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COMMENTARY

CD163, a Hemoglobin/Haptoglobin Scavenger Receptor, After Intracerebral Hemorrhage: Functions in Microglia/Macrophages Versus Neurons

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Abbreviations

ADAM-17	A disintegrin and metalloproteinase domain-17
ICH	Intracerebral hemorrhage
IL-10	Interleukin-10
sCD163	Soluble CD163
EV-CD163	Extracellular vesicle CD163
HO	Heme oxygenase
NeuN	Neuron-specific nuclear protein
SRCR	Scavenger receptor cysteine-rich

Introduction

Following intracerebral hemorrhage (ICH), clot-derived factors cause secondary brain damage. In particular, hemoglobin released during erythrolysis has been the focus of a large body of research due to its iron-containing heme cofactors. When not bound to O_2, heme-bound iron exists in a ferrous (Fe^{2+}) state capable of performing radical chemistry to create dangerous reactive oxygen species. Several studies have highlighted the hemoglobin-induced damage caused to neuronal populations during hemorrhagic stroke, suggesting the existence of pathways by which hemoglobin-associated iron can enter neurons and inflict oxidative injury [1–3]. While there are several known mechanisms by which free iron and heme can enter cells, the number of known hemoglobin uptake mechanisms is limited. The best characterized is CD163, long thought to be a marker of monocytes/macrophages. Recent evidence shows that CD163 can also be expressed in neuronal populations following ICH, providing a mechanism for hemoglobin uptake into neurons [2, 4]. This commentary discusses the potential beneficial roles of CD163 in microglia/macrophages after ICH and compares them to potentially detrimental effects the receptor may have in neurons.

CD163 Function

A 130-kDa member of the scavenger receptor cysteine-rich (SRCR) superfamily, CD163 is a single membrane-pass protein with nine extracellular domains [5]. Kristiansen et al. identified CD163 as a scavenger receptor for haptoglobin-hemoglobin complexes [6]. Haptoglobin binds hemoglobin with exceptionally high affinity (K_d~1 pM), and in addition to being involved in the cellular uptake of hemoglobin via CD163, it also markedly reduces the ability of hemoglobin to participate in oxidative reactions [7]. Systemically, the CD163-mediated internalization of haptoglobin-hemoglobin complexes into macrophages is very important in the clearance of hemoglobin released after red blood cell lysis [6]. There is some evidence that CD163 can bind free hemoglobin that has not yet been scavenged by haptoglobin, albeit at a lower affinity. This may occur during hemolytic conditions where haptoglobin is saturated [8] or, potentially, in tissues with low haptoglobin expression (e.g., brain).

CD163 has additional functions that may be distinct from its ability to scavenge hemoglobin from the extracellular space. CD163 is involved in anti-inflammatory signaling following binding of certain forms of haptoglobin, including triggering interleukin-10 (IL-10) responses via phosphatidylinositol-3 kinase-dependent Akt signaling [9]. IL-10 is involved in the polarization of macrophages to the

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M2 (CD163$^+$) phenotype [10, 11], resulting in a positive feedback loop of IL-10 and CD163 expression. However, pro-inflammatory signals such as tumor necrosis factor-α, interferon-γ, transforming growth factor-β, and lipopolysaccharide lead to decreased levels of CD163 expression [5].

In addition, CD163 may have roles in host defense. For instance, Fabriek et al. found a role of macrophage CD163 in binding of Gram-positive and Gram-negative bacteria [12]. Van Gorp et al. found that macrophage CD163 also bound porcine reproductive and respiratory syndrome virus [13].

Changes in the Hemoglobin-Haptoglobin-CD163 System After ICH

In ICH, hemoglobin can be released into the hematoma itself following red blood cell lysis and then diffuse into the brain parenchyma. Thus, following ICH, there is a gradual loss of hemoglobin from the hematoma [14]. The brain may be particularly susceptible to hemoglobin-induced injury because normal brain levels of the hemoglobin scavenger haptoglobin are very low; the CSF haptoglobin concentration is ~800 μg/L in humans, compared to 1.4 g/L in serum [15]. It should be noted that during ICH some haptoglobin will enter the brain from circulation. This haptoglobin may be involved in binding hemoglobin that is released soon after ICH. In addition, there is an upregulation of haptoglobin expression in the brain after ICH, particularly by oligodendrocytes [16] that may also serve to scavenge hemoglobin released from the hematoma.

CD163 expression in hematoma and perihematomal increases with time after ICH [14, 17, 18]. In humans, Liu et al. [17] found a progressive increase in the number of perihematomal CD163-positive cells between 6 and 72 h after ICH. Lively and Schlicter [18] also found a progressive increase in brain CD163 mRNA expression between 6 h and 7 days after ICH in aged rats. Interestingly, this upregulation was shorter in young rats. The signals that regulate CD163 expression after ICH (e.g., clot-derived factors) are unknown.

CD163 in Microglia/Macrophages After ICH

CD163 expression is induced primarily on M2 or "alternatively activated" macrophages [8], which have been described as the reparatory or healing macrophage polarity [19, 20]. As such, CD163 expression is significantly increased in the hematoma within 24 h [14]. This is most likely due to a combination of migration of M2 microglia into the hematoma, infiltration of new M2 macrophages from the circulation, and gene upregulation in microglia/macrophages. After binding either hemoglobin or a hemoglobin-haptoglobin complex, CD163 mediates clathrin-coated vesicle endocytosis. In macrophages, the inducible heme oxygenase (HO)-1 protein degrades heme to release Fe(II), biliverdin, and carbon monoxide inside endosomes. The Fe(II) is then transported out of the endosome by divalent metal transporter (DMT-1) into the cytosol. This iron is subsequently scavenged by cytosolic ferritin for oxidation to Fe(III) and storage (Fig. 1) [21]. Both HO-1 and ferritin are markedly upregulated in microglia/macrophages after ICH [22, 23].

In this way, macrophages and microglia are well equipped to internalize free hemoglobin and sequester the heme-bound iron. CD163 in macrophages therefore plays a key role in clearance of toxic blood components. CD163 levels in the brains of patients with ICH are positively correlated to hematoma absorption and neurological recovery [24]. Clearly, microglia/macrophage-CD163 is important for minimization of and recovery from secondary damage following hemorrhage [25].

The ectodomain of CD163 can be shed from the cell surface by the protease ADAM17 (a disintegrin and metalloproteinase domain-17) [26]. This shedding occurs near a palindromic RSSR amino acid sequence near the outer membrane of the cell [27]. This ectodomain forms soluble CD163 (sCD163) which is used as a marker of inflammation and inflammatory diseases related to M2 macrophage polarization. ADAM17 is involved in the cleavage of a variety of important signal molecules including the pro-inflammatory tumor necrosis factor-α. Given that ADAM17 is induced by inflammation, it seems likely that sCD163 plays further roles in inflammation perhaps as an inflammatory signal. It has been suggested that sCD163 serves as a siderophore to sequester iron from bacteria [27], but its role in hemorrhagic inflammation is not clear. Intriguingly, a recent study demonstrated that CD163 can be shed in extracellular vesicles (EV-CD163) in addition to the previously mentioned proteolytic cleavage [28]. It is possible that this EV-CD163 continues to mediate hemoglobin clearance and sequestration in exosomal

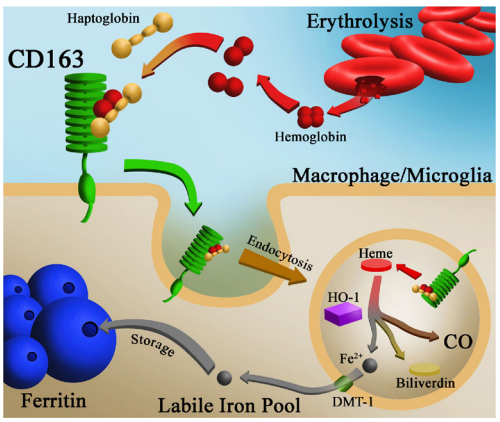

Fig. 1 A diagram of the hemoglobin-haptoglobin-CD163 pathway in microglia/macrophages. Following erythrolysis, hemoglobin dissociates into $\alpha\beta$ dimers before being scavenged by haptoglobin. This complex is then endocytosed by CD163. Heme is released from hemoglobin and is degraded by the inducible HO-1 to notably form Fe^{2+}. This ferrous iron is released into the cytoplasmic labile iron pool via DMT-1 and safely chelated by ferritin

bodies. The function of sCD163 and EV-CD163 is not yet clear following hemorrhage, however, and thus warrants further investigation.

CD163 in Neurons After ICH

Neuronal damage following hemorrhage is well documented, yet the mechanisms of that injury have not been fully elucidated [29–32]. After ICH, hemoglobin can be found within neurons at 72 h [33]. While it is possible, this may be due to hemoglobin synthesis within neurons, it is more likely that this is due to neuronal uptake of extracellular hemoglobin. Until recently, the mechanism of hemoglobin entry into neurons was unknown. Long thought to be expressed solely in monocytes and macrophages, CD163 has been treated as a marker for those cell types. However, several recent studies have reported evidence of $CD163^+$ neurons following hemorrhage. Our lab documented the first finding of CD163 expression in neurons [2, 4]. A neonatal rat model was used to simulate intraventricular hemorrhage, after which CD163 was found in hippocampal pyramidal neurons. Following intraventricular hemoglobin injection, CD163 was co-localized to neuron-specific enolase. Furthermore, we used RT-PCR to demonstrate upregulation of CD163 mRNA in neuronal primary cell cultures from fetal rats following incubation in hemoglobin. This demonstrated transcriptional upregulation of CD163 in neurons accompanied by actual expression of the protein in rat hippocampal neurons. Similarly, a study by Chen-Roetling et al. demonstrated the expression of CD163 in cortical mouse neurons in vitro via phase-contrast microscopy by utilizing the phase-bright and well-defined bodies of neurons in addition to co-localization with NeuN [3]. The authors confirmed this immunohistochemical finding by using antibodies both against the extracellular and intracellular domains of CD163, as well as using mixed and pure neuronal cultures. CD163 was stained primarily on the neuronal soma, but after treatment with hemoglobin, CD163 was also observed in the neuropils surrounding the cell body. This neuronal CD163 endocytosed hemoglobin-haptoglobin complexes, demonstrating its ability to operate as a pathway for hemoglobin entry into neuronal populations [3]. Importantly, Chen-Roetling et al. found that after endocytosis, the hemoglobin was toxic to the neurons, in a haptoglobin-dependent manner. This suggests that the improved ability of CD163 to bind hemoglobin-haptoglobin complexes was involved in the hemoglobin endocytosis and illustrates the potentially detrimental effect of CD163 expression. Even more recently, Dang et al. [34] found neuronal CD163 in basal ganglia neurons in adult rats as evidenced by NeuN-CD163 co-localization. As of yet, no study has examined neuronal CD163 expression in humans.

The findings of CD163 expression in neurons are potentially pathological in nature. In neurons, heme is broken down by the constitutively expressed HO-2, rather than the inducible HO-1 [35]. Neurons lack sufficient iron sequestering systems to handle the iron overload that is present during intracranial hemorrhage [36, 37]. This may result in an increase in intracellular Fe(II) that cannot be properly sequestered. The ferrous iron is therefore free to react with available hydrogen peroxide to form radical oxygen species via the Fenton reactions. This could result in the release of cytochrome c from the mitochondrion and induction of apoptotic cascades and cell death (Fig. 2).

Conclusion

The role(s) of CD163 in microglia/macrophages and in neurons remain to be fully elucidated. The receptor is clearly critical to the hematoma clearance and removal of dangerous iron from the extracellular space when it is expressed in microglia/macrophages. Its roles in anti-inflammatory signaling also suggest its importance in recovery from hemorrhagic secondary damage. However, when expressed in neurons, it results in increased toxicity as neurons are ill-equipped to handle iron overload. This raises a difficult but potentially important therapeutic problem: investigations into CD163-directed therapy must identify a method of specifically targeting neurons without affecting neighboring microglia/macrophages. Furthermore, any attempts to target CD163 must grapple with the presence of sCD163 in the blood stream and CSF; although, the relatively low level of sCD163 compared to the membrane-bound form may mitigate this issue [38]. Regardless, CD163 represents a potentially key step in the mechanism of neuronal injury after ICH. Further study is necessary to understand how neuronal CD163 functions and whether it could be a target in attempts to alleviate hemoglobin-induced neuronal cell death.

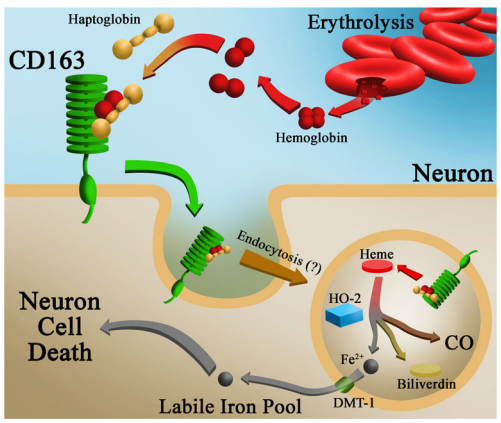

Fig. 2 A probable diagram of the hemoglobin-haptoglobin-CD163 pathway in neurons. Notably, neurons use the constitutive HO-2 to degrade heme, but lack the sufficient levels of ferritin needed to keep Fe^{2+} from performing oxidative chemistry, resulting in neuronal cell death

Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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