Acta Biochimica Polonica

Vol. 53 No. 2/2006, 257–268

on-line at: www.actabp.pl

Review

Haemoglobin scavenger receptor: function in relation to disease

Jolanta Zuwała-Jagiełło✉

Department of Pharmaceutical Biochemistry, Wrocław Medical University, Wroclaw, Poland;
✉e-mail: jagiellodr@interia.pl

Received: 08 February, 2006; revised: 01 May, 2006; accepted: 22 May, 2006
available on-line: 12 June, 2006

Highly efficient systems remove the toxic and proinflammatory haemoglobin from the circulation and local sites of tissue damage. Macrophages are major haemoglobin-clearing cells; CD163 was recently recognized as the specific haemoglobin scavenger receptor (HbSR). It is tightly involved in both physiological as well as pathophysiological processes, such as cytoprotection and inflammation. Haemoglobin functions as a double-edged sword. In moderate quantities and bound to haptoglobin, it forms a ligand for haemoglobin scavenger receptor CD163/HbSR, but when unleashed in large amounts, it can become toxic by mediating oxidative stress and inflammation. CD163/HbSR plays a crucial role in the control of inflammatory processes, probably in part through its effects on both ferritin induction and subsequent induction of antiinflammatory pathways through interleukin-10 and haem oxygenase. Besides the observation that the haemoglobin scavenger receptor provides a promising target for new treatment possibilities, it offers a novel view on the aetiology of diverse physiological as well as pathophysiological processes. In addition, monocyte CD163/HbSR and soluble CD163/HbSR are potential diagnostic tools in a variety of disease states, such as inflammation, atherosclerosis, transplant rejection, and carcinoma.

Keywords: haemoglobin scavenger receptor, CD163, haem oxygenase, inflammation, atherosclerotic lesions

INTRODUCTION

Haemoglobin released from erythrocytes into the circulation by intravascular haemolysis binds immediately with haptoglobin, a serum glycoprotein, and forms a stable haemoglobin–haptoglobin (Hb-Hp) complex. Hb binding by haptoglobin is thought to be important in the rapid hepatic clearance of haemoglobin from the plasma and in the inhibition of glomerular filtration of haemoglobin. The presence of specific receptors on liver parenchymal cells that recognize and endocytose the Hp–Hb complex has led to a widely held belief that the major function of Hb binding by Hp is to target plasma Hb for rapid clearance and degradation in the liver (Oshiro & Nakajima, 1988; Okuda *et al.*, 1992; Oshiro *et al.*, 1992). However, this belief is not consistent with other observations that haptoglobin binding has no effect on hepatic clearance and uptake of free haemoglobin from the plasma that isolated liver parenchymal cells can take up free Hb at a faster rate than that of the Hb–Hp complex and that free Hb is cleared at a faster rate from the circulation than that of the Hb–Hp complex (Keene & Jandl, 1965; Weinstein & Segal, 1984; Osada & Nowacki, 1989).

During severe intravascular haemolysis or transfusion of Hb solution, Hb precipitates in the renal tissues leading to acute renal failure and a concomitant depletion of Hp. Thus Hp retards the passage of Hb through glomeruli into the renal tubular cells, hence protecting against peroxidative kidney injury. The increased susceptibility to haemoglobin-driven lipid peroxidation demonstrated in conditions of anhaptoglobinemia or hypohaptoglobinemia in Hp-deficient mice and in humans supports this hypothesis (Panter *et al.*, 1985; Gutteridge, 1987; Miller

Abbreviations: AGE, advanced glycation endproducts; GHb, glycated haemoglobin; Hb, haemoglobin; Hp, haptoglobin; HbSR, haemoglobin scavenger receptor; HO, haem oxygenase; Hp–Hb, haptoglobin–haemoglobin complex; IL-10, interleukin-10; LPS, lipopolysaccharide; sHbSR, soluble plasma form of haemoglobin scavenger receptor; SR, scavenger receptor; SRCR, scavenger receptor cysteine-rich domain; TBARS, thiobarbituric acid-reactive substances.

et al., 1997; Delanghe *et al.*, 1998; and reviewed in Lim *et al*., 2001).

Tissue macrophages are a major part of the mononuclear phagocyte system and remove haemoglobin, generated by intravascular haemolysis, in a complex with haptoglobin (Wada *et al.*, 1970). The macrophage receptor has been of major interest in the study of the clearance mechanisms linking haptoglobin, free haemoglobin and tissue macrophages over the last years. CD163, previously described as RM3/1 antigen or M130, belongs to the cysteine-rich scavenger receptor superfamily type B and has recently been identified as a haemoglobin scavenger receptor (CD163/HbSR) for the Hp–Hb complex (Kristiansen *et al*., 2001). This specific receptor-ligand interaction explains the depletion of circulating Hp in individuals with increased intravascular haemolysis. Moreover, the CD163/HbSR-mediated endocytosis may represent a major pathway for the uptake of iron in tissue macrophages. The biological role of CD163/HbSR might be related: clearance of Hb and a potential immunoregulatory (anti-inflammatory) function (Graversen *et al*., 2002). The clearance of the pro-inflammatory Hb from sites of local tissue destruction after trauma or during inflammation may be a crucial role played by a subpopulation of anti-inflammatory macrophages. The CD163 receptor has been reported to be expressed by macrophages accumulating during the down-regulation of inflammatory reactions and during wound healing (Zwadlo *et al*., 1987; Djemadji-Oudjiel *et al*, 1996).

In this review the significance of the haemoglobin scavenger receptor is presented in relation to various biological and pathological processes, with special reference to inflammation. In addition, the CD163/HbSR is discussed in relation to atherosclerotic lesions and cancer.

GENERAL PROFILE OF SCAVENGER RECEPTOR FAMILY

Several new members of the scavenger receptor (SR) family have been cloned on the basis of their ability to recognise modified lipoproteins. The members are classified as follows: SR class A consists of SR-AI, SR-AII, SR-AIII; class B consists of SR-BI, CD36; class C contains only *Drosophila* SR-C.

The class A scavenger receptors (SR-As) are trimeric, integral membrane glycoproteins (Brown & Goldstein, 1983). There are three forms of the receptor derived by alternative splicing of a single gene (Gough *et al.*, 1998). The three isoforms each contain six predicted structural domains: cytoplasmic, transmembrane, spacer, α-helical coiled coil, collagenous, and a type-specific carboxyl terminus. Type I SR-A has a 110–amino acid scavenger receptor cysteine-rich (SRCR) domain, a highly conserved protein motif found in many other immunological proteins. Type II SR-A has a short carboxyl-terminal domain that is relatively nonconserved between species. Type III SR-A has a truncated form of the scavenger receptor cysteine-rich domain and has been shown to have dominant negative properties (Resnick *et al*., 1994; Gough *et al.*, 1998).

SR-A have been implicated in various macrophage functions, including endocytosis, adhesion, phagocytosis, and intracellular signalling (Platt *et al*., 1996). Both type I and type II SR-A bind a diverse array of macromolecules, including modified lipoproteins (acetylated or oxidised LDL), bacterial surface lipids (endotoxin and lipoteichoic acid) and proteins modified by advanced glycation (advanced glycation endproducts, AGEs) (Suzuki *et al.*, 1997).

Scavenger receptor class B type I (SR-BI), a member of the CD36 superfamily, is predominantly expressed in the liver and steroidogenic tissues, where it mediates selective uptake of cholesteryl esters from HDL. Hepatic SR-BI plays an important role in the late stages of reverse cholesterol transport and protects mice against the development of atherosclerosis (Krieger, 2001). SR-BI is also expressed in macrophages, including tissue macrophages, monocyte-derived macrophages, and macrophages in atherosclerotic lesions. Macrophage SR-BI is involved in the initial steps of reverse cholesterol transport and therefore might be protective against atherogenesis (de la Llera-Moya *et al.*, 2001). However, direct evidence that macrophage SR-BI expression is antiatherogenic is lacking. Interestingly, SR-BI and CD36 significantly differ in their ability for selective lipid uptake. SR-BI, which binds HDL and selectively transfers cholesteryl esters and phospholipids, could potentially mediate LPS transfer into the cell as well (Yu *et al.*, 1997). Thus, the class B SRs could indirectly have an effect on mediating an inflammatory response.

The scavenger receptor cysteine-rich (SRCR) superfamily was recognized during an analysis of the structure of the type I macrophage scavenger receptor. Molecules with SRCR domains are divided into two groups based on the localization and number of cysteine residues. All members of group A have six cysteine residues and lack cysteine residues at positions 1 and 4. Members of group B have either eight or six cysteine residues, but the cysteine residues at positions 1 and 4 are always present. Group A SRCR domains are encoded by two exons, whereas group B SRCR domains are encoded by a single exon (Resnick *et al*., 1994).

On the basis of their structure, sequence homologies, and domain organization, members of the group B SRCR family can be divided into three subgroups. The first subgroup comprises CD5, CD6

and SPα (Jones *et al.*, 1986; Aruffo *et al.*, 1991; Gebe *et al.*, 1997). CD5 and CD6 are predominantly expressed by mammalian T cells and some specialized B cells, while Spα is found in lymphoid tissue. Spα is a structurally different protein since it has no transmembrane domain and is supposed to be secreted. The second subgroup within the SRCR group B molecules includes gp-340 (Holmskov *et al.*, 1999), *DMBT1* gene (Mollenhauer *et al.*, 1997), and their murine and rabbit counterparts CRP-Ductin (Cheng *et al.*, 1996), and Ebnerin (Li & Snyder, 1995). The most extensively studied subgroup comprises WC1 and CD163. The *WC1* gene is expressed by T cells. WC1 is composed of eleven SRCR domains, a transmembrane region, and a cytoplasmic domain (Burton & Kehrli, 1996). Law *et al.* (1993) identified CD163 as a 130-kDa human macrophage-associated antigen defined by five different antibodies. Their results showed that the membrane protein contains a leader peptide of 40 residues and a putative extracellular domain of 1003 residues, followed by a hydrophobic segment of 24 residues and a cytoplasmic domain of 49 residues. The extracellular domain has nine repeating elements, of about 110 residues, that are similar to those of the scavenger receptor superfamily. Other results indicate that the *CD163* gene contains 17 exons and spans over 35 kb (Ritter *et al.*, 1999). Each of the nine SRCR domains is encoded by a separate exon, similar to other members of the group B SRCR subfamily. Two cytoplasmic variants of CD163 arise from alternative splicing of intron 15, while a truncated and an extracellular variant result from alternative splicing of intron 5 or intron 7, respectively (Ritter *et al.*, 1999). Kristiansen *et al.* (2001) also identified an acute phase-regulated and signal-inducing macrophage protein, CD163, as a receptor that scavenges haemoglobin by mediating endocytosis of Hp–Hb complexes. Specific CD163/HbSR-mediated endocytosis of Hp–Hb complexes was measurable in cells transfected with CD163 cDNA and in CD163-expressing myelomonocytic lymphoma cells.

STRUCTURE AND REGULATION OF THE HAEMOGLOBIN SCAVENGER RECEPTOR

Release of haemoglobin into plasma is a physiological phenomenon associated with intravascular haemolysis occurring during destruction of senescent erythrocytes (Fig. 1). Haptoglobin is the plasma protein with the highest binding affinity for haemoglobin. It is generally accepted that stable Hp–Hb complexes are subsequently delivered to the reticuloendothelial system by CD163 receptor-mediated endocytosis (Kristiansen *et al.*, 2001; Graversen *et al.*, 2002). Nevertheless, several investigators suggest the existence of another system involved in the recovery of free haemoglobin (Fig. 1). Kino *et al.* (1982) and Oshiro and Nakajima (1988) studied ^{125}I-haemoglobin–Hp complex uptake *in vitro* and *in vivo* by rat liver cells. They reported that ^{125}I-haemoglobin–Hp complex was bound to liver parenchymal cells, and that an excess of the unlabelled Hb–Hp complex greatly reduced the binding. Moreover, Oshiro and collaborators (1992) showed that, after internalization of Hb–Hp *via* receptor-mediated endocytosis into liver parenchymal cells, organelles containing the complex distribute in the microsome fraction where the complex dissociates into two subunits that are subsequently degraded. Recently, Fagoonee *et al.* (2005) presented data that convincingly supported the idea of a specific receptor for the Hp–Hb complex on liver parenchymal cells. Autoradiography showed that haemoglobin accumulated in hepatocytes and Kupffer cells in the liver in wild-type and Hp-deficient mice. The labelling of hepatocytes in wild-type mice suggests the existence of a receptor for the Hp–Hb complex as reported by *in vivo* and *in vitro* experiments on hepatocytes and hepatica cell lines (Kino *et al.*, 1982; Oshiro & Nakajima, 1988; Oshiro *et al.*, 1992; Okuda *et al.*, 1992). However, detection of labelled haemoglobin in the same cell types of Hp-null mice suggests the existence of yet another system able to take up haemoglobin. These data are in agreement with our reported results showing that isolated hepatocytes are capable of taking up free Hb at a faster rate than that of the Hp–Hb complex (Zuwała-Jagiełło & Osada, 1998). Taken together, these results support the idea that the Hp-independent system is specific for hepatocytes and may be responsible for Hb recovery under conditions of Hb overload when the buffering capacity of circulating Hp is saturated.

Excessive haemolysis or transfusion of Hb solution has been shown to result in Hp depletion and subsequent renal failure, in particular acute tubular necrosis (Glassock, 1995). Hp directs haemoglobin principally to the liver and spleen and prevents its glomerular filtration. Uptake of haemoglobin from glomerular filtrate in Hp-null mice is accounted for by megalin- and cubilin-mediated endocytosis (Gburek & Osada, 2000; Gburek *et al.*, 2002). Megalin and cubilin are multiligand endocytic receptors expressed at the apical membrane of proximal tubules (Fig. 1). Their primary function is to reabsorb small molecules that pass the glomerular filtration barrier (reviewed in Christensen & Birn, 2002). In tubular cells, haemoglobin is degraded in the endosomal compartment and haem is metabolized by haem oxygenase (Gburek *et al.*, 2002; Fagoonee *et al.*, 2005).

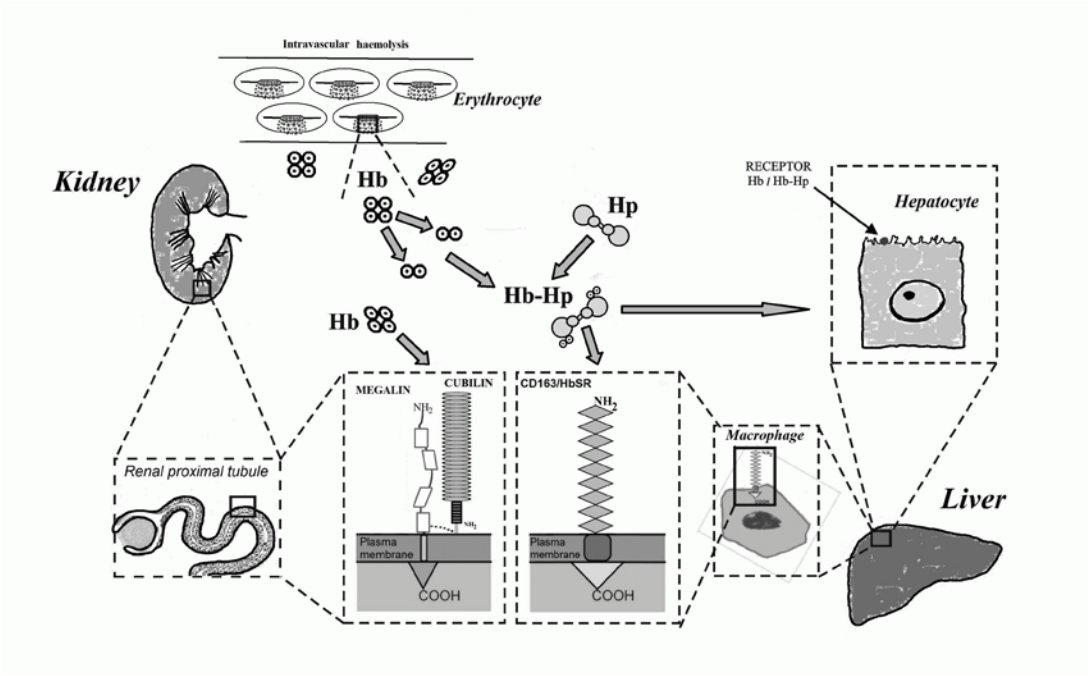

Figure 1. **Schematic representation of the principal extracellular pathways of haemoglobin (Hb)**.
Haemoglobin released from erythrocytes into the circulation by intravascular haemolysis binds immediately with haptoglobin, and forms a stable Hb–Hp complex. The Hp–Hb complex in plasma is rapidly cleared by the reticuloendothelial system in the liver. The presence of specific receptors on liver parenchymal cells that endocytose the Hp–Hb complex has led to the conclusion that a major function of the complex formation is the hepatic clearance and degradation of free haemoglobin. Hp directs haemoglobin principally to the mononuclear phagocyte system in the liver and spleen and prevents its glomerular filtration. Tissue macrophages are a major part of the mononuclear phagocyte system and remove haemoglobin in a complex with haptoglobin. CD163 belongs to the cysteine-rich scavenger receptor superfamily type B, and has recently been identified as the haemoglobin scavenger receptor (CD163/HbSR) for the Hp–Hb complex. This specific receptor–ligand interaction explains the depletion of circulating Hp in individuals with increased intravascular haemolysis. Moreover, the CD163 receptor may be central to the homeostatic functions of tissue macrophages to prevent haemoglobin toxicity. Uptake of haemoglobin from glomerular filtrate in Hp-null mice is accounted for by megalin- and cubilin-mediated endocytosis. Their primary function is to reabsorb small molecules that pass the glomerular filtration barrier (for more detail, see text).

Beside Hp, haemopexin binds Hb and haem with high affinity (Fagoonee *et al*., 2003)[1]. Experiments in cultured monocytes showed that haemopexin–haem taken up by these cells triggered the induction of haem oxygenase (Hvidberg *et al*., 2005). This study also demonstrated that haemopexin releases the haem into COS cells of monkey kidney origin only after internalization of the haemopexin–haem complex by the low-density lipoprotein receptor-related protein (LRP)/CD91. Thus Hp and haemopexin are important factors modulating renal excretion of Hb and its targeting to a receptor-mediated pathway.

As mentioned earlier, CD163 is a monocyte/macrophage-restricted receptor involved in the clearance of haptoglobin–haemoglobin complexes. The monoclonal antibody RM3/1 recognizes CD163, a membrane glycoprotein of 130 kDa, which is a cell-surface protein belonging to the scavenger receptor cysteine-rich (SRCR) protein superfamily, as previously reported (Högger *et al*., 1998; Schaer *et al*., 2001). In particular, CD163 comprises a large, extracellular region with nine scavenger receptor cysteine-rich class B domains, a transmembrane segment, and a short cytoplasmic tail. The first 42 amino acids after the membrane-spanning segment are in common for the three isoforms with different length of the cytoplasmic tails (Graversen *et al*., 2002).

Several different CD163 mRNAs, all arising from alternative splicing of a single *CD163* gene, have been described (Law *et al*., 1993; Ritter *et al*., 1999). Three of these encode CD163 proteins with different C-terminal cytoplasmic tails: the CD163 short tail variant and two long tail ones. Alternative splicing of cytoplasmic domains as a means of modulating receptor subcellular distribution has been reported for the human CD163 receptor (Nielsen *et al*., 2006). Surface expression was pronounced for the

[1]Fagoonee S, Gburek J, Hirsch E, Marro S, Moestrup SK, Christensen E, Silengo L, Altruda F, Tolosano E (2003) Plasma proteins haptoglobin and haemopexin modulate renal esponsiveness to pathological haemoglobin overload. *5 Convegno of Federazione Italiana Scienze della Vita, Rimini, Italy*, pp 237.

CD163 short tail variant, whereas the long tail variants were abundant in the *trans*-Golgy network area and endosomes. The CD163 cytoplasmic tail variants display divergent endocytic activities as a result of differences in their subcellular localization patterns. The cytoplasmic domain sequence contains consensus sequences for internalisation and phosphorylation by protein kinase C and casein kinase II. All three variants are phosphorylated by protein kinase C, whereas only the CD163 short tail variant and the CD163 long tail variant 1 are phosphorylated by casein kinase II *in vitro* (Ritter *et al*., 2001). Alternative splicing of the CD163 cytoplasmic region may therefore modulate the phosphorylation status of the intracellular tail and possibly, intracellular signalling mediated by CD163 (Nielsen *et al*., 2006).

The scavenger receptor cysteine-rich domain is an extracellular domain containing 110 amino-acid residues. The class A domain has six conserved cysteines, whereas the class B domain has eight cysteines. The structure of this domain is suggested to be a valid template for the structure for both class A and class B SRCR domains (Graversen *et al*., 2002). Class A variants have been found in a wide range of phyla ranging from metazoa to mammals, whereas class B have been found only in vertebrates. Based on the spacing of the cysteine residues in the CD163 molecule, it has been assigned to group B of the SRCR family (Resnick *et al*., 1994).

Experiments in CD163/HbSR-transfected Chinese hamster ovary cells clearly established that the protein functions as a high-affinity receptor mediating the uptake of Hp–Hb complexes (Kristiansen *et al*., 2001). Internalisation of Hp–Hb in macrophages is followed by lysosomal proteolysis of globin and conversion of haem to iron and bilirubin. There is no *a priori* reason to believe that the haptoglobin pathway is an important limiting factor in Hb homeostasis. One reason is that the vast majority of haemoglobin does not pass through this pathway; it has been estimated that the flow that can be attributed to free haemoglobin represents only 10% of the total haemoglobin normally catabolized (Deiss, 1999). Surprisingly, Schaer *et al.* (2006) have demonstrated that Hb interacts efficiently with CD163/HbSR in the absence of Hp. Free haemoglobin is internalised into an endosomal compartment by CD163/HbSR as a result of active receptor-dependent endocytosis, and also inhibits the uptake of Hp–Hb complexes. One likely explanation of these results is that, in human macrophages, Hp–Hb complex formation critically enhances Hb uptake at low (1 μg/ml), but not at high (greater than 100 μg/ml), ligand concentrations.

The expression of both CD163/HbSR and Hp is up-regulated by the acute phase mediator interleukin-6. In addition, interleukin-6 and glucocorticoids together with the anti-inflammatory factor interleukin-10 strongly induce CD163/HbSR expression, whereas the proinflammatory lipopolysaccharide (LPS) and interferon-γ down-regulate the expression (Buechler *et al*., 2000). These and other results indicate that during inflammatory conditions this system might be coordinately induced, thereby enhancing the mechanism preventing accumulation of Hb in the plasma. CD163/HbSR is a monocyte/macrophage differentiation antigen and tissue macrophages (e.g., in liver, spleen, lymph nodi) have a substantially higher expression compared to monocytes. The increased expression of CD163/HbSR is part of the maturation of the monocyte to a phagocytic macrophage (Sanchez *et al*., 1999) and is tightly regulated. Therefore, the regulation of CD163/HbSR by the acute phase mediators may suggest an immunoregulatory function. It has thus been shown that antibody-mediated cross-linking of CD163/HbSR mimics a putative ligand-induced cross-linking (Van den Heuvel *et al*., 1999).

In humans, there are two alleles of the haptoglobin gene. The biophysical and biochemical properties of the polymeric haptoglobin molecules resulting from the three possible combinations (Hp 1-1, 2-1, or 2-2) of these two alleles are dramatically different (reviewed in Langlois & Delanghe, 1996). Kristiansen *et al.* (2001) have demonstrated that Hp 2-2 binds with a 10-fold higher affinity to CD163 compared with Hp 1-1. Complexes of haemoglobin and multimeric Hp 2-2 exhibited higher functional affinity for CD163 than did complexes of haemoglobin and dimeric Hp 1-1. This is probably due to clustering of several binding sites in the multimeric ligand complex. It has also been suggested that the binding of an Hp 2-2–Hb complex might lead to a higher degree of CD163/HbSR cross-linking than the binding of an Hp 1-1–Hb complex (Kristiansen *et al*., 2001). Asleh *et al*. (2003) assessed the scavenging function of Hp using radiolabelled Hp in cell lines stably transfected with CD163 and in macrophages expressing endogenous CD163. Consistent with prior studies (Kristiansen *et al*., 2001), they found that the affinity of CD163 for the Hp–Hb complex in these cells was 8-fold higher for Hp 2-2 compared with Hp 1-1. They have also demonstrated that Hp 1-1–Hb complexes are more rapidly cleared than the Hp 2-2–Hb complexes, which they proposed would result in significantly less oxidative damage within the vessel wall in diabetic individuals with Hp 1-1 compared with diabetic individuals with Hp 2-2. Indeed, the Hp allelic variants differ dramatically in their shape and size and consequently in their sieving potential to enter the subendothelial space from the serum and bind free Hb that may be released at sites of vascular injury directly into the vessel wall (Melamed-Frank *et al*., 2001).

HAEMOGLOBIN SCAVENGER RECEPTOR MEDIATES ANTI-INFLAMMATORY EFFECTS

After endocytosis of Hp–Hb, the rate-limiting haem oxygenase (HO) enzymes degrade the haem subunit of Hb. Three isoforms of HO (HO-1, HO-2 and HO-3) have been described in mammals as products of separate genes. The constitutively expressed HO-2 is unresponsive to any of the known HO-1 inducers. The HO-3 isoenzyme is nearly devoid of catalytic activity and serves mainly as a haem-sensing/binding protein (Elbirt & Bonkovsky, 1999). The haem oxygenase activity oxidizes haem to biliverdin, which is then converted to bilirubin by biliverdin reductase, CO and free iron. HO-1 is rapidly induced in response to cytokines, NO and peroxynitrite, reactive oxygen species, etc. Overexpression of HO-1 confers protection against oxidative injury and can lead to anti-inflammatory effects, which could be due to biliverdin and bilirubin production, CO or ferritin induction (reviewed in Alcaraz *et al*., 2003).

It has been discovered that carbon monoxide mediates potent anti-inflammatory effects. Both *in vivo* and *in vitro*, carbon monoxide at low concentrations differentially and selectively inhibited the expression of the lipopolysacharide-induced pro-inflammatory cytokines tumor necrosis factor-α, interleukin-1β, and macrophage inflammatory protein-1β and increased the lipopolysaccharide-induced expression of the anti-inflammatory cytokine interleukin-10 (IL-10) (Otterbein *et al.,* 2000).

It has been shown that exogenous CO reduces inflammatory responses in several models of oxidant injury, which is consistent with the results observed with HO-1 overexpression (Abraham *et al*., 1995; Amersi *et al*., 1999). Although CO acts in many ways that are similar to NO, CO has additional functions in signal transduction pathways. CO inhibits pro-inflammatory genes while augmenting anti-inflammatory cytokine production by selective activation of several p38 mitogen-activated protein kinase (MAPK) signalling pathways in a guanylyl cyclase-independent manner (Otterbein *et al*., 2000; Brouard *et al*., 2002; Sarady *et al*., 2002; Song *et al*., 2003 and reviewed in Dulak & Józkowicz, 2003).

HO-1 can be induced by various stimuli, one of which is interleukin-6 (IL-6) (reviewed in Tosaki & Das, 2002). IL-6 is also a known stimulator of the synthesis/expression of both haptoglobin and CD163 (Kristiansen *et al*., 2001) pointing to a coregulation of Hp, CD163, and HO-1. Furthermore, HO-1 expression is up-regulated in macrophages during the resolution phase of inflammation and also in murine macrophages stimulated by IL-10 (Willis *et al*., 1996; Lee & Chau, 2002; Wagener *et al*., 2003). IL-10 has important regulatory effects on immunological and inflammatory responses because of its capacity to down-regulate class II MHC expression and to inhibit the production of proinflammatory cytokines by monocytes (reviewed in Spits & de Waal Malefyt, 1992). Philippidis *et al*. (2004) suggested also that Hp–Hb binding to CD163/HbSR on human monocyte-macrophages isolated *in vitro* and *in vivo* elicits a direct anti-inflammatory effect *via* the secretion of IL-10. This link between HO-1 synthesis in macrophages and Hp–Hb binding *via* CD163 may enable macrophages to coordinate haemoglobin scavenging and breakdown with anti-inflammatory activity. In another report, Hamann *et al.* (1995) suggest that, up-regulation of CD163/HbSR is associated with the release of a novel anti-inflammatory factor produced by RM3/1 macrophages derived from glucocorticoid-treated human monocytes. The biologic significance of CD163/HbSR such as the anti-inflammatory role has been summarized in a recent review article (Moestrup & Möller, 2004).

RELATION TO DISEASE

The haemoglobin scavenger receptor CD163/HbSR is tightly involved in both physiological as well as pathophysiological processes (Table 1), such as cytoprotection and inflammation. A better understanding of the haemoglobin scavenger receptor system may provide us with novel tools to combat diverse conditions, such as inflammation, atherosclerosis, transplant rejection and cancer.

It has been that CD163/HbSR can be shed from the cell membrane of glucocorticoid-stimulated monocytes (Droste *et al*., 1999) after an inflammatory stimulus and that CD163/HbSR is a normal component in the plasma of healthy donors (Sulahian *et al*., 2001; Möller *et al*., 2002). In particular, phorbol 12-myristate 13-acetate, lipopolysaccharide (LPS), and cross-linking of the Fc receptor for immunoglobulin G have been reported to induce shedding of CD163 (Droste *et al*., 1999; Hintz *et al*., 2002; Sulahian *et al*., 2004). Very recently, oxidative stress or 8-iso-prostaglandin F (2α) was identified as physiological activators of CD163 shedding that is consistently observed under inflammatory conditions (Timmermann & Hogger, 2005).

CD163/HbSR circulates in the plasma (1–3 mg/L) as a soluble protein with a size identical to that of the nine extracellular SRCR domains (Möller *et al*., 2002). The function of the extracellular domain is unknown, but it has been claimed to have an anti-inflammatory role. One can speculate that the plasma level of soluble CD163/HbSR (sCD163/HbSR) reflects the total pool of membrane-bound CD163/HbSR, which may be increased in case of proliferation of cells of myelomonocytic origin or in case of

Table 1. Selection of diseases/conditions associated with CD163/HbSR expression

Pathological conditions	References
Acute myeloid leukemia	Möller *et al.*, 2002
Atherosclerosis	Li *et al.*, 2004; Aristoteli *et al.*, 2006
Diabetic cardiovascular disease	Torres *et al.*, 2004
Endotoxemia	Hintz *et al.*, 2002
Fulminant hepatic failure	Hiraoka *et al.*, 2005
Gastrointestinal disease	Demetter *et al.*, 2005
Gauchesr's disease	Möller *et al.* 2004
Haemophagocytic syndrome	Schaer *et al.*, 2005
Melanoma	Woodward *et al.*, 2004
Multiple sclerosis brain lesions	Fabriek *et al.*, 2005
Rheumatoid arthritis	Matsushita *et al.*, 2002
Sarcoma	Vos *et al.*, 2005; Nguyen *et al.*, 2005
Spondylarthropathy synovitis	Baeten *et al.*, 2004
Symptoms of preterm delivery	Vogel *et al.*, 2005
Transplant rejection	Funding *et al.*, 2005
Tuberculosis	Knudsen *et al.*, 2005
Wound healing	Djemadji-Oudjiel *et al.*, 1996

up-regulation of CD163/HbSR expression by acute phase mediators. Indeed, highly increased levels of sCD163/HbSR are seen in patients with myelomonocytic leukemias and infections (Möller *et al.*, 2002). Hintz *et al.* (2002) suggests a likely mechanism for the endotoxemia-associated rise in plasma sCD163/HbSR. CD163/HbSR is rapidly mobilised in response to bacterial endotoxin. In addition, haemoglobin can bind lipopolysaccharide and enhance its toxicity. Although the experiments in isolated cells clearly have established that LPS and Fcγ receptor stimulation in short-term cultures suppresses CD163 mRNA expression, long-term cultures of monocytes treated with LPS resulted in an interleukin-10-dependent recovery of surface CD163 expression (Sulahian *et al.*, 2004). This may be important in infections by haemolytic bacteria, or in autoimmune haemolytic anaemia, in which immune complexes and high levels of free Hb are present. Möller *et al.* (2004) investigated the levels of soluble haemoglobin scavenger receptor in patients with Gaucher's disease, an inherited lysosomal storage disorder characterised by hepato- and splenomegaly due to excessive accumulation of macrophages. Their results showed that plasma level of the macrophage-derived soluble haemoglobin scavenger receptor was increased and positively correlate with severity in Gaucher's disease.

During sepsis and other conditions affecting macrophage activity, the level of sCD163/HbSR may raise many-fold (Möller *et al.*, 2004; Schaer *et al.*, 2005; Hiraoka *et al.*, 2005). Serum levels of the macrophage haemoglobin scavenger receptor were found to be highly increased in patients with reactive haemophagocytic syndrome, which is a disease of overwhelming macrophage activity triggered by infection, malignancy or autoimmune disorders (Schaer *et al.*, 2005). Results of other studies strongly indicate that serum levels of sCD163/HbSR are a sensitive and reliable marker to monitor activated macrophages in synovitis from both rheumatoid arthritis and spondylarthropathy patients (Matsushita *et al.*, 2002; Baeten *et al.*, 2004). In a work that casts new light on the role of the macrophage scavenger receptors, Demetter and colleagues (2005) have recently reported CD163/HbSR expression localised to activated macrophages in colon mucosa of patients both with spondyloarthritis (SpA) and Crohn's disease. SpA and Crohn's disease were also associated with large numbers of $CD68^+$ macrophages. CD68 is a lineage restricted, lysosomal-associated glycoprotein that could play a role in specialised phagocytic activities of tissue macrophages. These results implicate an important role for CD163 in suppression of inappropriate inflammatory and immunological reactions as well as the generation of cytocidal and anti-microbial mediators. Based on the above considerations, one can hypothesise that the haemoglobin scavenger receptor may be a macrophage-specific marker in patients with disorders of macrophage activation. In addition, the immunoregulatory properties of CD163/HbSR and its presence in the plasma are interesting aspects which might be exploited in anti-inflammatory therapy.

The levels of several cytokines and chemokines are elevated in various diseases, and activated macrophages may have a role in the production of these immune modulators. Moreover, the soluble sCD163/HbSR is released from activated macrophages. Very recently, it has been shown that products of activated macrophages may be involved in the pathogenesis of fulminant hepatic failure (FHF) (Hiraoka *et al.*, 2005). A kinetic study revealed that the levels of sCD163/HbSR decreased in all liver diseases, whereas the levels of sCD163/HbSR progressively increased in nonsurvivors of fulminant hepatic failure. It is tempting to speculate that these levels appeared to be an independent predictor of survival in verified FHF patients. In another report it has been demonstrated that both sCD163/HbSR and IL-6 are present in high levels in the aqueous humour from patients with rejection of corneal grafts, but neither IL-6 nor sCD163/HbSR were related to the outcome of the corneal rejection (Funding *et al.*, 2005). In contrast, sCD163/HbSR may show prognostic importance in tuberculosis (Knudsen *et al.*, 2005). It may thus be concluded that sCD163/HbSR may be a valuable laboratory parameter in monitoring diseases with increased macrophage activity.

CD163/HbSR and soluble CD163/HbSR may be potential diagnostic tools in atherosclerosis. Ar-

istoteli *et al.* (2006) have recently found in a study of more than 100 persons that sCD163 may act as a plasma marker of coronary atherosclerotic burden. Specifically, plasma sCD163/HbSR was non-parametrically distributed, being significantly higher in patients with coronary atherosclerosis than in a control group and was independent of conventional risk factors (i.e., age, hypercholesterolemia, hypertension, and current smoking). In another report, Fernandez *et al*. (2001) demonstrated that CD163/HbSR is expressed on macrophages in atherosclerotic plaques, and free Hb promotes atherogenesis by oxidising low-density lipoproteins (LDL). Diabetes is an example of a condition producing oxidative stress that is related to activation of inflammatory cells and haemolysis and provides an opportunity to identify vascular events that initiate LDL oxidation and predispose individuals to an increased risk of atherosclerosis (Basta *et al*., 2004). When large amounts of free haemoglobin or glycated haemoglobin (GHb) (locally) accumulate the scavengers get overwhelmed or are unable to reach them. In our studies, several independent assays for LDL oxidation-conjugated diene formation, and production of thiobarbituric acid-reactive substances (TBARS) — all suggested that oxidative modification of lipoprotein was taking place in plasma containing GHb or advanced glycation endproducts-modified haemoglobin (AGE-Hb) (Zuwała-Jagiełło *et al*., 1999)[2]. The results of these studies do suggest that products of reactions between GHb/AGE-Hb and LDL are distinctly cytotoxic and could well account for some of the endothelial damage observed in *in vitro* experiments.

It has been shown that Hb is a strong lipid oxidant, especially in an acidic environment. A pH as low as 3.6 has been recorded at the surface of an activated macrophage and as macrophages in human atherosclerotic lesions are known to be selectively activated they may also acidify their extracellular space, especially as they tend to occur in clusters (Aronson & Rayfield, 2002). We observed that when LDL was oxidized by GHb, there was a large decrease in the lag phase (which is due to the presence of antioxidants in LDL) and an increase in the rate of formation of conjugated dienes and TBARS as the acidity was increased, even modestly to pH 6.5. When LDL was incubated with AGE-Hb, its initial oxidation was slowed down at acidic pH, as judged by the formation of conjugated dienes and TBARS. In our research, the inhibition of LDL peroxidation in the presence of AGE-Hb after incubation with Hp 1–1 or Hp 2–2 was rather low about 14% and about 10%, respectively (Zuwała-Jagiełło & Kość, 2003)[3]. One can speculate that in the development of artery atherosclerosis as a complication of diabetes, the cooperation of GHb and AGE-Hb plays an essential role not only through a direct oxidant activity of both forms of haemoglobin, but also through retaining the oxidant activity of AGE-Hb bound in a complex with haptoglobin. Moreover, a decreased uptake of Hp–GHb complex through the macrophage CD163/HbSR contributes to its slower catabolism (Asleh *et al*., 2003). CD163/HbSR expression appeared in both early and advanced atherosclerotic lesions (Li *et al*., 2004). Furthermore, Hb catabolism by macrophages contributes to iron deposition and ferritin induction in human atheroma. The involvement of CD163/HbSR during ferritin induction may play an important role in modulating inflammatory processes in atherogenesis.

The susceptibility to cardiovascular disease is markedly influenced by the Hp phenotype and the clearance rate of Hp–Hb complexes by CD163 (Levy *et al*., 2002; Asleh *et al*., 2003). Interestingly, intimal haemoglobin scavenging may attenuate the inflammatory mechanisms underlying atherosclerosis. The atheroprotection not only depends on the removal of the pro-oxidant Hb from the vessel wall by both Hp and CD163 but also on the subsequent induction of anti-inflammatory pathways through IL-10 and HO-1 (Philippidis *et al*., 2004; Schaer *et al*., 2006). The newly identified link between HO-1 synthesis in macrophages and Hp–Hb binding *via* CD163/HbSR may enable macrophages to coordinate haem scavenging and breakdown with the anti-inflammatory activity. If CD163/HbSR has also the same important regulatory properties on plasma haemoglobin levels in humans that have been in HEK293 cells (Schaer *et al*., 2006), it could be a possible target for drug intervention in atherosclerosis.

As early as in 2003, the Schoedon group investigated expression of the haemoglobin scavenger receptor as an immunophenotypic marker of monocytic lineage in acute myeloid leukaemia (Walter *et al*., 2003). The results of these studies show that the restriction of CD163/HbSR expression to cells committed to the monocytic lineage is preserved beyond malignant transformation; this lineage-restricted pattern of antigen expression may thus be useful for the immunophenotypic subclassification of leukemias. Vos and co-authors (2005) described expression of CD163/HbSR as an immunophenotypic marker of histiocytic sarcoma. CD163/HbSR appears to be a specific marker of the histiocytic lineage and a promising diagnostic tool for evaluating histiocytic neoplasms. Similar results were reported by Nguyen

[2]Zuwała-Jagiełło J, Milczarska J, Osada J (1999) *7th Conference on Cell Biology.* Kraków, Poland, Abstracts, *Folia Histochem Cytobiol* **37** (Suppl. 1) p 88.
[3]Zuwała-Jagiełło J, Kość A (2003) *39th Meeting of the Polish Biochemical Society*. Gdańsk, Poland, Abstracts, *Acta Biochim Polon* **50** (Suppl. 1) p 83–84.

et al. (2005) who also tested the expression of the CD163 protein in histiocytic sarcoma, littoral cell angioma, Langerhans cell histiocytosis and Rosai-Dorfman disease. Staining of these tumors illustrated primary localization of CD163/HbSR to human malignancies. In contrast, CD163/HbSR reactivity was not observed in normal tissues, lymphomas, carcinomas, and in a majority of mesenchymal neoplasms. One year earlier Woodward *et al.* (2004) investigated the expression of CD163/HbSR using single- and double-labelling immunohistochemistry, cultured cells and paraffin sections, which detects macrophage (CD163, CD68) markers in patients with uveal melanoma. It is quite remarkable that melanoma cells themselves contribute at least in part to the haemoglobin scavenger receptor overexpression in melanoma lesions. Taken together, these results suggest that CD163 may have significant diagnostic utility in separating specific tumors with monocytic and histiocytic derivation from other entities in their differential diagnosis.

The membrane CD163/HbSR receptor-mediated endocytosis or internalization of the haptoglobin–haemoglobin complex and the haemoglobin scavenger receptor constitute the major route of Hb uptake. This efficient cellular uptake pathway might be exploited for the site-specific delivery not only of anticancer drugs and proteins, but also of therapeutic genes into proliferating malignant cells that overexpress the CD163/HbSR receptors. This might be achieved either chemically by conjugation of haptoglobin with therapeutic drugs, proteins, or genetically by infusion of therapeutic peptides or proteins into the structure of haptoglobin.

REFERENCES

Abraham NG, da Silva JL, Lavrovsky Y, Stoltz RA, Kappas A, Dunn MW, Schwartzman ML (1995) Adenovirus-mediated haem oxygenase-1 gene transfer into rabbit ocular tissues. *Invest Ophthalmol Vis Sci* **36**: 2202–2210.

Alcaraz MJ, Fernandez P, Guillen MI (2003) Anti-inflammatory actions of the haem oxygenase-1 pathway. *Curr Pharm Des* **9**: 2541–2551.

Amersi F, Buelow R, Kato H, Ke B, Coito AJ, Shen XD, Zhao D, Zaky J, Melinek J, Lassman CR *et al.* (1999) Up-regulation of haem oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. *J Clin Invest* **104**: 1631–1639.

Aristoteli LP, Möller HJ, Bailey B, Moestrup SK, Kritharides L (2006) The monocytic lineage specific soluble CD163 is a plasma marker of coronary atherosclerosis. *Atherosclerosis* **184**: 342–347.

Aronson D, Rayfield EJ (2002) How hyperglycemia promotes atherosclerosis?: molecular mechanisms. *Cardiovasc Diabetol* **1**: 1–10.

Aruffo A, Melnick MB, Linsley PS, Seed B (1991) The lymphocyte glycoprotein CD6 contains a repeated domain structure characteristic of a new family of cell surface and secreted proteins. *J Exp Med* **174**: 949–952.

Asleh R, Marsh S, Shilkrut M, Binah O, Guetta J, Lejbkowicz F, Enav B, Shehadeh N, Kanter Y, Lache O, Cohen O, Levy NS, Levy AP (2003) Genetically determined heterogeneity in haemoglobin scavenging and susceptibility to diabetic cardiovascular disease. *Circ Res* **92**: 1193–1200.

Baeten D, Möller HJ, Delanghe J, Veys EM, Moestrup SK, De Keyser F (2004) Association of CD163+ macrophages and local production of soluble CD163 with decreased lymphocyte activation in spondyloarthropathy synovitis. *Arthritis Rheum* **50**: 1611–1623.

Basta G, Schmidt AM, De Caterina R (2004) Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovasc Res* **63**: 582–592.

Brouard S, Berberat PO, Tobiasch E, Seldon MP, Bach FH, Soares MP (2002) Haem oxygenase-1 derived carbon monoxide requires the activation of the transcription factor NF-kB to protect endothelial cells from TNF-alpha mediated apoptosis. *J Biol Chem* **277**: 17950–17961.

Brown MS, Goldstein JL (1983) Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem* **52**: 223–261.

Buechler C, Ritter M, Orso E, Langmann T, Klucken J, Schmitz G (2000) Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and anti-inflammatory stimuli. *J Leukoc Biol* **67**: 97–103.

Burton JL, Kehrli ME (1996) Effects of dexamethasone on bovine circulating T lymphocyte populations. *J Leukoc Biol* **59**: 90–99.

Cheng H, Bjerknes M, Chen H (1996) CRP-ductin: a gene expressed in intestinal crypts and in pancreas and hepatic ducts. *Anat Rec* **244**: 327–334.

Christensen EI, Birn H (2002) Megalin and cubilin: multifunctional endocytic receptors. *Nat Rev Mol Cell Biol* **3**: 256–266.

de la Llera-Moya M, Connelly MA, Drazul D (2001) Scavenger receptor class B type I affects cholesterol homeostasis by magnifying cholesterol flux between cells and HDL. *J Lipid Res* **42**: 1969–1978.

Deiss A (1999) Destruction of erythrocytes. In *Wintrobe's Clinical Hematology* (Lee RG, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, eds) pp 267–299, Williams and Wilkins, Baltimore.

Delanghe JR, Langlois MR, De Buyzere ML (1998) Congenital anhaptoglobinemia versus acquired hypohaptoglobinemia [Letter]. *Blood* **91**: 3524.

Demetter P, De Vos M, Van Huysse JA, Baeten D, Ferdinande L, Peeters H, Mielants H, Veys EM, De Keyser F, Cuvelier CA (2005) Colon mucosa of patients both with spondyloarthritis and Crohn's disease is enriched with macrophages expressing the scavenger receptor CD163. *Ann Rheum Dis* **64**: 321–324.

Djemadji-Oudjiel N, Goerdt S, Kodelja V, Schmuth M, Orfanos CE (1996) Immunohistochemical identification of type II alternatively activated dendritic macrophages (RM 3/1+3, MS-1+/-, 25F9-) in psoriatic dermis. *Arch Dermatol Res* **288**: 757–764.

Droste A, Sorg C, Hogger P (1999) Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family. *Biochem Biophys Res Commun* **256**: 110–113.

Dulak J, Jozkowicz A (2003) Carbon monoxide — a "new" gaseous modulator of gene expression. *Acta Biochim Polon* **50**: 31–47.

Elbirt KK, Bonkovsky HL (1999) Heme oxygenase: recent advances in understanding its regulation and role. *Proc Assoc Am Physicians* **111**: 438–447.

Fabriek BO, Van Haastert ES, Galea I, Polfliet MM, Dopp ED, Van Den Heuvel MM, Van Den Berg TK, De Groot CJ, Van Der Valk P, Dijkstra CD (2005) CD163-positive perivascular macrophages in the human CNS express molecules for antigen recognition and presentation. *Glia* **51**: 297–305.

Fagoonee S, Gburek J, Hirsch E, Marro S, Moestrup SK, Laurberg JM, Christensen EI, Silengo L, Altruda F, Tolosano E (2005) Plasma protein haptoglobin modulates renal iron loading. *Am J Pathol* **166**: 973–983.

Fernandez AZ, Lopez F, Tablante A, Romano E, Hurt-Camejo E, Camejo G, Apitz-Castro R (2001) Intravascular haemolysis increases atherogenicity of diet-induced hypercholesterolemia in rabbits in spite of haem oxygenase-1 gene and protein induction. *Atherosclerosis* **158**: 103–111.

Funding M, Vorum H, Nexo E, Moestrup SK, Ehlers N, Möller HJ (2005) Soluble CD163 and interleukin-6 are increased in aqueous humour from patients with endothelial rejection of corneal grafts. *Acta Ophthalmol Scand* **83**: 234–239.

Gburek J, Osada J (2000) Haemoglobin binding sites on renal brush-border membranes. *Biochemie* **82**: 1135–1142.

Gburek J, Verroust PJ, Willnow TE, Fyfe JC, Nowacki W, Jacobsen C, Moestrup SK, Christensen EI (2002) Megalin and cubilin are endocytic receptors involved in renal clearance of haemoglobin. *J Am Soc Nephrol* **13**: 423–430.

Gebe JA, Kiener PA, Ring HZ, Li X, Francke U, Aruffo A (1997) Molecular cloning, mapping to human chromosome 1 q21–q23, and cell binding characteristics of Spα, a new member of the scavenger receptor cysteine-rich (SRCR) family of proteins. *J Biol Chem* **272**: 6151– 6158.

Glassock RJ (1995) Hematuria and pigmenturia. In *Textbook of Nephrology* (Massry SG, Glasock RJ, eds) p 557, Williams and Wilkins, Baltimore.

Gough PJ, Greaves DR, Gordon S (1998) A naturally occurring isoform of the human macrophage scavenger receptor (SR-A) gene generated by alternative splicing blocks modified LDL uptake. *J Lipid Res* **39**: 531–543.

Graversen JH, Madsen M, Moestrup SK (2002) CD163: a signal receptor scavenging haptoglobin-haemoglobin complexes from plasma. *Int J Biochem Cell Biol* **34**: 309–314.

Gutteridge JMC (1987) The antioxidant activity of haptoglobin towards haemoglobin-stimulated lipid peroxidation. *Biochim Biophys Acta* **917**: 219–223.

Hamann W, Floter A, Schmutzler W, Zwadlo-Klarwasser G (1995) Characterisation of a novel anti-inflammatory factor produced by RM3/1 macrophages derived from glucocorticoid treated human monocytes. *Inflamm Res* **44**: 535–540.

Hintz KA, Rassias AJ, Wardwell K, Moss ML, Morganelli PM, Pioli PA, Givan AL, Wallace PK, Yeager MP, Guyre PM (2002) Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte haemoglobin scavenger receptor CD163. *J Leukoc Biol* **72**: 711–717.

Hiraoka A, Horiike N, Akbar SM, Michitaka K, Matsuyama T, Onji M (2005) Soluble CD163 in patients with liver diseases: very high levels of soluble CD163 in patients with fulminant hepatic failure. *J Gastroenterol* **40**: 52–56.

Holmskov JU, Mollenhauer J, Madsen J, Vitved L, Gronlund J, Tornoe I, Kliem A, Reid KB, Poustka A, Skjodt K (1999) Cloning of gp-340, a putative opsonin receptor for lung surfactant protein D. *Proc Natl Acad Sci USA* **96**: 10794–10799.

Högger P, Dreier J, Droste A, Buck F, Sorg C (1998) Identification of the integral membrane protein RM3/1 on human monocytes as a glucocorticoid-inducible member of the scavenger receptor cysteine rich family (CD163). *J Immunol* **161**: 1883–1890.

Hvidberg V, Maniecki MB, Jacobsen C, Hojrup P, Möller HJ, Moestrup SK (2005) Identification of the receptor scavenging hemopexin-heme complexes. *Blood* **106**: 2572–2579.

Jones NH, Clabby ML, Dialynas DP, Huang HJ, Herzenberg LA, Strominger JL (1986) Isolation of complementary DNA clones encoding the human lymphocyte glycoprotein T1/Leu-1. *Nature* **323**: 346–349.

Keene WR, Jandl JH (1965) The sites of hemoglobin catabolism. *Blood* **26**: 705–719.

Kino K, Tsunoo H, Higa Y, Takami M, Nakajima H (1982) Kinetic aspects of haemoglobin-haptoglobin-receptor interaction in rat liver plasma membranes, isolated liver cells, and liver cells in primary culture. *J Biol Chem* **257**: 4828–4833.

Knudsen TB, Gustafson P, Kronborg G, Kristiansen TB, Moestrup SK, Nielsen JO, Gomes V, Aaby P, Lisse I, Möller HJ, Eugen-Olsen J (2005) Predictive value of soluble haemoglobin scavenger receptor CD163 serum levels for survival in verified tuberculosis patients. *Clin Microbiol Infect* **11**: 730–735.

Krieger M (2001) Scavenger receptor class B type I is a multiligand HDL receptor that influences diverse physiologic systems. *J Clin Invest* **108**: 793–797.

Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, Moestrup SK (2001) Identification of the haemoglobin scavenger receptor. *Nature* **409**: 198–201.

Langlois MR, Delanghe JR (1996) Biological and clinical significance of haptoglobin polymorphism in humans. *Clin Chem* **42**: 1589–1600.

Law SK, Micklem KJ, Shaw JM, Zhang X-P, Dong Y, Willis AC, Mason DY (1993) A new macrophage differentiation antigen which is a member of the scavenger receptor superfamily. *Eur J Immunol* **23**: 2320–2325.

Lee TS, Chau LY (2002) Haem oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. *Nat Med* **8**: 240–246.

Levy AP, Hochber I, Jablonski K, Resnick HE, Lee ET, Best L, Howard BV (2002) Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the strong heart study. *J Am Coll Cardinol* **40**: 1984–1990.

Li XJ, Snyder SH (1995) Molecular cloning of Ebnerin, a von Ebner's gland protein associated with taste buds. *J Biol Chem* **270**: 17674–17681.

Li W, Xu LH, Yuan XM (2004) Macrophage haemoglobin scavenger receptor and ferritin accumulation in human atherosclerotic lesions. *Ann N Y Acad Sci* **1030**: 196–201.

Lim SK, Ferraro B, Moore K, Halliwell B (2001) Role of haptoglobin in free haemoglobin metabolism. *Redox Rep* **6**: 219–227.

Matsushita N, Kashiwagi M, Wait R, Nagayoshi R, Nakamura M, Matsuda T, Hogger P, Guyre PM, Nagase H, Matsuyama T (2002) Elevated levels of soluble CD163 in sera and fluids from rheumatoid arthritis patients and inhibition of the shedding of CD163 by TIMP-3. *Clin Exp Immunol* **130**: 156–161.

Melamed-Frank M, Lache O, Enav BI, Szafranek T, Levy NS, Ricklis RM, Levy AP (2001) Structure/function analysis of the anti-oxidant properties of haptoglobin. *Blood* **98**: 3693–3698.

Miller Y, Altamentova S, Shaklai N (1997) Oxidation of low-density lipoprotein by haemoglobin stems from a haem-initiated globin radical: antioxidant role of haptoglobin. *Biochemistry* **36**: 12189–12198.

Moestrup SK, Möller HJ (2004) CD163: a regulated haemoglobin scavenger receptor with a role in the anti-inflammatory response. *Ann Med* **36**: 347–354.

Mollenhauer J, Wiemann S, Scheurlen W, Korn B, Hayashi Y, Wilgenbus KK, von Deimling A, Poustka A (1997) DMBT1, a new member of the SRCR superfamily, on chromosome 10q25.3-26.1 is deleted in malignant brain tumours. *Nat Genet* **17**: 32–39.

Möller HJ, Peterslund NA, Graversen JH, Moestrup SK (2002) Identification of the haemoglobin scavenger receptor/CD163 as a natural soluble protein in plasma. *Blood* **99**: 378–380.

Möller HJ, de Fost M, Aerts H, Hollak C, Moestrup SK (2004) Plasma level of the macrophage-derived soluble CD163 is increased and positively correlates with severity in Gaucher's disease. *Eur J Haematol* **72**: 135–139.

Nguyen TT, Schwartz EJ, West RB, Warnke RA, Arber DA, Natkunam Y (2005) Expression of CD163 (haemoglobin scavenger receptor) in normal tissues, lymphomas, carcinomas, and sarcomas is largely restricted to the monocyte/macrophage lineage. *Am J Surg Pathol* **29**: 617–624.

Nielsen MJ, Madsen M, Möller HJ, Moestrup SK (2006) The macrophage scavenger receptor CD163: endocytic properties of cytoplasmic tail variants. *J Leukoc Biol* **79**: 837–845.

Okuda M, Tokunaga R, Taketani S (1992) Expression of haptoglobin receptors in human hepatoma cells. *Biochim Biophys Acta* **1136**: 143–149.

Osada J, Nowacki W (1989) Elimination of goat haemoglobin and its complexes with goat haptoglobin from goat and rat circulation. *Acta Biochim Polon* **36**: 365–369.

Oshiro S, Nakajima H (1988) Intrahepatocellular site of the catabolism of heme and globin moiety of hemoglobin-haptoglobin after intravenous administration to rats. *J Biol Chem* **263**: 16032–16038.

Oshiro S, Yajima Y, Kawamura K, Kubota M, Yokofujita J, Nishibe Y, Takahama M, Nakajima H (1992) Catabolism of haemoglobin-haptoglobin complex in microsome subfractions. *Chem Pharm Bull* **40**: 1847–1851.

Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, Choi AM (2000) Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* **6**: 422–428.

Panter SS, Sadrzadeh SMH, Hallaway PE, Haines JL, Andersen VE, Eaton JW (1985) Hypohaptoglobinemia associated with familial epilepsy. *J Exp Med* **161**: 748–754.

Philippidis P, Mason JC, Evans BJ, Nadra I, Taylor KM, Haskard DO, Landis RC (2004) Haemoglobin scavenger receptor CD163 mediates interleukin-10 release and haem oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses *in vitro*, in resolving skin blisters *in vivo*, and after cardiopulmonary bypass surgery. *Circ Res* **94**: 119–126.

Platt N, Suzuki H, Kurihara Y, Kodama T, Gordon S (1996) Role for the class-A macrophage scavenger receptor in the phagocytosis of apoptotic thymocytes in vitro. *Proc Natl Acad Sci USA* **93**: 12456–12460.

Resnick D, Pearson A, Krieger M (1994) The SRCR superfamily: a family reminiscent of the Ig superfamily. *Trends Biochem Sci* **19**: 5–8.

Ritter M, Buechler C, Langmann T, Schmitz G (1999) Genomic organization and chromosomal localization of the human CD163 (M130) gene: a member of the scavenger receptor cysteine-rich superfamily. *Biochem Biophys Res Commun* **260:** 466–474.

Ritter M, Buechler C, Kapinsky M, Schmitz G (2001) Interaction of CD163 with the regulatory subunit of casein kinase II (CKII) and dependence of CD163 signalling on CKII and protein kinase C. *Eur J Immunol* **31**: 999–1009.

Sanchez C, Domenech N, Vazquez J, Alonso F, Ezquerra A, Dominguez A (1999) The porcine 2A10 antigen is homologous to human CD163 and related to macrophage differentiation. *J Immunol* **162**: 5230–5237.

Sarady JK, Otterbein SL, Liu F, Otterbein LE, Choi AM (2002) Carbon monoxide modulates endotoxin-induced production of granulocyte macrophage colony stimulating factor in macrophages. *Am J Respir Cell Mol Biol* **27**: 739–745.

Schaer DJ, Boretti FS, Hongegger A, Poehler D, Linnscheid P, Staege H, Muller C, Schoedon G, Schaffner A (2001) Molecular cloning and characterization of the mouse CD163 homologue, a highly glucocorticoid-inducible member of the scavenger receptor cysteine-rich family. *Immunogentics* **53**: 170–177.

Schaer DJ, Schleiffenbaum B, Kurrer M, Imhof A, Bachli E, Fehr J, Möller HJ, Moestrup SK, Schaffner A (2005) Soluble haemoglobin-haptoglobin scavenger receptor CD163 as a lineage-specific marker in the reactive hemophagocytic syndrome. *Eur J Haematol* **74**: 6–10.

Schaer DJ, Schaer CA, Buehler PW, Boykins RA, Schoedon G, Alayash AI, Schaffner A (2006) CD163 is the macrophage scavenger receptor for native and chemically modified haemoglobins in the absence of haptoglobin. *Blood* **107**: 373–380.

Song R, Ning W, Liu F, Ameredes BT, Calhoun WJ, Otterbein LE, Choi AM (2003) Regulation of IL-1beta-induced GM-CSF production in human airway smooth muscle cells by carbon monoxide. *Am J Physiol Lung Cell Mol Physiol* **284**: L50–L56.

Spits H, de Waal Malefyt R (1992) Functional characterization of human IL-10. *Int Arch Allergy Immunol* **99**: 8–15.

Sulahian TH, Hintz KA, Wardwell K, Guyre PM (2001) Development of an ELISA to measure soluble CD163 in biological fluids. *J Immunol Methods* **252**: 25–31.

Sulahian TH, Pioli PA, Wardwell K, Guyre PM (2004) Cross-linking of Fc gamma R triggers shedding of the haemoglobin-haptoglobin scavenger receptor CD163. *J Leukoc Biol* **76**: 271–277.

Suzuki H, Kurihara Y, Takeya M, Kamada N, Kataoka M, Jishage K, Ueda O, Sakaguchi H, Higashi T, Suzuki T, Takashima Y, Kawabe Y, Cynshi O, Wada Y, Honda M, Kurihara H, Aburatani H, Doi T, Matsumoto A, Azuma S, Noda T, Toyoda Y, Itakura H, Yazaki Y, Horiuchi S, Takahashi K, Kruijt JK, Van Berkel TJC, Steinbrecher UP, Ishibashi S, Maeda N, Gordon S, Kodama T (1997) A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. *Nature* **386**: 292–296.

Timmermann M, Hogger P (2005) Oxidative stress and 8-iso-prostaglandin F(2alpha) induce ectodomain shedding of CD163 and release of tumor necrosis factor-alpha from human monocytes. *Free Radic Biol Med* **39**: 98–107.

Torres SH, De Sanctis JB, de L Briceno M, Hernandez N, Finol HJ (2004) Inflammation and nitric oxide production in skeletal muscle of type 2 diabetic patients. *J Endocrinol* **181**: 419–427.

Tosaki A, Das DK (2002) The role of haem oxygenase signaling in various disorders. *Mol Cell Biochem* **232**: 49–57.

Van den Heuvel MM, Tensen CP, van As JH, Van den Berg TK, Fluitsma DM, Dijkstra CD, Dopp EA, Droste A, Van Gaalen FA, Sorg C, Hogger P, Beelen RH (1999) Regulation of CD 163 on human macrophages: cross-linking of CD163 induces signaling and activation. *J Leukoc Biol* **66**: 858–866.

Vogel I, Grove J, Thorsen P, Moestrup SK, Uldbjerg N, Möller HJ (2005) Preterm delivery predicted by soluble CD163 and CRP in women with symptoms of preterm delivery. *BJOG* **112**: 737–742.

Vos JA, Abbondanzo SL, Barekman CL, Andriko JW, Miettinen M, Aguilera NS (2005) Histiocytic sarcoma**:** a study of five cases including the histiocyte CD163. *Mod Pathol* **18**: 693–704.

Wada T, Oara H, Watanabe K, Kinoshita H, Yachi A (1970) Autoradiographic study on the site of uptake of the haptoglobin–haemoglobin complex. *J Reticuloendothel Soc* **8:** 185–193.

Wagener FA, Van Beurden HE, Von Den Hoff JW, Adema GJ, Figdor CG (2003) The haem-haem oxygenase system: a molecular switch in wound healing. *Blood* **102**: 521–528.

Walter RB, Bachli EB, Schaer DJ, Ruegg R, Schoedon G (2003) Expression of the haemoglobin scavenger receptor (CD163/HbSR) as immunophenotypic marker of monocytic lineage in acute myeloid leukemia. *Blood* **101**: 3755–3756.

Weinstein MB, Segal HL (1984) Uptake of free hemoglobin by rat liver parenchymal cells. *Biochem Biophys Res Commun* **123**: 489–496.

Wijngaard PL, Metzelaar MJ, MacHugh ND, Morrison WI, Clevers HC (1992) Molecular characterization of the WC1 antigen expressed specifically on bovine CD42 CD82 g dT lymphocytes. *J Immunol* **149**: 3273–3277.

Willis D, Moore AR, Frederick R, Willoughby DA (1996) Haem oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* **2**: 87–90.

Woodward J, Sisley K, Reeves G, Nichols C, Parsons MA, Mudhar H, Rennie I (2004) Evidence of macrophage and lymphocyte, but not dendritic cell, infiltration in Posterior uveal melanomas, whilst cultured uveal melanomas demonstrate pluripotency by expressing CD68 and CD163. *Int J Exp Pathol* **85**: 35–43.

Yu B, Hailman E, Wright SD (1997) Lipopolysaccharide binding protein and soluble CD14 catalyze exchange of phospholipids. *J Clin Invest* **99**: 315–324.

Zuwała-Jagiełło J, Osada J (1998) Internalization study using EDTA-prepared hepatocytes for receptor-mediated endocytosis of haemoglobin-haptoglobin complex. *Int J Biochem Cell Biol* **30**: 923–931.

Zwadlo G, Voegeli R, Osthoff KS, Sorg C (1987) A monoclonal antibody to a novel differentiation antigen on human macrophages associated with the down-regulatory phase of the inflammatory process. *Exp Cell Biol* **55**: 295–304.