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Forum Review

Heme Degradation and Human Disease: Diversity Is the Soul of Life

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ABSTRACT

We all depend on molecular oxygen and heme for our life, as evident from the pigments in blood and daily wastes. About 80% of serum bilirubin is derived from hemoglobin of senescent erythrocytes, which have finished their mission of 120 days and have been phagocytized by macrophages in the reticuloendothelial system. Here we present an overview of the heme degradation processes and relevant disorders by focusing on heme oxygenase-1 (HO-1), a key enzyme in heme catabolism. HO-1 cleaves the porphyrin macrocycle of heme at the expense of molecular oxygen to release a linear tetrapyrrole biliverdin, carbon monoxide, and ferrous iron; biliverdin is rapidly reduced to bilirubin. Bilirubin is transported to the liver (hepatocytes), conjugated with glucuronic acid by bilirubin UDP-glucuronosyltransferase, and excreted into bile. Genetic diversity, a strategy in the host defense, is seen in the human *ho-1* and UDP-glucuronosyltransferase genes. Moreover, striking interspecies variations are noted in the regulation of HO-1 expression by hypoxia, heat shock, or interferon-γ, each of which mainly represses HO-1 expression in human cells. Implications of such a variety are discussed in relevance to the pathogenesis of severe malaria caused by *Plasmodium falciparum*, the most ancient foe of humans. *Antioxid. Redox Signal.* 4, 593–602.

INTRODUCTION

THE HISTORY OF THE MODERN HUMAN SPECIES began several million years ago when the human lineage diverged from a great ape chimpanzee (17). The time of origin of modern humans, *Homo sapiens*, is estimated to be at least 130,000 years ago in sub-Saharan Africa, and the dispersion from Africa occurred within the last 100,000. The most important environmental factors, affecting evolution of the human genome, include dietary components (91), as seen in the diversity of cytochrome P450 heme-containing monooxygenases, and infections, such as severe malaria caused by *Plasmodium falciparum*, cholera, and tuberculosis. Among these pathogens, *Plasmodium* parasites successfully evade our immune system, as they live and multiply within erythrocytes that are not easily attacked by immune cells (35). Consequently, the selective pressure by *Plasmodium falciparum* is seen in globins, such as hemoglobin S (sickle cell anemia and trait) and thalassemias, and in some erythrocyte enzymes, such as glucose-6-phosphate dehydrogenase deficiency. In these examples, affected individuals may enhance the clearance of parasitized erythrocytes by further shortening the life span of erythrocytes, which may confer protection from death from severe malaria. It is therefore not surprising to find polymorphisms in the human genes encoding proteins related to the heme degradation processes, including haptoglobin (29), heme oxygenase-1 (HO-1) (23), and bilirubin UDP-glucuronosyltransferase (87). These three proteins cooperate to metabolize hemoglobin and heme in response to intravascular hemolysis, a clinical feature associated with malaria.

Haptoglobin is a polymorphic plasma protein and a member of the acute-phase proteins that increase during acute inflammation or after exposure to toxic stimuli (29). It is noteworthy that haptoglobin polymorphism is unique to humans and not seen in other mammals. HO-1 is an essential enzyme in heme breakdown and catalyzes the oxidative cleavage of the α-methene bridge of heme to form biliverdin IXα, ferrous

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iron and carbon monoxide (CO). Recent attention has focused on the biological functions of these heme degradation products, but these molecules should be considered as metabolic double-edged swords because of their potential cell toxicity (69). Bilirubin UDP-glucuronosyltransferase is a member of phase 2 xenobiotic-metabolizing enzymes and shows extensive genetic diversity.

Here we present an overview of the heme degradation processes and relevant disorders by focusing on HO-1 and, to a lesser extent, bilirubin UDP-glucuronosyltransferase. We then discuss topics of current interest to us: interindividual and interspecies variations in the regulation of HO-1 expression and repression of HO-1 expression observed in human cells, subjects that have been largely ignored in the field of *ho-1* gene regulation.

HEME OXYGENASE

Isozymes and reaction

To date, two isozymes of HO are known, HO-1 and HO-2 (33). HO activity is higher in those tissues such as the spleen, liver, and bone marrow, where senescent erythrocytes are sequestered and degraded (85). Characteristically, HO-1 is highly induced by its substrate heme (70, 71, 85) and by various nonheme substances (2, 22, 71, 72, 78, 84). In contrast, HO-2 is a constitutively expressed isozyme (33, 74). A putative isozyme, HO-3, was isolated from the rat brain by cDNA cloning, but its enzyme activity was not detected, despite the fact that it shares 90% identity with HO-2 (34). Further studies are required to establish the identity of HO-3.

HO (either HO-1 or HO-2) binds heme at an equimolar ratio and cleaves heme by using the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH), three molecules of oxygen, and at least seven electrons provided by NADPH-cytochrome P450 reductase (96). Heme cleavage by the HO system proceeds in an autocatalytic fashion on the heme bound to HO, in which the bound heme serves as both a prosthetic group and a substrate (95). HO catalyzes the consecutive steps of monooxidation of the hemin via α-mesohydroxyheme, α-verdoheme, and the ferric iron-biliverdin IXα complex. During the oxidative conversion of α-mesohydroxyheme to α-verdoheme, the α-methene carbon of heme is released as CO. The HO reaction is completed by the release of ferrous iron and biliverdin IXα. This final step is accelerated by the presence of biliverdin IXα reductase (32). The released ferrous iron is efficiently chelated by apoferritin and stored as a ferric state within ferritin molecules or transported to the bone marrow via transferrin for recycling in the bone marrow. CO is transported in blood plasma as carboxyhemoglobin to the lung, where CO is discarded into exhaled air. The bilirubin IXα produced is in the unconjugated form (called indirect bilirubin), which is transported to the liver for conjugation and excretion (Fig. 1). Unless otherwise specified, biliverdin and bilirubin represent IXα isomers in this review.

HO deficiency

The physiological importance of HO-1 has been established by the phenotypic consequences of HO-1 deficiency in mice (54, 55). Mating between heterozygous mice showed

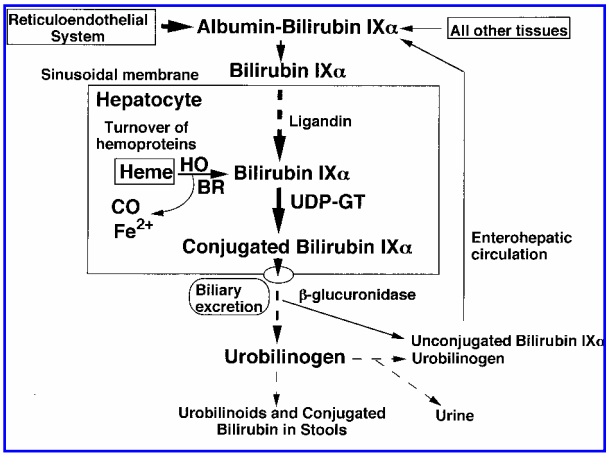

FIG. 1. Hepatic metabolism of bilirubin. About 80% of serum bilirubin is derived from senescent erythrocytes phagocytized by macrophages (reticuloendothelial system), and the rest of bilirubin is derived from turnover of hemoproteins in all tissues. Bilirubin is bound by ligandin and transported to the endoplasmic reticulum. Heme oxygenase (HO) and UDP-glucuronosyltransferase (UDP-GT) are located on the endoplasmic reticulum. BR represents biliverdin reductase. Conjugated bilirubin is excreted into bile.

partial prenatal lethality of the *ho-1*$^{(-/-)}$ mice, and homozygous mating pairs did not yield viable litters. The adult *ho-1*$^{(-/-)}$ mice developed an anemia associated with low serum iron levels, increased serum ferritin levels, and iron deposits in both Kupffer cells and hepatocytes and in renal proximal cortical tubules. Adult *ho-1*$^{(-/-)}$ mice were more vulnerable to mortality and hepatic necrosis when challenged with endotoxin, indicating that the induction of HO-1 represents a defense mechanism to protect cells from oxidative damage. In contrast, HO-2-deficient mice showed mild phenotypes; they are fertile and survive normally for at least 1 year (56), but cerebral HO activity was markedly reduced (98). A subsequent study revealed the ejaculatory abnormalities in male *ho-2*$^{(-/-)}$ mice (8). Thus, the function of HO-2 is not necessarily compensated by HO-1.

The first human case of HO-1 deficiency was identified in Japan (92). A 26-month-old boy was admitted to a hospital for recurrent fever, marked growth retardation, and generalized erythematous rash. Low serum bilirubin levels associated with persistent hemolytic anemia led the physicians to suspect a defect in heme catabolism. The patient's siblings and parents are healthy, except that the mother had experienced two intrauterine fetal deaths. Molecular defects of the *ho-1* gene were identified as compound mutations: a deletion of exon 2 of the maternal allele and a two-base deletion within exon 3 of the paternal allele (Fig. 2). Both mutant alleles encode truncated HO-1 proteins because of the frame shift. Thus, the patient has no functional HO-1 protein, and his parents are heterozygous carriers for each mutant allele. The patient died of intracranial hemorrhage at 6 years of age (49). Iron deposits in the liver and kidney, as well as increased ferritin levels, support the essential role for HO-1 in iron metabolism in humans. Persistent proteinuria and hematuria caused by renal tubular injury also provide proof for a crucial role of HO-1 in the kidney. Most of the symptoms are

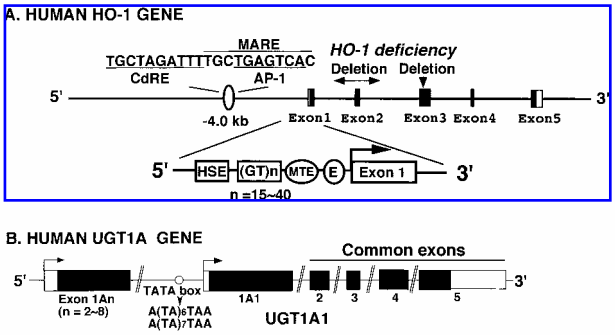

FIG. 2. Structural organization of the human *ho-1* gene (A) and the human UDP-glucuronosyltransferasegene (B). Open and closed boxes indicate the untranslated and protein-coding exons, respectively. The two mutant *ho-1* alleles are indicated. The composite enhancer, containing cadmiun-responsive element (CdRE) (83), and the proximal *cis*-acting elements (42, 64) of the *ho-1* gene are schematically shown. MARE, Maf recognition element. Also shown are heat shock element (HSE), the polymorphic (GT)n repeat, and E boxes in the *ho-1* promoter (A) and the TATA box polymorphism in the UDP-glucuronosyltransferase gene promoter (B). Note that Exon 1An represents at least seven first exons located in the upstream region from exon 1A1 of the UDP-glucuronosyltransferasegene.

similar to those observed in the HO-1-deficient mice (54, 55). The clinical and laboratory findings of this patient are detailed in the original articles (49, 92).

Here we summarize some of the invaluable lessons learned from the HO-1-deficient patient. (a) The patient lacked the spleen, which may in part account for his birth and survival for 6 years. Otherwise, the HO-1 deficiency may lead to fetal death. (b) High serum contents of heme (490 μM) were associated with undetectable levels of hemopexin and increased haptoglobin, indicating that the haptoglobin production exceeds the consumption of haptoglobin for detoxification of hemoglobin, derived from persistent hemolysis. (c) Hyperlipidemia, characterized by increased triglyceride and cholesterol, could be related to the high serum heme contents, because lipoprotein may be involved in hemin transport (36). (d) There are at least two inactive *ho-1* alleles in the Japanese population. Especially, the allele lacking exon 2 may be generated by the homologous recombination mediated by the *Alu* sequence (62), raising the possibility that this type of deletion may be relatively common in the human genome.

CO: a direct marker for heme catabolism

Under steady-state conditions, the pulmonary CO excretion rate largely reflects the rate of heme catabolism. Therefore, measurement of CO in exhaled air would be a good means to evaluate the degree of inflammation in patients with various disorders. Increased CO in exhaled air has been reported in patients with inflammatory lung diseases, such as asthma (99) and cystic fibrosis (53). Recent progresses in the physiological role of CO are discussed in an article of this issue (19).

Bilirubin IXα: a chain-breaking antioxidant

In healthy subjects, serum bilirubin concenrations are determined by its production and hepatic metabolism of bilirubin (uptake, conjugation, or biliary excretion). Unconjugated bilirubin is a nonpolar molecule and circulates as a noncovalent complex with albumin. Large population studies have shown that low serum bilirubin is a risk factor for coronary artery disease (16, 67). Unconjugated bilirubin IXα reacts with peroxyl radicals and lipid peroxides (77). In addition, bilirubin was shown to inhibit adhesion of neutrophils elicited by ischemia–reperfusion or exposure to hydrogen peroxide (15). These results suggest a role of bilirubin in the prevention of oxidative damages associated with cardiovascular diseases. It was also reported that bilirubin formed by activation of HO-2 protected neurons against oxidative stress injury (10).

Bilirubin oxidative metabolites have been established as markers for the chain-breaking antioxidative activity of bilirubin (94); bilirubin reacts with reactive oxygen species, generating tripyrroles, termed biotripyrrin-a and biotripyrrin-b. In fact, bilirubin oxidative metabolites are increased in human urine after surgery (86) and in cerebrospinal fluid of patients with Alzheimer's disease (24). These observations indicate that bilirubin is good for life.

UDP-GLUCURONOSYLTRANSFERASE

Serum unconjugated bilirubin is rapidly removed by the hepatocyte via diffusion or active transport across the sinusoidal membrane (20) (Fig. 1). Bilirubin is bound to a cytosolic protein, ligandin (glutathione *S*-transferase B), and transported to the endoplasmic reticulum, where bilirubin is conjugated with glucuronic acid by an isoform of UDP-glucuronosyltransferases, termed UDP-glucuronosyltransferase 1A1 (UGT1A1) (87). Conjugated bilirubin is then excreted into bile through the canalicular membrane by an energy-dependent concentration process. Conjugated bilirubin is rapidly converted to urobilinogen and other soluble reduction products by the action of intestinal bacteria. In the intestinal lumen, conjugated bilirubin is unstable and hydrolyzed to unconjugated bilirubin, which is reabsorbed by the intestinal mucosa to return to the liver via the portal circulation.

Glucuronidation of small lipophilic compounds by UDP-glucuronosyltransferases represents an important detoxification process of a large number of substrates, such as steroids, bilirubin, dietary constituents, and various xenobiotics. Accordingly, the UDP-glucuronosyltransferase gene, termed the *UGT1A* locus, encodes multiple UGT1A proteins (87); namely, the human *UGT1A* gene contains at least eight first exons, each of which codes for the amino-terminal half of a given UGT1A protein (Fig. 2). For example, the amino-terminal half of UGT1A1 (286 amino acids) is encoded by exon 1A1 (58). In contrast, all of the UGT1A proteins share the same carboxyl portion of 246 amino acids, which is encoded by common exons 2 to 5.

Gilbert's syndrome

Gilbert's syndrome is a common disorder (~8% of the general population), characterized by mild fluctuating hyperbilirubinemia, usually <3 mg/dl. Gilbert's syndrome is associated with either a homozygous promoter polymorphism or heterozygous missense mutations in the *UGT1A1* gene (4, 7). The promoter polymorphism is the insertion of extra TA

residues in the TATA box of the *UGT1A1* gene, giving rise to the $A(TA)_7TAA$ allele rather than the common $A(TA)_6TAA$ allele (Fig. 2). The promoter activity was shown to decrease with the increased number of (TA) repeats, suggesting that the promoter polymorphism may cause different expression levels of UGT1A1 protein (6). But this promoter polymorphism alone is not sufficient to cause hyperbilirubinemia (7). In addition, several heterozygous missense mutations associated with Gilbert's syndrome have been identified in the coding exons (25). Thus, Gilbert's syndrome is inherited as a recessive or dominant trait. Individuals affected with Gilbert's syndrome do not require treatment, and may enjoy the advantage of unconjugated hyperbilirubinemia.

Crigler–Najjar syndrome

Crigler–Najjar syndrome type I and type II are also attributable to UDP-glucuronosyltransferase deficiencies (allelic heterogeneity), and are characterized by severe and mild unconjugated hyperbilirubinemia, respectively. To date, >50 mutations have been reported (18). Mutations associated with Crigler–Najjar syndrome type I cause a premature stop codon, a shift of the reading frame, or a single substitution of a critical amino acid. Thus, these patients have no functional enzyme and require immediate liver transplantation. Crigler–Najjar syndrome type II is a relatively common disorder and is always caused by a single amino acid substitution, which only reduces the enzyme activity.

Neonatal jaundice

Virtually all human neonates, even healthy term infants, show unconjugated hyperbilirubinemia during the first several days after birth. In the United States, 60% of the 4 million newborns become clinically jaundiced each year (3). This condition is called neonatal jaundice or physiologic jaundice, but the parents are concerned with the jaundice of their babies, because of bilirubin encephalopathy, also called kernicterus and manifested as cerebral palsy. This is a reason why bilirubin has been considered as a toxic waste. It should be noted that the current tendency of early hospital discharge of neonates has resulted in a reemergence of kernicterus (60).

Neonatal jaundice is caused by three major factors characteristically seen in newborns: overproduction of bilirubin from the catabolism of fetal hemoglobin heme, insufficient activity of bilirubin UDP-glucuronosyltransferase in the newborn liver, and increased reabsorption of unconjugated bilirubin through the gastrointestinal tract. In fact, the erythrocyte life span in neonates is 70–90 days, which is significantly shorter than that in the adult (120 days). Furthermore, the activity of the intestinal β-glucuronidase is ~10 times higher in neonates than in adults (13). Birth is a first venture for newborns to survive under the air (pO_2 ~160 mm Hg), compared with the hypoxic state *in utero,* and the antioxidant activity of bilirubin may be beneficial to neonates.

INTERINDIVIDUAL VARIATION IN HO-1 EXPRESSION

The human *ho-1* gene promoter contains a (GT)n repeat (73), which is highly polymorphic, known as microsatellite polymorphism (23) (Fig. 2A). Analysis of the Japanese population revealed that the numbers of (GT)n repeats vary from 15 to 40, and two common repeats are 23 and 30 (93). This genetic marker, located on chromosome 22q12 (26, 27), should allow us to study the possible involvement of HO-1 in certain human diseases. The long (GT)n repeats may form Z-DNA and influence basal activity or inducibility of the *ho-1* gene promoter. Indeed, transient transfection assays suggest that the promoter activity decreases with increasing (GT) repeat numbers. Furthermore, long (GT)n repeats (n > 31) are associated with higher risk of emphysema caused by cigarette smoking (93). In individuals with long (GT)n repeats, the degree of HO-1 induction may not be sufficient to protect the tissue damage caused by smoking.

The (GT)n repeat polymorphism can be categorized as alleles that cause diminished or increased transcription of the human *ho-1* gene, leading to interindividual variation of HO-1 activity. In addition, the first case of HO-1 deficiency has revealed the two inactive *ho-1* alleles. It appears that the human *ho-1* gene has acquired the regulatory means to fine-tune its transcription level, thereby modulating the production of CO, iron, and bilirubin. It should be noted that the (GT)n repeat is not present at the equivalent positions in the rat and mouse *ho-1* genes. This difference may contribute at least in part to the interspecies variation in the regulation of HO-1 expression, as discussed below.

REPRESSION OF HO-1 EXPRESSION: MORE IS NOT ALWAYS BETTER

Here we focus on the repression of human *ho-1* gene expression by hypoxia, heat shock, or interferon-γ (IFN-γ). Incidentally, these factors show opposing effects on the regulation of HO-1 expression, depending on species or cell types, and are also involved in the pathogenesis of severe malaria (35, 88).

Hypoxia

We depend on oxygen for our life, but life in air is challenging. Maintenance of arterial oxygen tension depends on the intact respiratory and cardiovascular systems and healthy erythrocytes of normal hemoglobin contents. Anemia decreases the absolute amount of oxygen transported per unit volume of blood, resulting in anemic hypoxia. Hypoxia is involved in the pathogenesis of severe malaria, which is associated with anemia and impaired cerebral blood flow (cerebral malaria) (35), as discussed later.

HO-1 expression is induced by hypoxia in many cell types, including rat vascular smooth muscle cells (31, 40), Chinese hamster ovary cells (43), and human dermal fibroblasts (52). HO-1 mRNA levels in the cardiac ventricles were increased after 3 days of hypoxia (10% O_2 air) in a rat model of pulmonary hypertension *in vivo* (21). On the other hand, we observed down-regulation of HO-1 mRNA expression by hypoxia in human umbilical vascular endothelial cells, despite the induction of vascular endothelial growth factor and the functional activation of hypoxia-inducible factor-1 (45). The stability of HO-1 mRNA was not noticeably changed under hypoxic conditions (1% O_2) in human vascular endothelial

cells (45), whereas the stabilization of HO-1 mRNA is responsible for the hypoxic induction of HO-1 in human dermal fibroblasts (52). In fact, the half-life of HO-1 mRNA was ~3 h in human vascular endothelial cells (45), similar to the value measured in HeLa human uterine cervical cancer cells (79). Thus, there have been no reports showing that hypoxia activates transcription of the *ho-1* gene in human cells. Hypoxic repression of HO-1 mRNA was also observed in cultured human astrocytes and coronary artery endothelial cells (45). To our knowledge, this is the only report that shows the repression of HO-1 expression under hypoxia. Moreover, under our conditions of hypoxia, HO-1 mRNA expression is remarkably induced in rat and mouse cell lines (unpublished observations). Indeed, because the hypoxic induction of HO-1 was reported mostly in experimental animals and cultured rodent cells, there appears to be a species and cell-type difference in the mechanism sensing hypoxia or the response to hypoxia.

The heme breakdown catalyzed by HO-1 is an energy-consuming reaction, in which at least 3 mol of oxygen and 4 mol of NADPH are required to cleave 1 mol of heme. Thus, repression of HO-1 by hypoxia may reduce energy expenditure used for heme catabolism. Moreover, hypoxic repression of HO-1 may be beneficial to hypoxic cells, because CO may bind and inhibit the function of mitochondrial cytochrome oxidase or a hypothetical oxygen sensor, probably containing a heme molecule. We therefore propose that the hypoxic repression of HO-1 expression represents a defense strategy under hypoxia in certain human cell types.

Heat shock

Fever is an evolutionary conserved response in the host defense and essentially beneficial to the host. Fever in malaria (40°C or more) is known to be schizontocidal and contribute to synchronization of parasites' life cycle within erythrocytes, leading to production of the characteristic fever spikes (28). The rat *ho-1* gene promoter contains heat shock elements (HSEs) (41), the *cis*-acting element responsible for transcriptional activation of heat shock protein (HSP) genes by hyperthermia, and is transcriptionally induced by heat shock (42°C) (50, 63, 72). Thus, rat HO-1 has been established as HSP32. In theory, any reagents or conditions that are toxic or harmful to cells could increase *ho-1* gene expression in rat as a heat shock response. Consistent with this, hyperthermia was shown to lead to the remarkable induction of HO-1 mRNA and protein in the rat brain (12). On the other hand, HO-1 expression is also induced in the rat by a separate mechanism from the induction of HSP70, as seen in the brain following transient forebrain ischemia (81) and in the heart subjected to hemodynamic stress (21).

In rat cells, HO-1 mRNA levels and HO activity are increased by hemin or heat shock (72). In contrast, HO activity is not induced by heat shock in cultured cells derived from human, monkey, pig, and mouse, but is induced by hemin treatment in all the cells examined (68), indicating the interspecies difference in the regulation of HO-1 expression by heat shock. The human *ho-1* gene promoter contains a potentially functional HSE (38, 51; Fig. 2A), but there is a noticeable difference in the regulation of HO-1 expression by heat shock between human cell types. HO-1 is noninducible in many cell types (51, 78, 97), but inducible in Hep3B hepatoma cells (37). Strikingly, heat shock also inhibited the induction of HO-1 mRNA caused by cadmium or hemin (51). We have provided evidence that the sequence flanking the HSE may prevent heat-mediated activation of the human *ho-1* gene and have also suggested the involvement of the polymorphic (GT)n repeat in such a silencing function. Such a silencing effect is of particular significance in the brain, because heme degradation products are potentially toxic. If the human *ho-1* gene lacked such elements with silencing activity, HO-1 would be easily induced in the human brain under various conditions, as seen in rat brain (12). The human *ho-1* gene appears to have gained the silencer sequences to protect its harmful induction by heat shock. Such a repression mechanism may be related to the defense against malaria, a feverish infectious disease.

Cytokines

Cytokines and their receptors belong to members of host-defense proteins that show remarkable species differences in their structures between humans and rodents (44). For example, IFN-γ shows only 40% amino acid identity between the human and mouse sequences. Here we describe the effects of cytokines observed in cultured human cells. HO-1 expression is induced by interleukin-6 (IL-6) in Hep3B cells (39). In THP-1 human monocytic leukemia cells, IFN-γ plus lipopolysaccharide, a typical exogenous pyrogen, or tumor necrosis factor-α (TNF-α), an endogenous pyrogen, induces expression of HO-1, which is associated with the differentiation of THP-1 cells to macrophage-like cells (42). This type of HO-1 induction was seen only in monocyte-lineage cells.

In contrast, the expression levels of HO-1 mRNA were decreased by treatment with either IFN-γ or IL-1β but not by TNF-α in T98G glioblastoma cells (80). IFN-γ (100 U/ml) reduced the expression levels to ~10% of the control levels. IFN-γ also decreased HO-1 expression in a primary culture of human astrocytes. Pretreatment with IFN-γ also reduced the magnitude of induction of HO-1 mRNA by sodium nitroprusside, cadmium, or hemin (80). Thus, there is a difference in HO-1 expression in response to cytokines, depending on cell types. Down-regulation of HO-1 by IFN-γ may represent one protective mechanism in the brain against cell toxicity due to overproduction of heme degradation products.

HO-1 AND ITS RELEVANT DISORDERS

Cardiovascular and renal disorders

Free radical attack or oxidative stress is considered to be important in the pathogenesis of vascular diseases, such as atherosclerosis. As already discussed, bilirubin may act as antiatherogenic factor through antioxidant and vasodilatory actions (75). Prominent HO-1 expression was detected in endothelium and foam cells/macrophages in human atherosclerotic lesions (90). We have shown co-localization of HO-1 protein and bilirubin IXα in foam cells of the atherosclerotic lesions obtained from cholesterol-fed rabbits (46), suggesting that the HO-1 produced in the foam cells actually catalyzes heme breakdown.

The HO-1-deficient patient suffered from persistent proteinuria and hematuria caused by renal tubular injury, indicating an essential role of HO-1 in renal function (49, 92). HO-1 protein was detected immunologically in the renal tubules of a biopsy specimen of a patient with chronic tubulointerstitial disease due to paroxysmal nocturnal hemoglobinuria, whereas HO-1 was not detected in normal human kidneys (48). HO-1 expression is also increased in circulating endothelial cells and the kidney of patients with sickle cell disease (47) and in rat kidneys with heme protein-induced chronic renal inflammation (48). Overexpression of HO-1 may ameliorate the states of these diseases, probably through the antioxidant actions of heme degradation products. A more detailed discussion on the role of HO-1 in the kidney can be found in a comprehensive review (1).

Neurologic diseases

HO-1 and HO-2 mRNAs are expressed in various regions of the human brain (78) and in excised human brain tumors (14). In the brains of patients with Alzheimer's disease, HO-1 expression is significantly increased in both neuronal and nonneuronal cells closely associated with senile plaques and neurofibrillary tangles (57, 76). Furthermore, the level of tau protein, the major component of the intraneuronal lesions (neurofibrillary tangles) of Alzheimer's disease, was dramatically decreased in HO-1-overexpressing cells (82). In addition, HO-1 expression decreased β-amyloid peptide- and hydrogen peroxide-induced cytotoxicity in an immortalized neuronal cell line SN56 (30). β-Amyloid peptide has been implicated in generating free radicals and oxidative stress *in vitro* and *in vivo,* and plays a prominent role in the pathogenesis of cell injury and death in Alzheimer's disease. Thus, HO-1 expressed in the brains of Alzheimer's disease patients may protect neurons against oxidative stress-induced injury.

HO-1 may also be involved in the pathophysiology of Parkinson's disease and prion diseases. In patients with idiopathic Parkinson's disease, HO-1 expression was upregulated in the substantia nigra, suggesting that the affected tissue was being exposed to chronic oxidative stress (65). HO-1 was induced in mouse brains infected by scrapie, the animal phenotype of transmissible spongifrom encephalopathies (prion diseases) (9). In fact, prion protein fragment 106–126 induced HO-1 mRNA expression in cultured neurons and astroglial cells (59).

In summary, HO-1 may be involved in the pathophysiology of some nervous system diseases, which are associated with oxidative stress.

Malaria

Malaria is a world-wide protozoan infection, caused by one of four species of *Plasmodium.* Each year, 300–500 million people are infected, and 1–2 million people, especially children under 5 years of age, succumb to this infection. *Plasmodium falciparum*, which causes most malignant malaria, may have diverged from *Plasmodium reichenowi*, the causative parasite of chimpanzee malaria, ~8 million years ago, when the human lineage diverged from great apes (5). In contrast, the three other human parasites, *Plasmodia malariae*, *ovale*, and *vivax*, are remotely related to each other and to *Plasmodium falciparum.* Thus, *Plasmodium falciparum* is the most ancient foe of humans and has provided continuing selective pressure on the human genome.

Plasmodium parasites in erythrocytes digest globin portions of hemoglobin molecules for their growth but cannot efficiently utilize the heme moiety as a source of iron. Heme is therefore detoxified through polymerizing reactions and discarded into food vacuole as insoluble pigments, called hemozoin or malaria pigments. Paradoxically, large amounts of heme present in erythrocytes are not utilized by parasites, although parasites depend on iron for their growth and asexual proliferation. The tragedy in *falciparum* malaria is caused in part by the formation of knobs on the surface of parasitized erythrocytes (35). Through the knobs, parasitized erythrocytes are sequestered in the cerebral microvasculature, which may lead to coma, or cerebral malaria, in certain susceptible individuals. Parasitized erythrocytes remain attached to endothelial cells of brain capillaries, thereby escaping "death trap" in the spleen and leading to cerebral hypoxia. In this context, an adhesion molecule ICAM-1 is a receptor for retaining parasitized erythrocytes on the luminal surface, and its expression is induced in human umbilical vein endothelial cells by treatment with hemin or TNF-α (89).

In murine malaria, caused by *Plasmodium berghei,* heme catabolism is markedly enhanced, as measured by CO production (11). In another murine model of malaria caused by *Plasmodium yoelii*, HO activity was increased in the liver (61). The induction of HO-1 may reflect the pathogenesis of malaria. It should be noted, however, that there are no animal models for cerebral malaria.

The variability in pathological findings of the cases with cerebral malaria suggests that host factors may underlie the susceptibility to cerebral involvement (88). HO-1 is likely to be involved in the pathogenesis of malaria, because malaria is usually associated with intravascular hemolysis, anemia, and hyperbilirubinemia. Recently, the increased level of HO-1 protein was shown in Dürck's granuloma, a typical lesion of advanced cerebral malaria (66), but this observation is not surprising because the formation of Dürck's granuloma is preceded by hemorrhage (88). We are currently analyzing the potential association between the (GT)n polymorphism of the *ho-1* gene promoter and the susceptibility to cerebral malaria.

Pathogenesis of cerebral malaria is not fully understood, but the involvement of fever, hypoxia, and cytokines has been proposed. These factors exhibit differential effects on the regulation of HO-1 expression in various culture systems and animal models as already discussed. Such a variety may reflect the evolutionary strategy of humans against severe malaria caused by *Plasmodium falciparum.*

CONCLUSION AND PERSPECTIVES

All nucleated cells require heme for their survival. Heme must be synthesized and degraded within an individual cell, because heme cannot be recycled among different cells. Heme degradation products, bilirubin, CO, and iron, possess important physiological roles, but by themselves are cytotoxic if present at high concentrations. To cope with these universal molecules under certain selective pressure, variety

must be generated in the genes coding for relevant proteins and enzymes, leading to interindividual variations in protein functions, as well as in the regulation of gene expression. Animal models for human diseases are invaluable tools for medical research, but we must never forget the interspecies variations in the regulation of host-defense genes (44). In fact, there are no malarias in dogs, cats, cows, and horses that have high sodium erythrocytes (35).

Perhaps, many inflammatory diseases are associated with altered HO-1 expression. It may be rather difficult to find human diseases that are entirely independent of HO-1. Induction or down-regulation of HO-1 expression by pharmacological means will be a promising strategy for treatment of relevant disorders. Humans had successfully adapted to upright bipedal walking and will continue to go their ways in this and subsequent millennia by inducing or repressing HO-1.

ABBREVIATIONS

CO, carbon monoxide; HO, heme oxygenase; HSE, heat shock element; HSP, heat shock protein; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; UGT1A1, UDP-glucuronosyltransferase 1A1.

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