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Heme oxygenase-1 and anti-inflammatory M2 macrophages

Author names:

Yuji Naito^{a,*}, Tomohisa Takagi^a, and Yasuki Higashimura^{a,b}

Affiliations:

^aMolecular Gastroenterology and Hepatology, Graduate School of Medical Science,
Kyoto Prefectural University of Medicine, Kyoto, Kyoto 602-8566, Japan

^bDepartment of Food Factor Science, Kyoto Prefectural University of Medicine, Kyoto,
Kyoto 602-8566, Japan

*To whom correspondence should be addressed. Dr. Yuji Naito, Molecular
Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto
Prefectural University of Medicine, Kyoto, Kyoto 602-8566, Japan

Tel.: +81-75-2515508

Fax: +81-75-2510710

E-mail: ynaito@koto.kpu-m.ac.jp

Abstract

Heme oxygenase-1 (HO-1) catalyzes the first and rate-limiting enzymatic step of heme degradation and produces carbon monoxide, free iron, and biliverdin. HO-1, a stress-inducible protein, is induced by various oxidative and inflammatory signals. Consequently, HO-1 expression has been regarded as an adaptive cellular response against inflammatory response and oxidative injury. Although several transcriptional factors and signaling cascades are involved in HO-1 regulation, the two main pathways of Nrf2/Bach1 system and IL-10/HO-1 axis exist in monocyte/macrophage. Macrophages are broadly divisible into two groups: pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages. More recently, several novel macrophage subsets have been identified including Mhem, Mox, and M4 macrophages. Of these, M2 macrophages, Mhem, and Mox are HO-1 highly expressing macrophages. HO-1 has been recognized as having major immunomodulatory and anti-inflammatory properties, which have been demonstrated in HO-1 deficient mice and human cases of genetic HO-1 deficiency. However, the mechanism underlying the immunomodulatory actions of HO-1 remains poorly defined. This review specifically addresses macrophage polarization. The present current evidence indicates that HO-1 induction mediated by multiple pathways can drive the phenotypic shift to M2 macrophages and suggests that HO-1 induction in macrophages is a potential therapeutic approach to immunomodulation in widely diverse human diseases.

Keywords:

Heme oxygenase, Macrophage, Polarization, Inflammation

Abbreviations used: HO, heme oxygenase; CO, carbon monoxide; Nrf2, nuclear factor erythroid 2-related factor-2; BTB, broad complex-tramtrack-bric-a-brac; CNC, cap'n'collar; Bach1, BTB and CNC homolog 1; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; StRE, stress-responsive element; MARE, Maf-response element; ARE, antioxidant-response element; Keap1, Kelch-like ECH-associating protein 1; IL, interleukin; STAT, signal transducer and activator of transcription; Mhem, hemorrhage-specialist macrophages; Mox, macrophages generated with oxidized phospholipids; Hb, hemoglobin; LPS, lipopolysaccharides; MR, mannose receptor; Fizz1, resistin-like α ; Ym1, chitinase 3-like 3; TLR, toll-like receptor; Hp, haptoglobin; Arg1, arginase-1; AC, apoptotic cells; IBD, inflammatory bowel disease; Hx, hemopexin; AGOs, agaro-oligosaccharides; GSH, glutathione; GSSG, oxidized glutathione; IFN γ , interferon γ

1 **Introduction**

2 Heme oxygenase (HO) is the rate-limiting microsomal enzyme that catalyzes heme
3 degradation [1]. With the use of cytochrome P-450, nicotinamide adenine dinucleotide
4 phosphate (NADPH), and molecular oxygen, HO catabolizes heme to equimolar
5 amounts of carbon monoxide (CO), ferrous iron, and biliverdin [2-4]. Biliverdin is
6 converted immediately to bilirubin by biliverbin reductase [5]. These byproducts of HO
7 enzymatic activity are regarded as cytoprotective molecules because of their antioxidant
8 and anti-inflammatory properties [6]. Two distinct HO isozymes are known, HO-1 and
9 HO-2, which are encoded respectively by genes *HMOX1* and *HMOX2* [7,8]. A third
10 isoform, designated as HO-3, which has been identified only in rats, most likely
11 represents a pseudogene [9]. One such isoform, HO-1, is a stress-inducible protein with
12 expression induced by various stressors such as oxidative stress, ultraviolet irradiation,
13 inflammatory cytokine, and heavy metals [4,10,11]. Because of the strong adaptive
14 response of HO-1 to various stressors, HO-1 expression has been regarded as an
15 adaptive cellular response against inflammatory response and oxidative injury [12].

16 The direct link between HO-1 and inflammation was shown initially in an HO-1
17 knockout mouse model. These mice, which displayed increased embryonic lethality,
18 anemia, and chronic inflammatory disorders, are highly vulnerable to experimental
19 sepsis induced by the classical pro-inflammatory mediator endotoxin [13-15].
20 Importantly, phenotypical alterations in the only two known human cases of genetic
21 HO-1 deficiency are similar to those observed in HO-1 knockout mice [16-18]. In
22 contrast, up-regulation of HO-1 using chemical agents, functional food, and genetic
23 engineering has exhibited important beneficial effects in various experimental animal
24 models of inflammation. Consequently, the immune-regulatory function of HO-1 is well

1 established, although the precise molecular mechanism of its function remains under
2 intense investigation.

3 This review provides a comprehensive overview of the molecular mechanisms of
4 HO-1 regulation in monocyte/macrophage. Moreover, we present current evidence and
5 concepts suggesting that HO-1 modulates the immune system during homeostasis and
6 disease by regulating the function and phenotype of macrophages.

7 8 **Regulatory mechanism of HO-1 expression**

9 *HMOX1* gene expression is tightly regulated at the transcriptional level [19]. Several
10 transcriptional factors bind to the 5'-untranslated region of *HMOX1* gene [20,21],
11 including activator protein-1 [22], nuclear factor erythroid 2-related factor-2 (Nrf2)
12 [23,24], hypoxia-inducible factor-1 [25], nuclear factor-kappa B [26], and broad
13 complex-tramtrack-bric-a-brac domain (BTB) and cap'n'collar (CNC) homolog 1
14 (Bach1) [27]. Also, HO-1 expression is regulated by signaling cascades such as
15 mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase
16 (PI3K)/Akt [28]. In fact, Akt can phosphorylate HO-1 protein directly at Ser188 and
17 modulate its activity. These transcriptional factors and signaling cascades that are
18 putatively involved in HO-1 induction mutually differ depending on the stressor and
19 cell species [19]. In addition to the transcriptional regulation, a recent report has
20 described that HO-1 is subjected to post-translational regulation by the
21 ubiquitin-proteasome system through the endoplasmic-reticulum-associated degradation
22 pathway [29].

23 As described above, the well-established regulatory mechanism of HO-1 has been
24 reviewed extensively elsewhere [11,19,30,31]. Therefore, additional details of the two

main pathways of HO-1 induction in monocyte/macrophage are presented below.

Nrf2/Bach1 system

Nrf2 and Bach1, which belong to the CNC family of transcriptional factors, form heterodimers with small Maf proteins [32,33]. The resultant heterodimers regulate HO-1 transcription via interaction with the stress-responsive element (StRE), which is structurally and functionally similar to the Maf-response element (MARE) and the antioxidant-response element (ARE) [21]. Nrf2, which contains a transcription-activation domain, positively regulates transcription of *HMOX1* gene in response to various stimuli such as electrophiles and oxidative stress. Under normal conditions, Nrf2 localizes in the cytoplasm, where it interacts with the actin-binding protein, Kelch-like ECH-associating protein 1 (Keap1), and where it is degraded rapidly by the ubiquitin-proteasome pathway [34], indicating that Keap1 acts as a negative regulator of Nrf2. Various stimuli liberate Nrf2 from Keap1, enabling Nrf2 to translocate into the nucleus and enabling it to bind to the StRE. Under conditions of excess heme, intracellular heme promotes stabilization of Nrf2, leading to accumulation of Nrf2 [35]. Although Alam and colleagues [35] inferred that the generation of cellular oxidative stress is involved in the heme-mediated Nrf2 stabilization, details related to the mechanism remain unclear.

Under baseline conditions, Bach1 forms a heterodimer with small maf proteins that repress transcription of *HMOX1* gene by binding to the StRE. Under conditions of excess heme, increased heme binding to Bach1 causes a conformational change and causes a decrease in DNA-binding activity, which in turn engenders transcriptional activation of the *HMOX1* gene through the StRE [33]. Additionally, heme has been

1 shown to control the cellular Bach1 protein level via a mechanism that involves
2 proteasomal degradation of Bach1 [36]. This series of reports suggests that an
3 intracellular heme concentration displaces Bach1 from the StRE sequences by heme
4 binding, which then permits Nrf2 binding to the StRE. Consequently, intracellular heme
5 ultimately regulates HO-1 transcription via modification of Bach1 and Nrf2 statuses
6 [Fig. 1A].

7 8 *IL-10/HO-1 axis*

9 Several lines of evidence suggest that HO-1 expression is related to interleukin-10
10 (IL-10) signaling and vice-versa [37,38]. IL-10, which is known as an
11 anti-inflammatory cytokine, binds to a receptor tetramer complex that activates
12 phosphorylation of signal transducer and activator of transcription (STAT)-3 [39]. The
13 resultant phosphorylated STAT-3 translocates to the nucleus where it binds to STAT
14 binding elements in the promoters of various genes. Ricchetti *et al.* [39] demonstrated
15 that IL-10-mediated induction of HO-1 requires activation of STAT-3 and PI3K
16 pathways in macrophage cell lines. Cells that were infected with a STAT-3
17 dominant-negative-encoding adenovirus did not express HO-1 when stimulated with
18 IL-10, suggesting that STAT-3 is a pivotal element in IL-10-mediated HO-1 induction.
19 Also, HO-1 and CO modulate IL-10 production through the activation of p38 MAPK
20 [40]. Previous reports have described that lipopolysaccharide (LPS) stimulation induces
21 IL-10 production in human monocytes and mouse macrophages. The inductions were
22 significantly abrogated in the presence of the HO inhibitor, zinc protoporphyrin IX. In
23 contrast, cobalt protoporphyrin IX, which is the HO-1 inducer, enhanced LPS-induced
24 IL-10 production. These results indicate that HO-1 pathway is connected with IL-10

1 signaling complicatedly. This positive feedback circuit between HO-1 and IL-10 has
2 been shown to be functional, which might amplify the anti-inflammatory effects of
3 HO-1 in monocyte/macrophage [Fig. 1B].
4

5 **Polarization of macrophages**

6 Macrophages are key components of the innate immune system. They orchestrate
7 both the initiation and resolution of inflammation and can mediate either
8 pro-inflammatory or anti-inflammatory immune responses. Macrophages are broadly
9 divisible into two groups: classically (M1) and alternatively (M2) activated
10 macrophages [41]. Of these, M1 macrophages are characterized by the expression of
11 high levels of pro-inflammatory cytokines, high production of reactive oxygen and
12 nitrogen intermediates, promotion of Th1 response, and strong antimicrobial activity. In
13 contrast, M2 macrophages function in resolving inflammation while promoting cell
14 proliferation and wound healing. They largely reflect the Th2 response. Recently,
15 several novel macrophage subsets have been identified. The notion of macrophage
16 polarization was extended beyond a simple M1 versus M2 classification system. These
17 subsets include hemorrhage-specialist macrophages (Mhem)/hemorrhage-associated
18 macrophages [42], macrophages generated with oxidized phospholipids (Mox) [43], and
19 M4 macrophages [44] induced respectively by hemoglobin (Hb), oxidized
20 phospholipids, and chemokine ligand 4. Of these, M2 macrophages, Mhem, and Mox
21 are HO-1 highly expressing macrophages.

22 M2 macrophages are characterized by high expression of scavenging molecules, the
23 expression of mannose receptor (MR) encoded by gene *Mrc1*, and production of
24 ornithine and polyamines through the arginase pathway. They are associated with

1 responses to anti-inflammatory reactions, wound healing, and helminth infection [45].
2 Recently, Satoh *et al.* [46] demonstrated that F4/80⁺MR⁺ M2 macrophages controlled
3 by Tribble1 are crucially important for adipose tissue maintenance and the suppression
4 of metabolic disorders such as hypertriglyceridemia and insulin resistance. The
5 physiological and pathophysiological functions of M2 macrophages are currently under
6 intense investigation. They are being clarified to an increasing degree. In fact, M2
7 macrophages have at least three distinctly differing subpopulations: M2a, M2b, and
8 M2c macrophages [47]. The M2a macrophages can be induced by IL-4/IL-13 through
9 the activation of IL-4/IL-13 receptors. IL-4 activates STAT-6 signaling, which in turn
10 activates transcription of genes typical of M2a polarization [48]. In addition to *Mrc1*,
11 *resistin-like α (Retnla, Fizz1)* and *chitinase 3-like 3 (Chi3l3, Ym1)* expressions are
12 up-regulated in M2a macrophages [49], suggesting that these expressions are regarded
13 as selective markers of M2a macrophages. IL-4 also induces up-regulation of the
14 histone demethylase JMJD3, which alters chromatin modifications to promote
15 expression of M2 genes and inhibit M1 genes [50]. M2b macrophage polarization
16 induced by combined immune complexes with toll-like receptor (TLR) or IL-1 receptor
17 agonists needs Notch1 signaling [51,52]. Actually, IL-10, acting via IL-10 receptor,
18 skews the macrophage phenotype toward M2c. IL-10 activates STAT-3-mediated
19 expression of genes such as *IL-10*, *transforming growth factor-β*, and *Mrc1*, associated
20 with an M2c phenotype [53].

21 Mhem polarization induced by Hb is potentially associated with intra-plaque
22 hemorrhages. Mhem is characterized by increased expression of IL-10 and CD163,
23 which is a scavenger receptor for the Hb-haptoglobin (Hp) complex, and low expression
24 of human leukocyte antigen-DR [42]. Induction of this macrophage polarization was

also mediated by the transcription factor Nrf2 and up-regulation of HO-1 [54], suggesting that Mhem can play an anti-atherogenic role through the antioxidant and immunomodulatory functions of HO-1. As the feature of high expressions of IL-10 and CD163, Mhem apparently resembles M2c macrophages. As described above, M2 macrophages are characterized by the arginine metabolism. However, it remains unknown whether Mhem can produce ornithine and polyamines through the arginase pathway. Consequently, expression of *Arginase-1* (*Arg1*) may be the marker that distinguishes Mhem from M2c macrophages.

Treatment of macrophages with oxidized phospholipids led to a phenotypic shift of both M1 and M2 macrophages into the Mox macrophage, characterized by exclusive up-regulation of Nrf2-mediated expression of redox-regulated genes such as HO-1 and thioredoxin reductase [43]. It is particularly interesting that Mox macrophages are also characterized by decreased phagocytic capacity, suggesting that this phenotype plays an important role in atherosclerotic lesion development despite its high expression of HO-1 and other antioxidant genes [55].

HO-1 induction and M2 macrophages

In 2009, Weis *et al.* [56] first described the role of HO-1 in macrophage polarization, although several past reports in the literature described that HO-1-overexpressing macrophages had anti-inflammatory and cytoprotective abilities. Weis and colleagues [56] demonstrated that apoptotic cells (AC) polarized macrophages toward M2 phenotype, and that HO-1 induced by AC-derived sphingosine-1-phosphate is closely involved in the polarization. However, the expression levels of M1 and M2 markers were not measured in this study. Recently, some researchers have reported as we have

1 that HO-1 induction can affect macrophage polarization toward M2 phenotype [Table 1].

2 Therefore, more details of the role for HO-1 in the M2 polarization are discussed below.

4 *Adiponectin and M2 macrophages*

5 Adiponectin, known as an anti-inflammatory adipokine, suppresses macrophage
6 activity via several mechanisms [57]. For example, adiponectin suppresses phagocytic
7 activity and inhibits the induction of endothelial adhesion molecules in response to
8 inflammatory signals [58-60]. Mandal and colleagues [61,62] reported that adiponectin
9 engenders M2 polarization through the regulation of HO-1 expression and subsequent
10 suppression of LPS-stimulated cytokine production. Adiponectin is present in the
11 circulation as full-length form (fACrp) and globular form (gACrp), which can be
12 cleaved proteolytically from fACrp. gACrp, acting via the adiponectin R1 receptor,
13 elicits anti-inflammatory activity in Kupffer cells through the activation of
14 IL-10/STAT-3/HO-1 pathway, suggesting that gACrp participates in macrophage
15 polarization toward M2c phenotype. However, fACrp, acting via adiponectine R2
16 receptor, elicits anti-inflammatory activity through the activation of IL-4/STAT-6
17 pathway, suggesting that fACrp is involved in macrophage polarization toward M2a
18 phenotype. Knockdown of HO-1 only partially suppressed the induction of M2
19 macrophage markers in fACrp-treated Raw264 cells, suggesting that HO-1 partially
20 participated in the fACrp-mediated M2a polarization. It is particularly interesting that
21 circulating levels of adiponectin decrease concomitantly with increasing visceral obesity
22 and that they are lower in patients with type 2 diabetes, people with metabolic syndrome,
23 and people with cardiovascular disease than in controls matched by body mass index
24 [63], suggesting that adiponectin-mediated M2 polarization via HO-1 induction is

involved in the onset and progression of these diseases.

Bach1 deficiency and M2 macrophages

Bach1-deficient mice have dramatically higher HO-1 expression in the heart [64], lung [65], spinal cord [66], gastrointestinal tract [67], and liver [68], indicating a role for Bach1 in tonic suppression of HO-1 transcription. The mice reportedly have resistance to LPS-induced hepatic injury [68], hyperoxic lung injury [65], ischemia-reperfusion-induced myocardial injury [64], hypertensive cardiopathy [69], spinal cord injury [66], and atherosclerosis [70]. Recently, we investigated the role of Bach1 in the pathogenesis of gastrointestinal disorders using Bach1-deficient mice, which were gifted from Dr. Igarashi (Tohoku University, Japan). Results demonstrated that the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colonic inflammation, which is a mouse model of inflammatory bowel disease (IBD), is remarkably attenuated in Bach1-deficient mice. Furthermore, the increased expression of inflammatory cytokines and myeloperoxidase activity in the colonic mucosa after the TNBS administration is suppressed in Bach1-deficient mice [71]. In contrast, the administration of tin protoporphyrin, an HO inhibitor, potentiated the colonic damage as well as decreased HO-1 activity, indicating that these beneficial effects observed in Bach1-deficient mice are mediated by HO-1 activity. Similar results were obtained when a mouse model of small intestinal injury induced by indomethacin was used [72]. We also demonstrated that the isolated peritoneal macrophages from Bach1-deficient mice highly expressed HO-1 and manifested M2 macrophage markers such as *Arg1*, *Mrc1*, *Fizz1*, and *Ym1*, suggesting that deletion of Bach1 participates in macrophage polarization toward M2a phenotype. Most importantly, results show that transferring the

Bach1-deficient macrophages ameliorated colonic inflammation in mice, indicating that HO-1-overexpressing M2 macrophages from Bach1-deficient mice have an important anti-inflammatory function in intestinal disorders. In clinical studies, HO-1 expression is localized in the macrophages, but not in epithelium, in inflamed colonic mucosa in patients with active ulcerative colitis [73], suggesting HO-1-overexpressing M2 macrophages as a therapeutic target in IBD patients.

Hemin and M2 macrophages

Heme is well known as an HO-1 inducer. The pool of free heme is often increased dramatically following tissue injury because damaged myocytes and erythrocytes respectively contain high concentrations of heme in the form of myoglobin and Hb. Macrophages have two specific cell-surface receptor systems for heme uptake, which couple tissue injury to HO-1 induction and modulation of macrophage function [74]. Hb released by hemolysis forms a complex with Hp immediately. The resultant Hb-Hp complex binds to the CD163, a receptor that is expressed only on the surface of monocyte/macrophage, and which is internalized by receptor-mediated endocytosis. However, free heme, which is highly toxic, can be released at the injury site by heme-containing proteins including myoglobin. Free heme binds to hemopexin (Hx), which is a means for free heme clearance. The resultant heme-Hx complex undergoes receptor-mediated endocytosis upon binding to another cell-surface receptor CD91. The resultant intracellular heme activates HO-1 transcription through the Bach1 and Nrf2 systems as described above. It is particularly interesting that Figueired and colleagues [75] reported that heme led to HO-1 transcription via direct interaction with TLR-4. Consequently, HO-1 expression is regulated strictly by the heme concentration.

Several lines of evidence suggest that heme-mediated induction of HO-1 abated inflammation selectively enhanced the M2 macrophages while suppressing the M1 macrophages [76-77]. Choi *et al.* [78] demonstrated that hemin-mediated induction of HO-1 in MR⁺ M2 macrophages prevented gastroparesis, which is a well-recognized complication of diabetes. Additionally, hemin treatment suppressed pericardial adiposity in Zucker diabetic fatty rats, a model of insulin resistance, dyslipidemia, hyperglycemia, and diabetic cardiomyopathy, through macrophage polarization toward the M2 phenotype [77]. These reports describe that hemin-primed macrophages manifested *CD163* and *Mrc1* expressions, which express in both M2a and M2c macrophages. Therefore, the exact phenotype of hemin-primed macrophages remains to be established. Nakamichi *et al.* [79] reported that intraperitoneal hemin administration dramatically increased peritoneal and pancreas macrophages that overexpress HO-1 and protected pancreatitis induced by a choline-deficient diet. These hemin-primed peritoneal macrophages are capable of migrating to the pancreas. However, the associations of M2 polarization are not identified as a homing capacity of macrophages. Further investigations of this issue must be conducted.

Food factors and M2 macrophages

Emerging evidence suggests that dietary and other naturally occurring compounds stimulate HO-1 expression in various cell types [80]. In monocyte/macrophage, results demonstrated that several food factors such as curcumin [81], sulforaphane [82], resveratrol [83], and agaro-oligosaccharides (AGOs) [84], induce HO-1 expression and subsequent inhibition of pro-inflammatory responses. However, whether most of these factors participate in the macrophage polarization toward M2 phenotype or not remains

unclear. Of these, AGOs, which are hydrolysis products of agarose, have been investigated widely in terms of their structure and bioactivity [85-87]. We demonstrated previously that AGOs induced HO-1 expression in macrophages, along with the induction of HO-1 required Nrf2 function and the phosphorylation of c-jun N-terminal kinase [88]. Additionally, AGO-mediated induction of HO-1 manifested the expression of M2 macrophage markers such as *Arg1*, *Mrc1*, and *Ym1* [89], suggesting that AGOs participate in macrophage polarization toward an M2a phenotype.

Resveratrol, a natural phytoalexin, is a constituent of various edible plant products such as peanuts and grape skin [90]. Several lines of evidence suggest that resveratrol plays a role in the prevention of human pathological processes such as inflammation [91]. A report of a recent study described that resveratrol induces HO-1 expression in Raw264.7 cell line [83]. Additionally, Wan *et al.* [92] demonstrated that resveratrol skews macrophage phenotype toward M2, and the conditioned medium from macrophages polarized into M2 phenotype by resveratrol promotes M1 macrophage apoptosis. The apoptosis is mediated by IL-10 released from M2 macrophages. Nonetheless, no direct evidence indicates whether HO-1 participates in the resveratrol-mediated skewing of macrophages.

Perspective and conclusion

The biological significance of HO-1 up-regulation in macrophage phenotype and function remains to be fully elucidated. Recent reports have described that HO-1 and its enzymatic byproducts modulate several immunological events in macrophages such as inflammasome activation, bactericide, and efferocytosis [93-95]. Regarding macrophage polarization, HO-1 induction mediated by multiple pathways including

1 endogenous factor, genetic engineering, chemical agent, and food factors drives the
2 phenotypic shift to M2 macrophages [Fig. 2]. However, the molecular mechanism of the
3 phenotypic shift mediated by HO-1 remains to be established. One characteristic
4 distinguishing M2 macrophages from M1 macrophages is intracellular redox status,
5 which is monitored by the balance between intracellular reduced glutathione (GSH) and
6 oxidized glutathione (GSSG) [96]. The GSH/GSSG ratio was increased in interferon
7 gamma (IFN γ)-treated macrophages. In contrast, IL-4 stimulation decreased the
8 GSH/GSSG ratio, indicating that the intracellular status of M2 macrophages was
9 oxidized status. Because of the strong adaptive response of HO-1 to oxidative status,
10 HO-1 expression might become a marker protein of M2 macrophages, which might
11 reflect an intracellular redox status. Taken together, although further investigations of
12 this issue must be conducted, HO-1 induction in macrophages must be regarded as a
13 potential therapeutic approach to immunomodulation in many important human
14 diseases.

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23 **Conflict of interest**

24 Yasuki Higashimura is affiliated with the collaborative research department

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2 The other authors declare that they have no competing financial interest.

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Figure legends

Fig. 1. Regulation of *HMOX1* gene expression via Nrf2/Bach1 system and IL-10/HO-1 axis. **(A)** *HMOX1* gene expression is regulated via Nrf2 and Bach1, which have counter-regulatory functions. Under basal conditions, the transcription repressor Bach1 binds to StRE of the promoter region of *HMOX1* gene. An increase in heme levels alleviates Bach1-mediated repression through inhibition of its DNA-binding activity, making StRE available for Nrf2. **(B)** Evidence suggests that the HO-1 pathway is connected with IL-10 signaling complicatedly. IL-10, acting via IL-10 receptor, activates STAT-3 signaling, and subsequently induces HO-1 expression. HO-1 and CO also modulate IL-10 production through the activation of p38 MAPK. Nrf2, nuclear factor erythroid 2-related factor-2; Keap1, Kelch-like ECH associating protein 1; Bach1, broad complex-tramtrack-bric-a-brac and cap'n'collar homolog 1; StRE, stress-responsive element; IL-10, interleukin-10; IL-10R, interleukin-10 receptor; STAT-3, signal transducer and activator of transcription-3; SBE, STAT-binding element; CO, carbon monoxide.

Fig. 2. Macrophage polarization and HO-1 induction. M1 stimuli such as LPS and IFN γ signal engender the polarization of macrophages to the M1 phenotype. In contrast, IL-4/IL-13, immune complexes, and IL-10 respectively skew macrophages phenotypes toward M2a, M2b, and M2c. HO-1 induction mediated by multiple pathways drives the phenotypic shift to M2 macrophages. LPS, lipopolysaccharides; IFN γ , interferon gamma; IL, interleukin; HO-1, heme oxygenase-1.

Figure 1

Figure 2

Table 1
Heme oxygenase-1 induction and M2 macrophages.

HO-1 induction	Experimental models	Species	Effects	M2 marker	References
Adiponectin	Alcoholic liver disease	Rat	Prevent stimulated TNF- α expression in Kupffer cells	Arg1, MR, Ym1, IL-4 receptor α	Mandal <i>et al.</i> 2011 [62]
Bach1 ^{-/-}	Crohn's disease	Mouse	Ameliorate intestinal inflammation	Arg1, MR, Fizz1, Ym1	Harusato <i>et al.</i> 2013 [71]
Hemin	Hypertension	Rat	Potentiate insulin signaling	CD163, CD14, MR	Ndisang <i>et al.</i> 2013 [76]
Hemin	Diabetes	Rat	Inhibit pericardial adiposity and cardiac injury	CD163	Jadhav <i>et al.</i> 2013 [77]
Hemin	Diabetes	Mouse	Prevent diabetes-induced delayed gastric emptying	MR	Choi <i>et al.</i> 2010 [78]
AGOs	Intestinal injury	Mouse	Prevent indomethacin-induced intestinal injury	Arg1, MR, Ym1	Higashimura <i>et al.</i> 2014 [89]

HO-1, heme oxydase-1; TNF- α , tumor necrosis factor- α ; Arg1, arginase-1; MR, mannose receptor; Ym1, chitinase 3-like 3; IL-4, interleukin-4; Bach1, broad complex-tramtrack-bric-a-brac and cap'n'collar homolog 1; Fizz1, resistin-like a; AGOs, agaro-oligosaccharides.