intravascular hemolysis. CD163 is highly expressed on phagocytic macrophages. Upon hemolysis, released hemoglobin (Hb) is rapidly bound by the acute-phase protein Hp forming the haptoglobin–hemoglobin (Hp-Hb) complex. The complex is subsequently bound and removed from the circulation by CD163-positive macrophages in the liver, spleen,and bone marrow. The uptake of Hb by macrophages contributes to the recycling of iron and also to the inflammatory response. The uptake of Hb in macrophages and subsequent degradation of heme by heme oxygenase-1 (HO-1), produce the anti-inflammatory metabolites, Fe^{2+}, CO, and biliverdin. Biliverdin reductase converts biliverdin to bilirubin, which is secreted to the cell exterior. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

knock models (116). These data show that mice with a combined hemopexin and Hp knockout are far more sensitive to Hb-induced inflammation than mice with knockout of Hp or hemopexon alone. (109). In line with these data, the knockout of CD163 as described in an accompanying article of this issue seems to have no obvious effect on the mouse health (26). On the other hand, the same study shows striking differences between mouse and man in terms of the role of Hp in Hb metabolism. Most important, mouse Hp, which has less than a tenth of the Hp plasma concentration in humans, does not promote high-affinity binding of the complex to CD163. Interestingly, free mouse Hb binds with higher affinity to CD163 compared to human Hb. In view of these differences, it seems essential to take precautions when translating rodent findings in the Hb degradation pathway to human settings.

Humans also differ from other species by having two different Hp gene alleles encoding Hp1 and Hp2, where the Hp2 protein contains a duplicated α-chain that bridge to two other Hp α-chains. This provides the property of forming disulfide-linked Hp multimers (see (60) for review) in individuals heterozygous or homozygous for the Hp2 gene (the Hp2-1 and Hp2-2 phenotypes, respectively). Individuals with the Hp1-1 phenotype have dimeric Hp that is the basic Hp form in most mammalian species. All the human Hp phenotypes bind with high affinity to CD163 when complex Hb. *In vitro* experiments have shown that the multimeric Hp2-2 binds with a higher functional affinity to the immobilized receptor than the Hp1-1 form (57). The multivalency in terms receptor-binding sites leading to crosslinking of multiple receptors by the multimers may explain this observation.

Human and other old monkey primates also express the Hp-related protein (Hpr), which has only 23–30 amino acids in difference compared to Hp1-1. Hpr binds Hb with high affinity, but the Hpr-Hb complex does not bind to CD163 (84–86, 115). Hpr is instead associated to apolipoprotein L-containing lipoprotein particles that constitute a subspecies of high-density lipoprotein (HDL) particles. Hb binds to Hpr in the particles, which then are activated as an innate defense weapon against certain trypanosome parasites (Trypanosoma brucei brucei) causing animal sleeping sickness (Nagana) (84, 114). To sequester heme for enzymes, the parasites have a Hp-Hb receptor, but in contrast to CD163, this receptor also binds Hpr-Hb (84, 114, 115). Consequently, the parasites take up circulating HDL particles also containing Hp-Hb and apolipoprotein L. The latter has a strong membrane pore-forming effect that leads to rupture of the lysosomal membrane and to self-digestion of the parasites (12). Primates, including humans, therefore have an innate immunity against the trypanosoma brucei brucei infection, which is a great threat against domestic animals in a large part of the African continent (115). However, humans have no resistance against the human pathogenic Trypanosoma brucei rhodiense, and Trypanosoma brucei gambiense parasites are apparently accounted for by the presence of an apolipoprotein L inhibitor (119) and reduced haptoglobin–hemoglobin complex receptor expression (50), respectively.

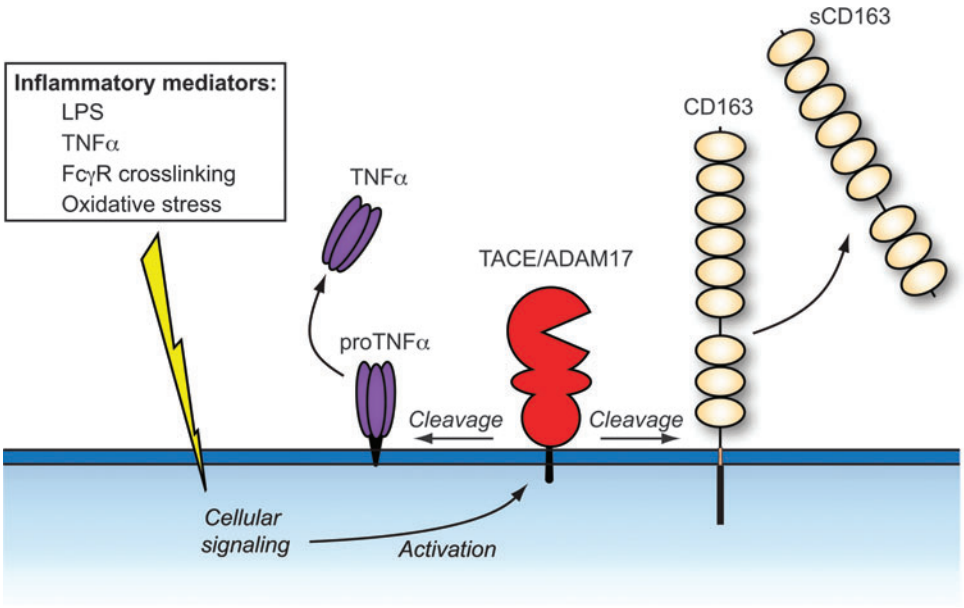

FIG. 3. A disintegrin and metalloproteinase 17 (ADAM17)/tumor necrosis factor (TNF)-α converting enzyme (TACE)-mediated shedding of CD163 and TNF-α upon stimulation by proinflammatory stimuli. CD163 and proTNF-α are rapidly cleaved from the surface of activated macrophages by an ADAM17/TACE-dependent mechanism. A number of proinflammatory substances induce ADAM17/TACE activation. The exact site of cleavage in CD163 awaits identification. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Other Functions of CD163

In addition to the established role of CD163 in Hb metabolism, other ligands for CD163 have been identified as reviewed in detail recently (112).

Early data on the rat ED2 antigen, which later have been identified as CD163, identified binding to rat erythroblast (11). A later follow-up study reported that CD163 has regulatory role during rat erythropoiesis (29).

Several reports (15, 74) have shown that TWEAK, a secreted cytokine belonging to the TNF-α superfamily, binds to CD163. CD163 is proposed as a scavenger receptor for TWEAK, preventing TWEAK from exerting its biological functions by sequestering it from the physiological environment.

Some bacteria and virus have been reported to bind human CD163. The first study on binding of the bacteria to CD163 reports that CD163 functions as sensor rather than an endocytic receptor for Streptococcus mutans, Eschericia coli, and Staphylococcus aureus. The expression of CD163 in monocytic cells promoted bacteria-induced production of proinflammatory cytokines, like TNF-α. Another study has reported binding of soluble CD163 to Staphylococcus aureus *via* binding of specific fibronectin peptides, which promotes recognition, phagocytosis, and killing of the bacteria (53).

African swine fever virus (ASFV) and the porcine reproductive and respiratory syndrome virus (PRRSV) are the two virus reported to bind CD163 (21, 101, 113). The binding of virus to CD163 seems important for the virus infection, but in different ways. ASFV is proposed to exploit CD163 for attachment and internalization (101), whereas PRRSV data suggest a role for CD163 during virus uncoating (113). Minor envelope glycoproteins GP2a and GP4 of PRRSV are reported to interact with CD163 (24).

Among the new ligands reported during the last decade, only the virus–CD163 interaction and TWEAK interactions have been reported by more than one laboratory. Further investigation should establish the physiological role and the consequences in human disease.

Finally, it is intriguing to speculate that CD163 might have other not yet identified ligands. Many endocytic scavenging receptors are multiligand receptors (56) and it would make sense if, for instance, other intracellular components, such as Hb, liberated to the plasma during cell rupture or extracellular waste products generated in the resolution phase of inflammation take advantage of the CD163 (or CD163b)-mediated scavenging in macrophages.

CD163 As Clinical Biomarker in Inflammation

A soluble form of CD163 ectodomain is present in normal plasma (81) and an increased plasma concentration of sCD163 is seen in diseases relating to macrophage activity, including acute and chronic inflammations (71). The physiological role of sCD163 is not defined. Soluble CD163 binds Hp-Hb complexes (81), but it is a poor competitor of CD163-mediated uptake of this ligand (80), probably because the ligand has no affinity gain in crosslinking to soluble receptors. It is possible that the soluble receptor has functions not related to Hb, such as the proposed role in opsonization of bacteria described above (53).

Soluble CD163 is a homogenous protein (80) spanning at least 94% of the entire CD163. Several studies (43, 66, 118) have pointed on a metalloproteinase as the enzyme responsible for cleavage of CD163 in macrophages, and recently the inflammation regulated a disintegrin and metalloproteinase 17 (ADAM17)/TNF-α–cleaving enzyme (TACE) was identified as the responsible enzyme using inhibitor and siRNA knockdown analyses (28) (Fig. 3). In view of the TNF-α-mediated inflammatory symptoms during conditions, such as sepsis and chronic inflammations (1), it is likely that the increased levels of sCD163 measured under these conditions are the outcome of concomitant ADAM17/TACE-induced release of CD163 and TNF-α in macrophages. Figure 4 shows the effect on the LPS and TNF-α levels in healthy subjects after an LPS injection, which instantly increases the plasma levels of TNF-α and CD163. In contrast to the fast clearance of TNF-α, the soluble CD163 level remains increased for days. In this context, soluble CD163 may be

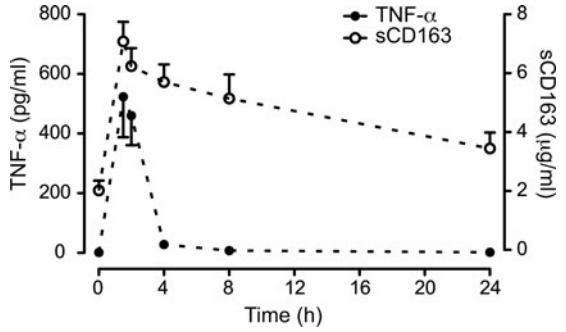

FIG. 4. Soluble CD163 is a long-circulating surrogate marker of TNF-α in experimental endotoxemia. Serum levels of sCD163 and TNF-α in healthy volunteers ($n = 8$) after receiving a bolus-injection of endotoxin. Serum analysis shows a fast increase in both markers, whereas TNF-α rapidly cleared increased levels sCD163 are still measured after 24 h. The figure is reproduced from (28) with permission from J. Leuk. Biology.

regarded as a long-circulating surrogate marker for TNF-α in conditions where LPS leads to shedding of CD163. Probably, this may extend to other inflammatory conditions, since several other stimuli, such as Fc receptor crosslinking *via* activation of toll-like receptors, cause TACE/ADAM17 activation and release of TNF-α and CD163 (104, 118).

Although the levels of soluble CD163 may increase many-fold by ADAM17/TACE activation, the amount of soluble CD163 in plasma is probably low compared to the amount of the membrane-bound form in macrophages. There are no data with exact comparison of the two forms in humans, which so far is the only species, where the soluble CD163 has been reported. However, comparison of the levels of receptor purified from human tissues (57) with the measured amount in plasma suggests that most of the body's CD163 is membrane-associated even when the soluble CD163 concentration is upregulated. The major pool of CD163 seems to be localized intracellularly (83) as seen for other endocytic receptors trafficking between the surface and endosomes (70), and it is therefore likely that a temporary shedding of CD163 will be followed by a fast mobilization of CD163 from the intracellular pool.

As reviewed in detail elsewhere (71), the level of CD163 has been thoroughly characterized in normal and diseased individuals (Table 2). The range in normal individual is 1–4 mg/l based on a widely used enzyme-linked immunosorbent assay (77) and there is a low intraindividual variation (78). Increased levels are seen in many diseases (71) involving macrophages with the highest levels measured in patients with hemophagocytosis (103) and the related macrophage activation syndrome (13). These conditions are characterized by an abnormal lymphohistiocytic activation of complex and partially unknown etiology leading to the systemic inflammatory response syndrome. Macrophage accumulation in the bone marrow, liver, or lymphoid tissues and overt phagocytosis of blood cells and their precursors are a pathognomonic feature of the syndrome, which has a high mortality. The high significant level of sCD163 in hemophagocytosis and macrophage activation syndrome has led to the proposal of including soluble CD163 as one of the diagnostic criteria (22, 25).

Several other diseases, where inflammation is an important component of the pathogenesis have increased levels of soluble CD163. This includes acute diseases, such as bacterial sepsis/infection (54, 76, 79), hepatitis (44), and malaria (58) as well chronic inflammation, such as rheumatoid arthritis (39, 66), Crohn's disease (76), scleroderma (82), coeliac disease (23), and atherosclerosis (4, 73) (Table 3). Generally, the increase in the CD163 level is much more pronounced in acute inflammations, such as bacteremia/sepsis, where the CD163 level also has negative correlation to survival (79).

The measurement of soluble CD163 in a general population cohort encompassing 8849 individuals followed for 18 years revealed that soluble CD163 is a risk marker for the development of type 2 diabetes and it correlates with indices of the metabolic syndrome (72). Previous data have shown that soluble CD163 correlates with the body fat mass (5, 106), which probably reflects the fat-induced low-grade inflammation in these individuals. Interestingly, the cohort study shows that soluble CD163 predicts diabetes, independently of body mass index and age, suggesting that soluble CD163 may be used to identify persons genetically predisposed for low-grade inflammation diabetes 2 (72). Two recent studies following up on the large cohort study have shown that soluble CD163 positively correlates to insulin resistance, which is the fundamental problem in diabetes 2 (90, 120).

In conclusion, soluble CD163 is increased in a number of diseases (and in particular, inflammatory diseases) with increased macrophage activity. The receptor will probably not be used as a single diagnostic marker of a disease, but it may gain use as a macrophage activity marker complementary to clinical findings and other laboratory tests. Furthermore, it

Table 2. Reported CD163 Binding Substances

Hp-Hb complexes (57)
Hb (102)
TWEAK protein (15, 74)
Porcine-pathogenic viruses (the African swine fever virus (ASFV) (101)
Porcine reproductive and respiratory syndrome virus (PRRSV) (21, 113)
Various gram-positive and gram-negative bacteria (30, 53)
Erythroblasts (11, 29)

Hp-Hb, haptoglobin–hemoglobin complex; TWEAK, TNF-like weak inducer of apoptosis.

Table 3. Examples of Inflammatory Diseases with Increased CD163 Plasma Levels

Acute inflammations (infections)		
Hepatitis	High increase	(44)
Bacteriemia/sepsis	High increase	(54, 76, 79)
Malaria	High increase	(58)
Chronic inflammations		
Rheumatoid arthritis	Moderate increase	(39, 66)
Mb Crohn	Low increase	(76)
Scleroderma	Low increase	(82)
Celiac disease	Low increase	(23)
Atherosclerosis	Low increase	(4, 73)
Low-grade chronic inflammation		
Diabetes II	Low increase	(71, 90, 120)
Other type of inflammation		
Macrophage activation syndrome/ Hemophagocytosis	Very high increase	(13, 103)

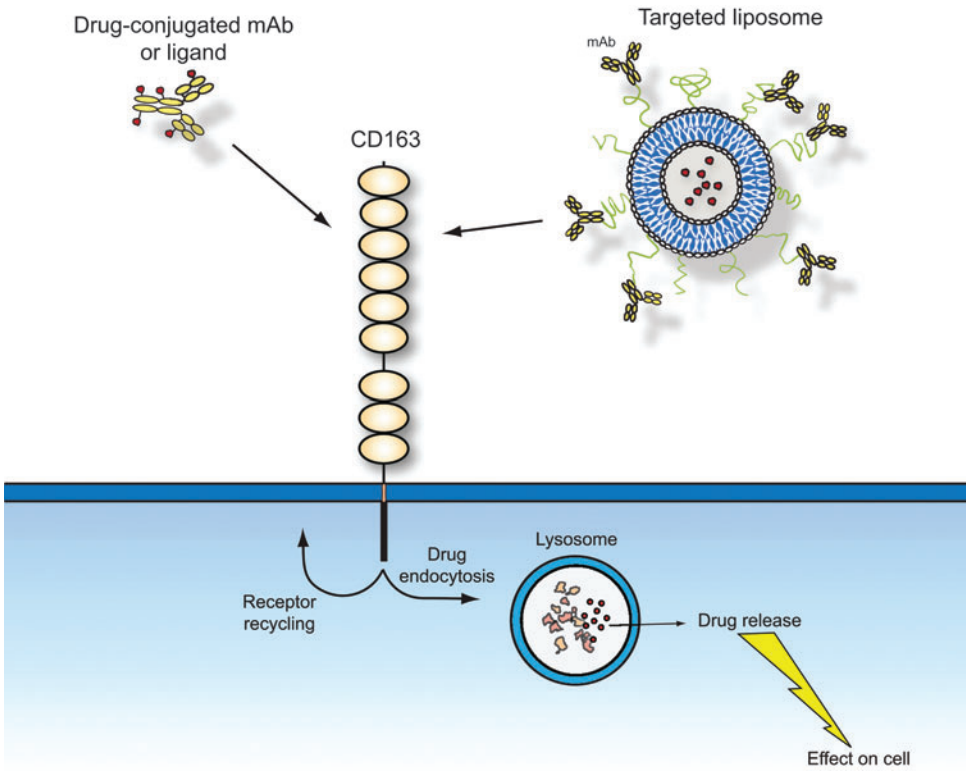

FIG. 5. Using CD163 as a target for directed drug delivery. The endocytic property of CD163 allows either ligand- or antibody-associated drugs to have an easy and specific access to cytosolic compartment of macrophages. The figure shows examples of two types of conjugates: CD163 antibody- or ligand-drug conjugates and drugs encapsulated in pegylated liposomes with a CD163-binding antibody or ligand linked to the phospholipid layer. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

may be used as a monitoring marker of single individuals during disease progression and treatment.

CD163 As a Receptor for Drug-Targeting Macrophages

The specific expression of CD163 in monocytes/macrophages makes the receptor an interesting gate for drug delivery. This may apply to different kinds of disease, including inflammatory disorders, certain cancers of myeolo-monocytic origin, infections, where the macrophages harbor the pathogen (*e.g.*, mycobacterium, HIV, or leishmania parasites), and rare genetic disorders (*e.g.*, Gaucher's disease affecting macrophages).

The pathological mechanisms of inflammation includes several types of leukocyte effector cells (monocytes–macrophages, T-lymphocytes, and granulocytes) and complex immune cell interplays such as those between macrophages and T helper cells. There are therefore many potential drug attack points in treatment of inflammation. Cytostatics (*e.g.*, methotrexate), glucocorticoids (*e.g.*, prednisolone and dexamethasone), and different biological drugs (*e.g.*, antibodies against TNF-α ad other cytokines) are potent drugs widely used in rheumatoid arthritis and other chronic inflammatory disease. However, serious side effects (*e.g.*, by glucorticoids) or loss of efficacy (*e.g.*, by methotrexate and biological drugs) keep up the need for development of new anti-inflammatory drugs.

The CD163-expressing macrophage is an interesting therapeutic target because CD163 macrophages are present at the site of inflammation where they, despite an overall anti-inflammatory function, also produce inflammatory cytokines, such as TNF-α. This cytokine is largely produced by macrophages and the efficacy of anti-TNF-α biological drugs (35) suggests that the macrophage is an obvious target for anti-inflammatory therapy. The proof of concept of this CD163-targeting therapy has recently been established in a recent study in rats using an antibody drug conjugate (ADC) consisting of average four glucocorticoid drugs (dexamethasone) linked to an anti-rat CD163 antibody (38). This glucocorticoid conjugate exhibited high affinity to CD163 comparable with that of nonconjugated antibody, and cell experiments revealed that the receptor mediates the endocytosis and transport of the drug to the cell interior. A rat model of acute sepsis-induced inflammation (LPS-induced TNF-α release) revealed about a 50-fold higher efficacy of the intravenously injected conjugate compared to free dexamethasone. Holding promise for the use of this new strategy in glucocorticoid treatment, the conjugate showed no major systemic effects/side effects measured as suppression of endogenous cortisol production and weight loss of whole body and thymus (indicates lymphocyte apoptosis). In contrast, the equipotent amount of free dexamethasone induced complete suppression of endogenous cortisol production and a substantial loss in body and thymus weight.

CD163 targeting may also be used for other type of conjugates, such as drugs directed linked to a physiological CD163 ligand (*e.g.*, Hp-Hb) or liposome-encapsulated liposomes with a CD163 targeting moiety in the membrane (Fig. 5). A recent rodent study has, for instance, designed liposomes encapsulating the fluorescent dye calcein in the interior and integrated Hb in the phospholipid layer (122). *In vitro* uptake analysis showed that they are taken by cultured macrophages. The CD163 specificity in terms of targeting CD163-positive macrophages of these Hb-liposomes remains to be investigated though. The potential alternative clearance

mechanism of Hb and the apparent absent Hp-promoting of Hb clearance in the mouse (25) suggest that mice, and perhaps also other rodents, should be used as the only test animals. An alternative type of conjugate vehicles targeting CD163 has recently been constructed and analyzed *in vivo* and *in vitro* (27). This type of conjugate vehicle is a pegylated liposome (stealth liposome) protected with polyethylene glycol that prevents nonspecific targeting and intravascular rupture. Anti-mouse CD163 is linked to the phospholipid layer by a hydrophobic linker. Loading the liposomes with the cytotoxic agent doxorubicin revealed strong CD163-dependent cytotoxic effects in cultured CD163-expressing cells. *In vivo* analysis of calcein-loaded anti-CD163 stealth liposomes showed accumulation in mainly the liver that contains the majority of the body macrophages. A much lower uptake was seen with nontargeting liposomes (27).

Using CD163 for drug delivery to macrophages could suggest an inhibitory effect of circulating soluble CD163 that may bind to the therapeutic ADC intended for targeting CD163 in macrophages. However, the dose for use in human therapy of ADC is far higher than the plasma amount of soluble CD163, thus suggesting that the soluble receptor is competed out in the first dose. Furthermore, soluble CD163 is a poor competitor for macrophage uptake of Hp-Hb complexes probably because the ligand gains functional affinity by crosslinking two CD163s (or more for the Hp2-1 and Hp2-2 phenotypes) in the membrane. The IgG moiety of the therapeutic ADC is also divalent and therefore likely also to have preference for crosslinking membrane-associated CD163.

Finally, CD163 may also indirectly be involved in therapy using Hp that may be administered during excessive hemolysis, where the body's Hp store has been consumed. A recent study in guinea pigs nicely demonstrates that such a Hp therapy attenuates the tissue damaging effects by Hb released during blood transfusion (7).

Conclusion

The present review summarizes information on the Hb scavenger receptor CD163 with focus on its pamphlet of relations to inflammation. Besides being an important biological link between Hb metabolism and the anti-inflammatory response elicited by HO-1 and the heme metabolites in macrophages, an increasing body of evidence from many laboratories have now evidenced that the level of soluble CD163 increases in several acute and chronic inflammatory disorders. Novel data have documented that this increased presence in plasma is owing to the stimulated activation of the ADAM 17/TACE metalloproteinase (27). The high concentration of soluble CD163 in systemic inflammatory hemophagocytosis, has led to a proposal of including soluble CD163 as a marker in the diagnosis of this disorder. Further studies and documentation may reveal whether soluble CD163 in the future may be included in clinical risk prediction, diagnosis, and disease monitoring in relation to other inflammatory diseases. The latest novel connection between CD163 and inflammation concerns the use of CD163 and a target for drug delivery to macrophages. By the use of targeting antibodies or ligands directly coupled to drugs or to liposomes encapsulating drugs, it is now possible to direct in principle any kind of drug, such as cystostatic and anti-inflammatory compounds, directly to macrophages in the inflammatory process. The first reports (26,37) using this technology seem rather promising for the development of conjugate glucocorticiods with far lower side effects and higher potency than the nonconjugated glucocorticoid used in therapy today.

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Abbreviations Used

ADAM17 = a disintegrin and metalloproteinase 17
ADC = antibody drug conjugate
ASFV = African swine fever virus
CO = carbon monoxide
CXCL4 = CXC-chemokine ligand 4
GM-CSF = granulocyte–macrophage colony-stimulating factor
Hb = hemoglobin
HDL = high-density lipoprotein
HO-1 = heme oxygenase-1
Hp = haptoglobin
Hp-Hb = haptoglobin–hemoglobin complex
Hpr = haptoglobin-related protein
IL = interleukin
LPS = lipopolysaccharide
PRRSV = porcine reproductive and respiratory syndrome virus
SRCR = scavenger receptor cysteine-rich
TACE = TNF-α converting enzyme
TNF-α = tumor necrosis factor-α
TWEAK = TNF-like weak inducer of apoptosis