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REVIEW

Signaling to Heme Oxygenase-1 and its Anti-Inflammatory Therapeutic Potential*

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ABSTRACT

Heme oxygenase (HO)-1 is the inducible isoform of the first and rate-limiting enzyme of heme degradation. Induction of HO-1 protects against the cytotoxicity of oxidative stress and apoptotic cell death. More recently, HO-1 has been recognized to have major immunomodulatory and anti-inflammatory properties, which have been demonstrated in HO-1 knockout mice and a human case of genetic HO-1 deficiency. Beneficial protective effects of HO-1 in inflammation are not only mediated via enzymatic degradation of proinflammatory free heme, but also via production of the anti-inflammatory compounds bilirubin and carbon monoxide. The immunomodulatory role of HO-1 is associated with its cell type-specific functions in myeloid cells (including macrophages and monocytes) and in endothelial cells, as both cell types are crucially involved in initiating inflammatory responses. This review covers the molecular mechanisms and signaling pathways that are involved in HO-1 gene expression. In particular, it is discussed how key nuclear factors such as the redox-dependent transcriptional activators NF-E2 related factor 2 (Nrf2), NF-κB and AP-1 along with the transcription repressor BTB and CNC homologue 1 (Bach1) mediate inducible HO-1 gene expression. The role of central pro- and anti-inflammatory cellular signaling cascades including p38 MAPK and phosphatidylinositol-3 kinase (PI3K)/Akt in HO-1 regulation is highlighted. Finally, we summarize emerging strategies that apply targeted pharmacological induction of HO-1 for therapeutic interventions in inflammatory conditions.

KEYWORDS

Gene expression, heme oxygenase-1, immunomodulation, inflammation, oxidative stress, signaling.

INTRODUCTION

Heme oxygenase (HO) catalyzes the first and rate-limiting enzymatic step of heme degradation and produces carbon monoxide (CO), iron and biliverdin [1-3], which is converted into bilirubin (BR) via biliverdin reductase. Two genetically distinct HO isozymes, HO-1 and HO-2, are known. HO-2 represents the constitutive non-inducible isoform and is primarily expressed in brain and testis [4]. By contrast, the inducible isoform HO-1, which exhibits low basal expression levels in most cells and tissues, is highly up-regulated by a wide variety of oxidative stress stimuli. Due to its regulatory pattern, induction of HO-1 has generally been considered to be an adaptive cellular response against the toxicity of oxidative stress [5-10]. More recently, HO-1 has also been recognized to exhibit important immunmodulatory and anti-inflammatory functions. A potential link between HO-1 and inflammation has initially been shown in an animal model, in which specific up-regulation of HO enzyme activity attenuated complement-dependent inflammation [11]. Shortly thereafter, it has been demonstrated in a HO-1 knockout mouse model that these animals develop a chronic inflammatory disease and are highly vulnerable to an experimental sepsis induced by the classical pro-inflammatory mediator endotoxin [12]. Importantly, phenotypical alterations in the only known human case of genetic HO-1 deficiency are highly similar to those observed in HO-1 knockout mice [13]. By contrast, HO-2 deficient mice have an intact immune system, but exhibit major neurological defects [14]. Independently, targeted overexpression of HO-1 has been shown to have beneficial effects in various experimental animal models of inflammation [2, 15]. Due to the critical role of HO-1 in immunological responses [16-18], the mechanisms of its immunomodulatory functions are currently under intense investigation.

In this review, we discuss the current understanding of how HO-1 may mediate its anti-inflammatory effects in myeloid and endothelial cells. Moreover, we summarize the regulatory role of major transcription factors (TFs) and signaling pathways that govern the inducible HO-1 gene expression. Finally, we highlight the therapeutic potential of targeted pharmacological induction of HO-1.

1. Cell-specific immunomodulatory functions of HO-1 in myeloid and endothelial cells

Inflammation is a complex reaction of the immune system in vascularized tissues at sites of an infection, toxin exposure or cell injury. Although HO-1 is expressed in all cells and tissues the salutary anti-inflammatory effects of HO-1 appear to be critically dependent on its cell type-specific functions in myeloid and endothelial cells.

1.1. Myeloid cells

Myeloid cells comprise monocytes, macrophages and dendritic cells, which play crucial regulatory roles in the innate and adaptive immune system [19]. For example, macrophages ingest and kill invading microorganisms as a first line of defense and are activated by various immunological stimuli such as microbial products and cytokines to initiate inflammatory immune responses [20]. In rodent macrophages, HO-1 has been shown to be up-regulated by lipopolysaccharide (LPS) [21-23], which then attenuates the expression of various proinflammatory genes including cyclooxygenase-2, inducible nitric oxide (NO) synthase (iNOS), tumor necrosis factor (TNF)-α or interleukin (IL)-6 [18, 24] (Figure 1). In contrast to the LPS-dependent induction of HO-1 in rodent macrophages, gene expression of HO-1 is down-regulated by treatment with LPS in human monocytes [25]. More recently, the myeloid cell-specific immunomodulatory functions of HO-1 have also been investigated in a conditional HO-1 knockout mouse model. Mice with myeloid cell-specific genetic HO-1 deficiency exhibited a defect of the interferon-β pathway along with pathological immune responses in experimentally induced infections and an experimental autoimmune encephalomyelitis [26]. Finally, HO-1 is also important for the function of dendritic cells, which are the main cell population for antigen-presentation and for intiation of adaptive immune responses. Specifically, targeted up-regulation of HO-1 has been shown to modulate maturation and cell-specific functions of dendritic cells in human and mouse models [17, 27].

In conclusion, HO-1 appears to have versatile functions via immunomodulation of myeloid cells.

1.2. Endothelial cells

Endothelial monolayers are intimately linked with inflammation, because they constitute a barrier between the peripheral blood stream and inflamed tissues. The endothelium regulates recruitment and transmigration of immunologically active blood cells such as polymorphonuclear leukocytes and T lymphocytes to the site of an inflammation [28, 29]. HO-1 has been shown to directly affect the cellular interactions of polymorphonuclear leukocytes with endothelial cells in an *in vivo* rat model, in which increased HO activity down-regulated the adhesion of these cells during experimental oxidative stress conditions [30]. Independently, major pathological alterations of the endothelium have been observed in HO-1 knockout mice, in which endothelial cells were more susceptible to apoptotic cell death and denudation from the extracellular matrix [31]. Independently, anti-inflammatory endothelial protection via HO-1 has been shown to be mediated via its ability to down-regulate TNFα-induced expression of various adhesion molecules [32, 33]. More recently, it has been reported that HO-1 was involved in the recruitment of endothelial progenitor cells to the site of an experimental vascular injury in various animal models [34].

Thus, HO-1 counteracts inflammatory reactions via modulation of various endothelial cell functions.

2. Anti-inflammatory functions of HO-1: degradation of proinflammatory free heme and production of the anti-inflammatory compounds BR and CO

The mechanisms that mediate the anti-inflammatory effects of HO-1 are not understood in detail. It has been appreciated in recent years, however, that the enzymatic degradation of proinflammatory free heme and the production of the anti-inflammatory compounds biliverdin/BR and CO may play major roles to counteract inflammatory reactions.

Heme consists of a tetrapyrrole ring with a central iron ion and is an abundant compound in mammalians with contradictory biological functions. On the one hand, heme plays a physiological role for oxygen and mitochondrial electron transport as an essential prosthetic group of hemoglobin, myoglobin and cytochromes [35, 36]. On the other hand, non-protein

bound free heme is highly toxic as it may cause oxidative stress. Due to the prooxidant properties of free heme, which have been shown in various animal and cell culture models, the enzymatic synthesis and degradation of this molecule is tightly controlled [35, 37, 38]. More recently, free heme has also been shown to have proinflammatory properties [10, 15, 39]. For example, heme has been demonstrated to be responsible for the increased influx of leukocytes into organs during an experimental inflammation *in vivo* [40]. Detrimental proinflammatory effects of free heme have also been shown in an animal model of experimental cerebral malaria, in which the heme-dependent detrimental effects were more pronounced in HO-1 deficient mice [41]. Thus, it is conceivable that the enzymatic degradation of proinflammatory free heme via HO-1 plays a critical role for the anti-inflammatory functions of HO-1 [10, 15, 37-39].

The role of BR as a beneficial compound with potent antioxidant and anti-inflammatory effects has only been appreciated in recent years [42, 43]. Protection against experimental inflammation via HO-1-derived biliverdin has been shown in animal models of proinflammatory cardiovascular [30] and gastrointestinal disorders [44]. Independently, beneficial effects of BR have directly been implicated in the protection against endothelial activation and dysfunction in human aortic endothelial cells [45]. Interestingly, BR has also been suggested to specifically reduce leukocyte transmigration to the site of an experimental inflammation via interaction with adhesion molecules [46].

Although CO is generally considered a toxic gas, it has been recognized to have major physiological functions as a signaling molecule [3, 47, 48]. Specifically, HO-1-derived CO has been shown to be involved in the regulation of apoptosis, vasodilation and inflammation. In an early report on the potential protective effects of this gas, administration of exogenous CO blocked the LPS-induced production of proinflammatory cytokines via modulation of p38 MAP kinase [24]. Similar to the signaling gas NO, CO up-regulates the production of cGMP and this mechanism has been implicated in other functions of CO such as vasodilation and blockage of smooth muscle cell proliferation. Major potential for future therapeutic

applications may have CO-releasing molecules (CORMs), which are compounds that deliver CO to its target sites without the toxicity of gaseous CO [49, 50].

In summary, HO-1 counteracts inflammatory responses via metabolic conversion of proinflammatory free heme and production of the anti-inflammatory compounds BR and CO.

3. Redox-dependent TFs mediate the inducible HO-1 gene expression

Targeted modulation of HO-1 for potential anti-inflammatory therapeutic interventions not only requires detailed knowledge of the immunomodulatory effects of HO-1. To achieve this goal, it is also necessary to precisely understand the mechanisms that regulate HO-1 gene expression.

HO-1 is induced by a plethora of physiological and pathological stimuli including oxidative stress signals, cytokines, bacterial compounds and growth factors. HO-1 expression is primarily regulated on the transcriptional level and multiple *cis*-acting regulatory elements (REs) of the HO-1 promoter have been shown to mediate the basal and inducible HO-1 gene expression in different species (reviewed in [2, 8, 9, 51]). Two upstream enhancer regions, which are termed E1 and E2, play major functional roles for redox-dependent induction of HO-1 [52, 53]. Both E1 and E2 enhancer regions contain several antioxidant response elements (AREs), which have also been identified in the promoters of other stress-inducible antioxidant and phase 2 detoxifying genes [54, 55]. An important difference between the rodent and human HO-1 genes with major biological relevance is a GT-microsatellite polymorphism, which is localized in the proximal human HO-1 gene promoter region. Lower numbers of GT repeats within this polymorphic sequence have been associated with higher inducibility of HO-1 gene expression in response to stress stimuli [56] and individuals with this allele seem to be protected against cardiovascular disorders (reviewed in [57]).

In the following, we highlight the critical role of the major redox-dependent TFs NF-E2-related factor 2 (Nrf2), BTB and CNC homologue 1 (Bach1), NF-κB and AP-1 in regulating the inducible HO-1 gene expression.

3.1. The Keap1/Nrf2 system

Redox-dependent transcriptional induction of HO-1 is primarily mediated through the cap'n'collar (CNC) TF Nrf2 [58], which was initially identified while screening for proteins that interact with the NF-E2 binding motif [59]. Nrf2 forms heterodimers with small Maf proteins and up-regulates a program of inducible protective genes via interaction with AREs [55, 60]. Activation of Nrf2 by oxidative stress is mainly controlled by the cytosolic inhibitor Kelch-like ECH-associated protein 1 (Keap1)[61, 62], also termed inhibitor of Nrf2 [63](Figure 2). Numerous prooxidant stimuli cause dissociation of Nrf2 from Keap1, which then permits subsequent nuclear translocation of Nrf2 [63, 64]. In a zebrafish model it has been demonstrated that various chemicals activate Nrf2 in a compound-specific manner via the modulation of various regulatory sites of Keap1 [65]. More recently, the Keap1/Nrf2 pathway has also been shown to be activated by the regulator protein p62 in experimental conditions that cause autophagy [66]. Thus, the Keap1/Nrf2 system appears to be a central sensor for a broad spectrum of unfavourable cellular conditions.

It is important to point out, that the Keap1/Nrf2 module may not only be regulated by prooxidant stimuli, but also by stress-independent signals such as glycogen synthase kinase (GSK)-3β-dependent phosphorylation (Figure 3). Finally, it is not clear whether Nrf2-dependent induction of HO-1 is part of a general Nrf2-regulated antioxidant response that includes other Keap1/Nrf2-regulated genes such as NAD(P)H:oxidoreductase or thioredoxin reductase-1. A potential mechanism that could mediate Nrf2-specific induction of HO-1 involves the *brahma-related gene 1* (BRG1). BRG1 has been shown to be necessary for the specific recruitment of Nrf2 to the promoter of the HO-1 gene, but not to that of other phase 2 detoxifying genes [67].

In conclusion, the redox-dependent Keap1/Nrf2 system plays a central role for HO-1 induction in response to oxidativ stress.

3.3. The transcription repressor Bach1

The transcription repressor Bach1 has been recognized to be a key regulator of the inducible HO-1 gene expression (Figure 2). Bach1 was initially identified as a regulator of globin gene expression in erythroid cells [68]. Similar to Nrf2, Bach1 belongs to the CNC family of TFs and forms heterodimers with small Maf proteins that bind to Maf recognition elements [60, 69] such as AREs of the HO-1 promoter. In contrast to Nrf2, Bach1 has six heme regulatory motifs, which are crucial for its regulatory functions. Bach1 has initially been shown to repress HO-1 gene expression in the presence of low levels of intracellular heme. When intracellular heme levels are elevated, Bach1 changes its conformation and dissociates from the HO-1 promoter, allowing Nrf2 to bind to the ARE and to activate HO-1 gene expression [70] (Figure 2). More recently, heme has also been shown to control cellular Bach1 protein levels via a mechanism that involves proteasomal degradation of this protein [71]. As free heme exhibits major proinflammatory effects, it is conceivable that Bach1 might play a regulatory role in inflammation. In line with this notion, a regulatory link between Bach1 and the proinflammatory cytokine IL-6 has recently been elucidated in Bach1 knockout mice in the context of hyperoxic lung injury [72].

Bach1 does not only regulate HO-1 gene expression by heme, but also by other prooxidant compounds such as cadmium [73], diamide [74] and sodium arsenite [75]. More recently, the regulatory role of Bach1 in stress-dependent HO-1 induction has also been shown to be dependent on the differentiation status of keratinocytes in cell culture [76].

The interplay between Bach1 and Nrf2 to regulate HO-1 gene expression is discussed controversially. As an example, sodium arsenite has been shown to cause Bach1-specific HO-1 induction independent from Nrf2, whereas sodium arsenite-dependent regulation via this mechanism was not observed for other ARE-regulated genes such as thioredoxin reductase-1 [75]. Similarly, it has recently been demonstrated that knockdown of Bach1 in human keratinocytes specifically up-regulated gene expression of HO-1, but not that of other Nrf2-regulated genes [77]. In contrast, other investigators have reported that Bach1 induced HO-1 gene expression in coordination with a subset of other Nrf2-regulated genes [78, 79].

In conclusion, the interplay of Bach1 and Nrf2 is crucial for the redox-dependent HO-1 induction and might give this system a high range of plasticity to adapt to adverse cellular conditions.

3.4. NF-κB

The NF-κB/Rel family of proteins comprises several TFs that regulate the inducible gene expression of various immunological and antioxidant protective responses including the up-regulation of major proinflammatory cytokines, adhesion molecules and antioxidant stress proteins [80, 81]. Under basal conditions NF-κB is contained in the cytoplasm by inhibitor of NF-κB (I-κB). In response to multiple signals the regulatory NF-κB subunits p50 and p65 dissociate from I-κB and subsequently translocate to the nucleus [82, 83]. Although NF-κB has been shown to be induced by stimuli that are also known to up-regulate HO-1 gene expression, the role of this TF in HO-1 gene regulation has been discussed controversially. This might be explained by the fact that functional κB-elements have remained elusive for a long while, because indirect approaches including computer-based sequence predictions, treatment with pharmacological NF-κB inhibitors and dominant negative mutants of I-κB have been applied in most studies.

In two recent reports functional binding sites for NF-κB of the promoters of the rat and mouse HO-1 genes have been identified. A κB element of the proximal rat HO-1 gene promoter region has been shown to control HO-1 up-regulation by the phorbol ester phorbol myrisate acetate (PMA), which is an activator of macrophages. PMA-dependent up-regulation of HO-1 gene expression was not observed in cells from mice, which were deficient for the NF-κB subunit p65 and was mediated via an I-κB-kinase-independent pathway [84]. Moreover, Li and colleagues have described a functional κB element in the mouse HO-1 promoter. These authors have shown that a mechanism involving the NF-κB subunits p50 and p65 as well as the inducible NO-synthase mediated HO-1 up-regulation *in vivo* [85].

In summary, NF-κB appears to be directly involved in the induction of HO-1 gene expression.

3.5. Activating protein-1 (AP-1)

The TF AP-1 is composed of structurally and functionally related members of the Jun (c-Jun, JunB and JunD), Fos (c-Fos, FosB, Fra1 and Fra2) and activating TF (ATF) protein families. Dimers of these proteins regulate gene expression via interaction with AP-1 sites, which are also known as TPA-responsive elements (TREs). Inducible gene expression via AP-1 has been shown to be involved in a diverse range of cellular responses including immunological and antioxidant stress responses [86, 87]. Similar to NF-κB, AP-1 is up-regulated by a wide variety of prooxidant and pro-inflammatory stimuli. Alam and colleagues have initially reported that AP-1 played a crucial role for the induction of the mouse HO-1 gene [53, 88]. Subsequently, various functional AP-1 sites, which mediated inducer-dependent gene expression of HO-1, have been identified in the promoter regions of the rat and human HO-1 genes [51, 88-91]. Elucidation of the molecular mechanisms that are involved in AP-1-dependent HO-1 gene regulation has turned out to be challenging for two major reasons. First, the classical AP-1 (TGATGCA) site is contained in the consensus sequence of AREs (TGCTGAGTCA), which are localized in the E1 and E2 regions of the HO-1 gene promoter and serve as major target sites for Nrf2 [58]. Cross-talk of Nrf2 with members of the AP-1 family, however, appears to be highly complex. As an example, c-Jun has recently been shown to directly interact with Nrf2 to activate the expression of the ARE-regulated genes NAD(P)H:quinone reductase and glutamate-cysteine ligase catalytic subunit by the chemical compound 4-hydroxy-2-nonenal [92]. Moreover, others have shown that Nrf2 might indirectly regulate the inducible expression of the glutamate-cysteine ligase catalytic subunit gene via activation of AP-1 [93]. Second, AP-1 sites in the HO-1 promoter mediate inducer-dependent HO-1 gene expression via mechanisms that involve synergistic cooperation of AP-1 with other TFs such as USF2 or SP-1. This has been shown for regulation of the rodent and human HO-1 genes in various cell culture models [94-97].

In summary, we have highlighted the role of various redox-dependent TFs that mediate the complex regulation of inducible HO-1 gene expression. Detailed overviews on this issue have also been given by others [2, 8, 51].

4. Signaling cascades that mediate HO-1 gene regulation

In general, activation of TFs is regulated by intracellular signaling cascades, which are controlled by modules of kinases/phosphatases and redox reactions. In the following, we highlight major signalling cascades that mediate HO-1 induction and are involved in the regulation of inflammatory immune responses.

4.1. p38 MAPK

It has been known for many years that activation of MAPKs plays a central role for the induction of HO-1 gene expression [51] (Figure 3). Three major subfamilies of MAPK are known: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK [98]. While ERK is primarily considered to be activated in response to hormones and growth factors, JNK and p38 are primarily induced by stress-related stimuli [98, 99]. Due to space limitations, it is not intended to give a comprehensive overview on how various MAPKs might mediate HO-1 gene regulation, but rather discuss the specific role of p38 MAPK for HO-1 regulation.

Similar to HO-1, p38 MAPK has been shown to be involved in antioxidant and anti-inflammatory responses. Not surprisingly, gene expression of HO-1 and p38 MAPK are activated by the same or similar stimuli. Numerous reports have demonstrated that inhibition of p38 activity by specific small molecule inhibitors, dominant negative mutants or gene silencing strategies blocked HO-1 induction in response to multiple stimuli [2, 9, 51] (Figure 3). Of note, the α and β isoforms of p38 play counter-regulatory roles to the p38 γ and δ isoforms, which has been demonstrated for sodium arsenite- and LPS-dependent induction of HO-1 [100, 101]. Unexpectedly and contradictory to what is generally thought on the activating role of p38 MAPK on HO-1 gene expression, inhibition of p38α has been demonstrated to up-regulate HO-1 gene expression in human hepatoma cells via interaction with the TF Nrf2 [102, 103]. These findings have essentially been confirmed in an independent report, in which pharmacological inhibition and genetic deficiency of p38α also up-regulated HO-1 gene expression [104]. Increased levels of HO-1 gene expression in

p38α-deficient cells might be explained by the fact that these cells contain increased levels of intracellular ROS as compared to wild type cells [105]. Because p38α is a sensor of ROS, cross-talk between HO-1 and p38α may have additional yet unknown functions in the context of oxidative stress and inflammation. It is also important to point out that p38α is not only an upstream regulator of HO-1, but that p38α is also a downstream target of HO-1. Silva and colleagues have demonstrated that increased HO-1 activity degraded p38α in endothelial cells suggesting that these two molecules might form a physiological relevant feedback loop to regulate apoptosis [106].

In summary, p38 and HO-1 make up a closely linked signaling module with major regulatory functions in antioxidant and anti-inflammatory cellular responses.

4.2. The phosphatidylinositol-3 kinase (PI3K)/Akt pathway

PI3K/Akt is an anti-apoptotic survival pathway and is regulated by a number of receptor-dependent mechanisms that are activated by growth factors and cytokines [107]. Moreover, in models of PI3K genetic deficiency an essential role of this kinase has been implicated in the regulation of inflammatory reactions [108]. Accumulating experimental evidence has indicated that activation of PI3K/Akt not only up-regulates HO-1 gene expression, but that the protective effects of this signaling cascade might be intimately linked with the salutary effects of HO-1 [109]. HO-1 gene expression has been shown to be up-regulated via PI3K/Akt in immunological cells in response to various signals such as prostaglandins or the pharmacological compounds [2, 94, 109, 110]. A more recent report has shown that HO-1 induction was mediated via activation of this signaling cascade by a mitochondrial redox-dependent pathway in vascular endothelial cells [111]. Finally, it has been suggested that PI3K/Akt and GSK3β may have counter-regulatory functions in HO-1 gene regulation. The complex interplay between these two kinases appears to involve mechanisms that control the nuclear localization of Nrf2 and Bach1[112, 113] (Figure 3). Details on the underlying regulatory mechanisms, however, are largely unknown.

In conclusion, the PI3K/Akt cascade is a key mediator of HO-1 induction in response to cytokines and growth factors.

4.3. IL-10 and the Jak-STAT pathway

The anti-inflammatory cytokine IL-10, which is a major inhibitor of activated macrophages and dendritic cells, has consistently been shown to induce HO-1 gene expression as reported by Lee and Chau [114] as well as by others [115-117]. Although the signaling cascades that couple HO-1 gene expression to the IL-10 receptor are not clear, activation of STAT3 has repeatedly been suggested to be involved in this regulatory pathway [116]. Jak-STAT signaling plays a key role in the immune system and mediates major cytokine-activated pathways. In endothelial cells STAT3 has been shown to be necessary to mediate the HO-1 dependent protection against hyperoxic lung injury [118]. Functional STAT3 elements have recently been identified in the promoter regions of the rat [119] and human [120] HO-1 genes. Interestingly, a positive feedback circuit between IL-10 and HO-1 has been shown to be functional, which might amplify the anti-inflammatory effects of IL-10 in LPS-stimulated macrophages [114].

Taken together, the IL-10 pathway is an important regulator of HO-1 gene expression in mononuclear cells.

4.4. The toll-like receptor (TLR)-4 pathway

HO-1 induction by LPS is mediated via (a) TLR-4 coupled pathway(s) and has been extensively studied in macrophages [21, 23]. Interestingly, Figueiredo and colleagues have recently demonstrated that heme led to HO-1 gene expression via direct interaction with TLR-4 and suggested that this interaction could at least partially explain the proinflammatory LPS-like effects of heme [121]. HO-1 gene expression by LPS in macrophages is not only induced via a TLR-4-dependent mechanism, but increased HO-1 activity has also been shown to have inhibitory effects on intracellular signaling, that is initiated by TLR-4-activation. The inhibitory effect of HO-1 on TLR-4 signaling could be regulated via HO-1-derived CO,

which blocked translocation of TLR-4 into lipid rafts [122] and/or the interaction of TLR-4 with the structural membrane protein caveolin-1 [123]. This regulatory interplay between TLR-4 and HO-1 appears to form a negative feedback loop, which might inhibit excessive activation of macrophages by LPS.

Taken together, activation of the central proinflammatory TLR-4 cascade leads to HO-1 gene activation and a negative feedback circuit in macrophages might be of major significance for the regulation of inflammatory responses.

5. Pharmacological induction of HO-1 as an anti-inflammatory therapeutic target

A promising near term approach to apply HO-1 for therapeutic interventions is the targeted induction of this inducible enzyme via pharmacological compounds. In the following, we discuss how pharmacological induction of HO-1 might be applicable for specific anti-inflammatory therapeutic interventions.

Metalloporphyrins such as cobalt protoporphyrin IX, which are prototypical inducers of HO-1 and are commonly used in experimental cell culture and animal models, do not seem to be applicable for clinical interventions, because they lack cell-specificity and are severely toxic. Similarly, the HO substrate heme, which is one of the most potent inducers of HO-1 and has been approved for the treatment of acute intermittent porphyria (heme arginate), only has limited potential for the treatment of inflammatory disorders. By contrast, a growing number of currently available pharmacologic compounds, which induce HO-1 and are applied in standard therapies, might be useful for clinical interventions in inflammatory disorders. As an example, statins, which have initially been introduced to prevent atherosclerosis via their cholesterol-lowering effects, have recently also been recognized to exhibit anti-inflammatory effects via HO-1 induction [124, 125]. Moreover, treatment with 5-aminosalicylic acid (5-ASA), which is one of the pharmacologic standard therapies of inflammatory bowel disease, has been shown to mediate its protective anti-inflammatory effects at least in part through up-regulation of HO-1 in an animal model of colitis [126]. Finally, various polyphenols, which are a group of antioxidant compounds and are currently investigated for their anti-

inflammatory and anticancer activities, have been shown to provide anti-inflammatory protection via the induction of HO-1 [127].

Due to the cell-type specific immunomodulatory effects of HO-1, targeted up-regulation of HO-1 in myeloid and endothelial cells seems to be a straightforward anti-inflammatory therapeutic option. In fact, accumulating evidence has demonstrated that specific HO-1 induction in these cells protects against inflammatory reactions. As an example, it has recently been demonstrated in an *in vivo* rat model that cell-specific up-regulation of HO-1 in liver tissue macrophages via the cytokine adiponectin protects against experimental ethanol-dependent inflammation [117]. Other compounds, which have been shown to mediate myeloid cell-specific induction of HO-1, are the cardiovascular hormone atrial natriuretic peptide [128] and the clinically applied antiprotease compound 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) [94]. Clearly, these compounds might be applicable to specifically induce HO-1 in myeloid cells for therapeutic interventions. In endothelial cells, quercetin and theaflavin have been shown to provide specific HO-1-dependent anti-inflammatory protection in an ApoE knockout mouse model of atherosclerosis [129]. Moreover, it has recently been demonstrated that the nonsteroidal anti-inflammatory drug celecoxib provided specific anti-inflammatory effects in endothelial cells via induction of HO-1 [111].

Finally, it is important to point out that targeted up-regulation of HO-1 has failed to provide anti-inflammatory protection, when induced after the onset of inflammation. This has been shown in animal models of inflammatory bowel disease and pancreatitis, respectively. In either case HO-1 was only protective when induced before the onset of experimental inflammation [130, 131]. These findings indicate that anti-inflammatory protection via HO-1 induction is questionable in established inflammation, but might be useful as a preventive measure.

In summary, targeted HO-1 induction in myeloid and endothelial cells has major anti-inflammatory therapeutic potential. Therefore, identification and characterization of

pharmacological compounds that induce HO-1 in a cell-specific and cell context-specific manner deserve further attention.

6. Conclusions

1. HO-1 plays key immunomodulatory and anti-inflammatory roles via its cell type-specific effects in myeloid and endothelial cells.

2. Critical functions of HO-1 are degradation of proinflammatory free heme and enzymatic production of the anti-inflammatory compounds CO and BR.

3. The inducible HO-1 gene regulation is mediated via an interplay of redox-dependent activating TFs and the transcription repressor Bach1, which are under the control of a complex network of signaling cascades.

4. Targeted induction of HO-1 in myeloid and endothelial cells has major therapeutic potential for the treatment of inflammatory disorders.

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FIGURE LEGENDS

Figure 1: Regulatory role of HO-1 in the inflammatory response of macrophages

Schematic presentation on how HO-1 and its products BR and CO might control the balance of pro- and anti-inflammatory cytokines in macrophages. This balance plays a major role in the activation of macrophages and may thus be critical for the regulation of inflammatory reactions. *Abbreviations:* BR, bilirubin; CO, carbon monoxide; Fe, ferrous iron; IL, interleukin; LPS, lipopolysaccharide; LTA, lipoteichoic acid; TNFα, tumor necrosis factor-α.

Figure 2: Regulation of HO-1 gene expression via the redox-dependent TFs Nrf2 and Bach1

HO-1 gene expression is regulated via the TFs Nrf2 and Bach1, which have counter-regulatory functions. Under basal conditions the transcription repressor Bach1 binds to AREs of the HO-1 promoter. When cellular heme levels are high and in response to stress stimuli, Bach1 is removed from the HO-1 promoter. In addition, stress stimuli cause dissociation of Nrf2 from Keap1, which activates HO-1 gene expression after nuclear translocation via binding to HO-1 AREs. Thus, the interplay between Bach1 and Nrf2 appears to be crucial for the regulation of inducible HO-1 gene expression. *Abbreviations:* ARE, antioxidant response element; Bach1, BTB and CNC homologue 1; HO-1, heme oxygenase-1; Keap1, Kelch-like ECH-associated protein 1; Nrf2, NF-E2-related factor 2; ROS; reactive oxygen species.

Figure 3: Signaling cascades that target Keap1/Nrf2 and Bach1 to regulate HO-1 gene expression

Schematic presentation of major signaling cascades that are involved in the regulation of HO-1 gene expression via interacting with Nrf2 and Bach1. The MAPK p38α has been shown to activate, but also to inhibit HO-1 gene expression via the TF Nrf2. GSK3β-mediated phosphorylation has been shown to regulate the activity of Nrf2 and Bach1. *Abbreviations:* ARE, antioxidant response element; Bach1, BTB and CNC homologue 1; ERK, extracellular-

regulated kinase; GSK3β, glycogen synthase kinase-3β; Keap1, Kelch-like ECH-associated protein 1; Nrf2, NF-E2-related factor 2; PI3-K, phosphatidylinositol-3 kinase.

Figure 1

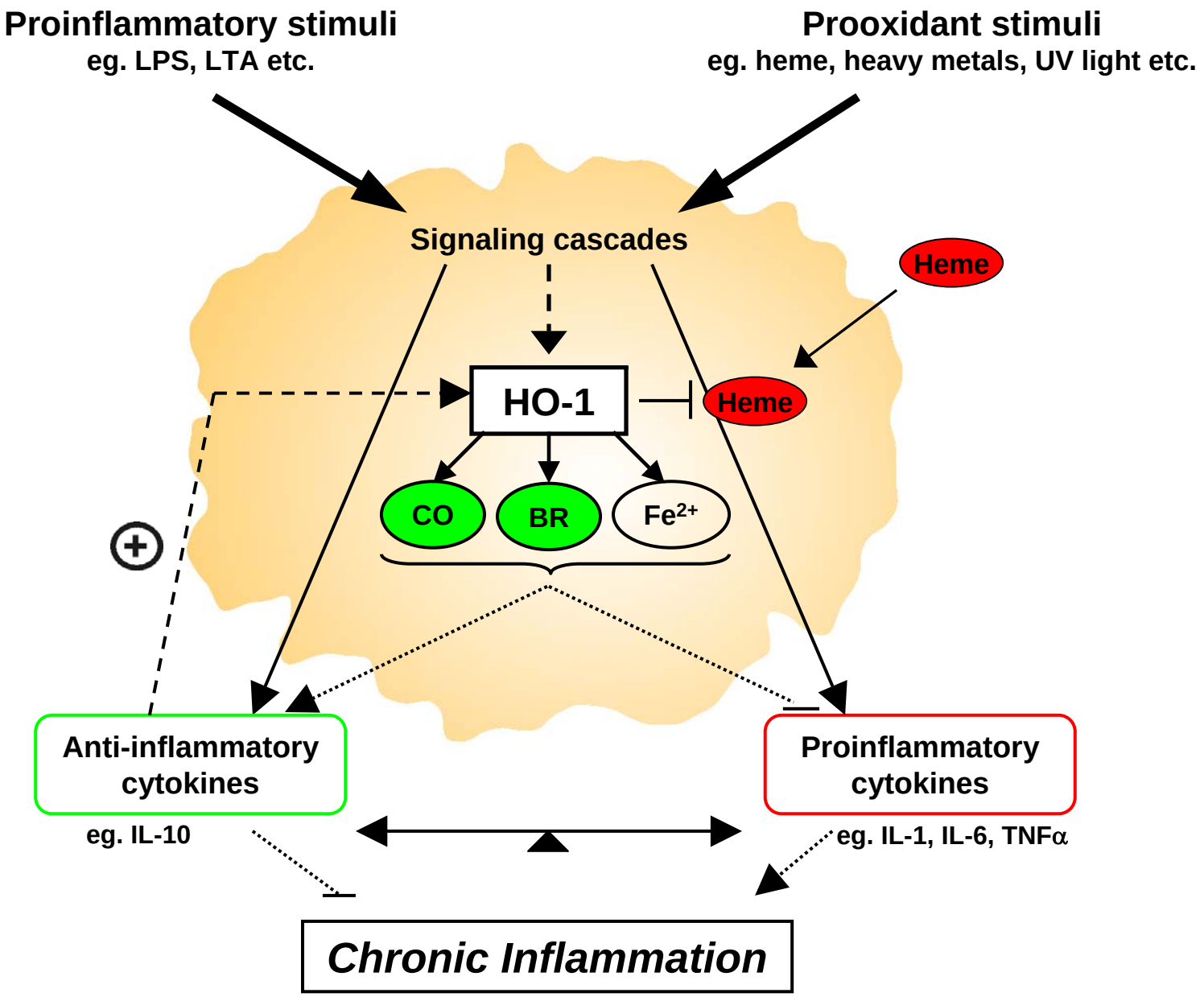

Proinflammatory stimuli
eg. LPS, LTA etc.
Prooxidant stimuli
eg. heme, heavy metals, UV light etc.
Signaling cascades
Heme
HO-1
Heme
CO
BR
Fe2+
Anti-inflammatory cytokines
eg. IL-10
Proinflammatory cytokines
eg. IL-1, IL-6, TNFα
Chronic Inflammation

Figure 2

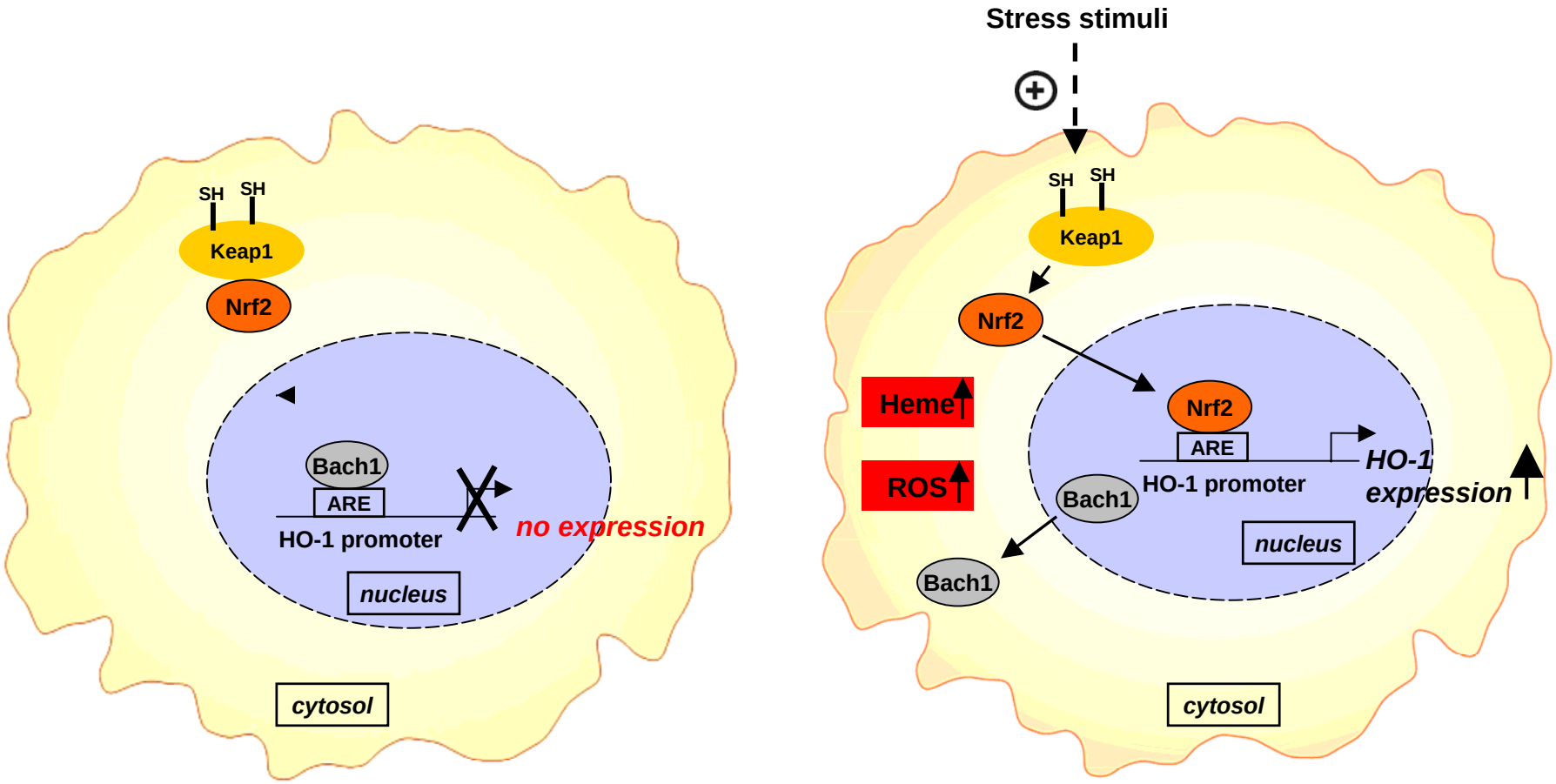
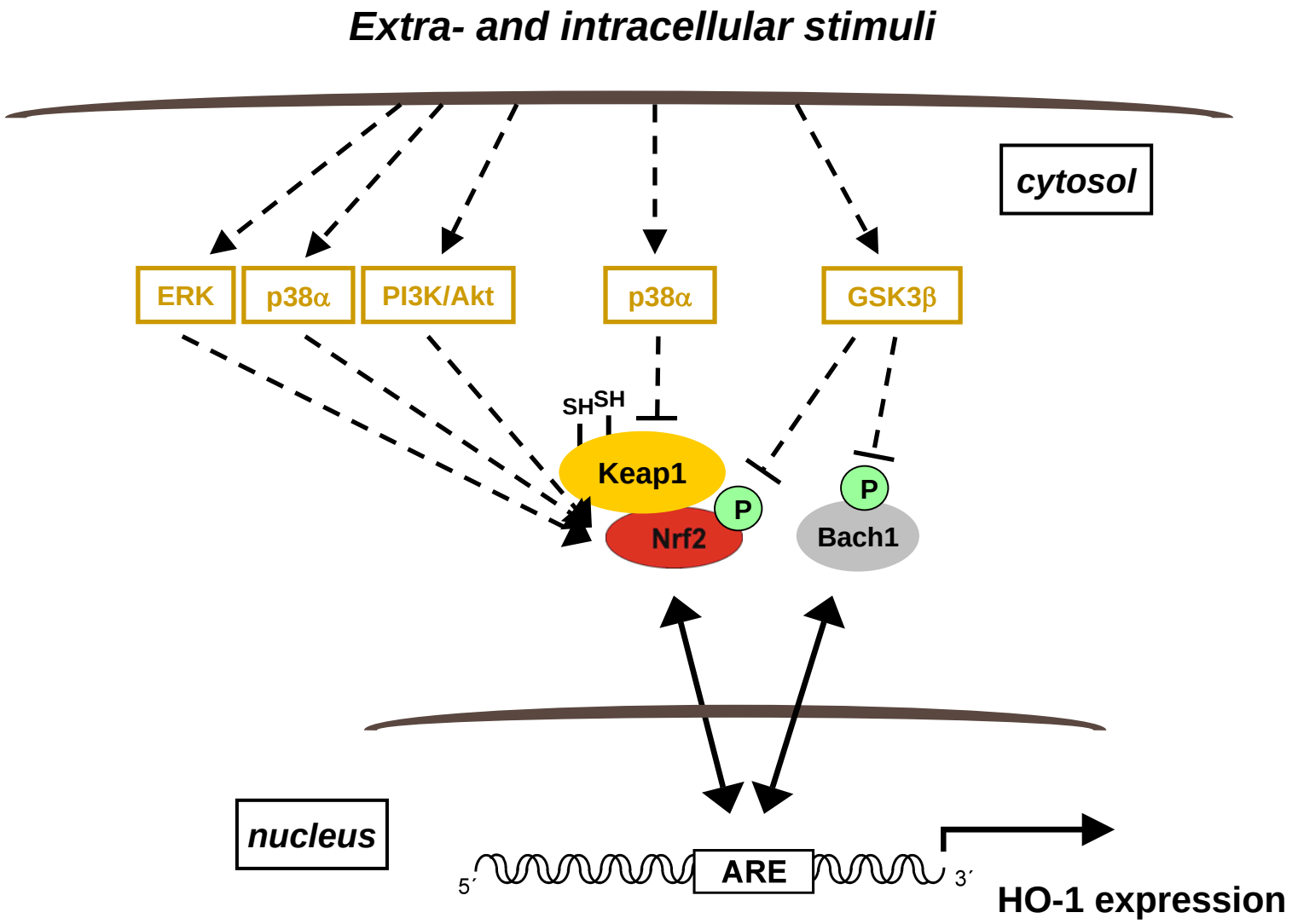

SH
SH
Keap1
Nrf2
Bach1
ARE
HO-1 promoter
no expression
nucleus
cytosol
Normal conditions
Stress stimuli
SH
SH
Keap1
Nrf2
Heme
ROS
Nrf2
ARE
HO-1 promoter
HO-1 expression
Bach1
nucleus
Bach1
cytosol
Prooxidant conditions

Figure 3


Extra- and intracellular stimuli
cytosol
ERK
p38α
PI3K/Akt
p38α
GSK3β
SH SH
Keap1
P
Nrf2
P
Bach1
nucleus
5´
ARE
3´
HO-1 expression

*Graphical Abstract
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