Heme Oxygenase-1 Deficiency

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Keywords
Heme oxygenase-1 · Oxidative stress · Asplenia · Hemolytic anemia · Endothelial injury

7.1 Case Report

A 26-month-old boy was admitted because of recurrent fever, generalized erythematous rash, and joint pain. The fever persisted, and the joint pain waxed and waned. The combination of fever, erythematous rash, and joint pain suggested that the patient suffered from a childhood chronic inflammatory illness such as systemic juvenile idiopathic arthritis (sJIA) or chronic infantile neurological cutaneous and articular syndrome (CINCA, alternatively called neonatal-onset multisystem inflammatory disease or NOMID). However, neither of the conditions could explain the unique clinical features and the extraordinary laboratory findings seen in this patient. The patient had characteristic facial features such as flat nasal bridge, frontal bossing, and prominent edema of the eyelids. Marked hepatomegaly was noted, but the spleen was absent. Unlike most of patients with asplenia, this patient did not have any form of congenital heart disease. His brother and sister were healthy. The mother had experienced two intrauterine fetal deaths.

The white cell count was 51,600/μL (normal range; 3300 to 8800), and the platelet count was 226 x 10^4/μL (normal range; 13–35). The marked leukocytosis and thrombocytosis both persisted throughout the course of the illness. He had significant anemia with erythrocyte count 1.48 x 10^6/μL (normal range; 4.30–5.50) and hemoglobin concentration 4.9 g/dL (normal range; 13.5–17.0). Peripheral blood smear showed numerous fragmented erythrocytes and erythroblasts (Fig. 7.1). Serum iron concentration was 64 μg/dL (normal range; 50–170). Lactate dehydrogenase (LDH) was 17,470 IU/L (normal range; 196–355), and aspartate aminotransferase (AST) was 442 IU/L (normal range; 9–42), but alanine aminotransferase (ALT) was within normal limits. Serum ferritin was 780 ng/mL (normal range; 26–280). Marked abnormalities were noted in parameters of both coagulation and fibrinolysis system, although he did not show apparent bleeding tendency or signs of accelerated coagulation. Fibrinogen was 109 mg/dL (normal range; 196–356), fibrin degradation product (FDP) was 300.1 μg/mL (normal range; <5), d-dimer was 186.1 μg/mL (normal range; <2.5), thrombin-

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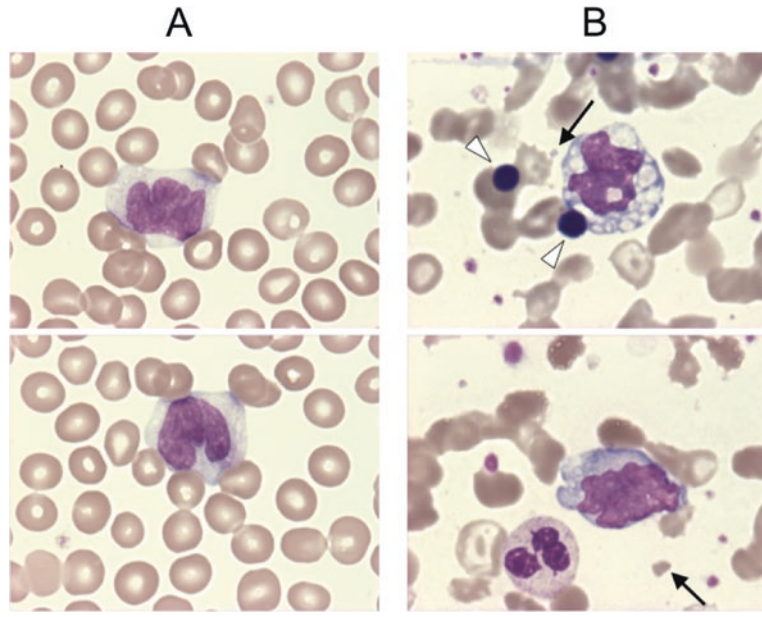

Fig. 7.1 May-Grünwald Giemsa staining of the control (**a**) and the patient (**b**) peripheral blood. Normal control smear shows intact erythrocytes and normal appearing monocytes, whereas the patient's smear shows numerous fragmented erythrocytes (arrows), erythroblasts (arrow heads), and monocytes with numerous vacuoles and basophilic cytoplasm

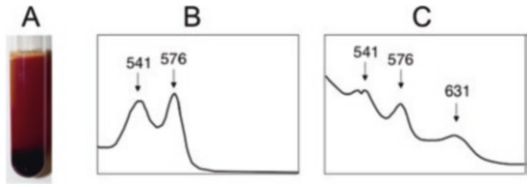

Fig. 7.2 Appearance of the centrifuged patient blood was highly specific. The hematocrit was significantly low, and the serum was turbid with dark brownish tint (**a**). Spectrophotometer analysis of the fresh hemolysate of the patient erythrocytes showed two distinct peaks of OxyHb at 541 nm and 576 nm (**b**). The serum analysis showed an additional peak of MetHb at 631 nm (**c**)

antithrombin complex was 202.2 μg/L (normal range; <3), and plasmin-α_2 plasmin inhibitor complex was 22.3 μg/mL (normal range; <0.8). Thrombomodulin was 12 ng/mL (normal range; <3.5) and von Willebrand factor was 580% (normal range; 60–170). Hyperlipidemia was another prominent finding, with triglyceride at 638 mg/dL (normal range; 32–115) and total cholesterol at 552 mg/dL (normal range; 32–115) with predominance of low-density lipoprotein cholesterol.

The appearance of his serum was peculiar. Freshly separated serum was always turbid with brownish tint (Fig. 7.2a). Hemolysate of the patient's erythrocytes showed two distinct peaks of oxyhemoglobin (OxyHb) at 541 nm and 576 nm (Fig. 7.2b). In addition to the two peaks of OxyHb, the patient's serum showed a third unique peak at 631 nm, which corresponded to methemoglobin (MetHb) (Fig. 7.2c). Both gross appearance of the serum and the absorption spectrum analysis suggested that heme in the form of OxyHb and MetHb was markedly increased in the patient's serum. These results indicated that either massive hemolysis is constantly taking place in vivo or that Hb accumulated in the serum due to defects in Hb catabolic pathway. Haptoglobin (Hp) concentration was extremely elevated at 800–1200 mg/dL (normal range; 19–170), and a large amount of the Hb-Hp complex was detected in the patient's urine sample. Repeated measurement of serum bilirubin concentration was always low at 0.1–0.3 mg/dL (normal range; 0.2–1.3). Hemopexin was undetectable by immunoelectrophoresis. Serum heme concentration was extremely high at 490 μM (normal range; <1 μM). Both direct and indirect Coombs' tests were negative on repeated occasions. Putting all these data together, it was highly suggested that there is a certain abnormality in the process of hepatic uptake of Hb-Hp complex or in heme degradation pathway (Fig. 7.3).

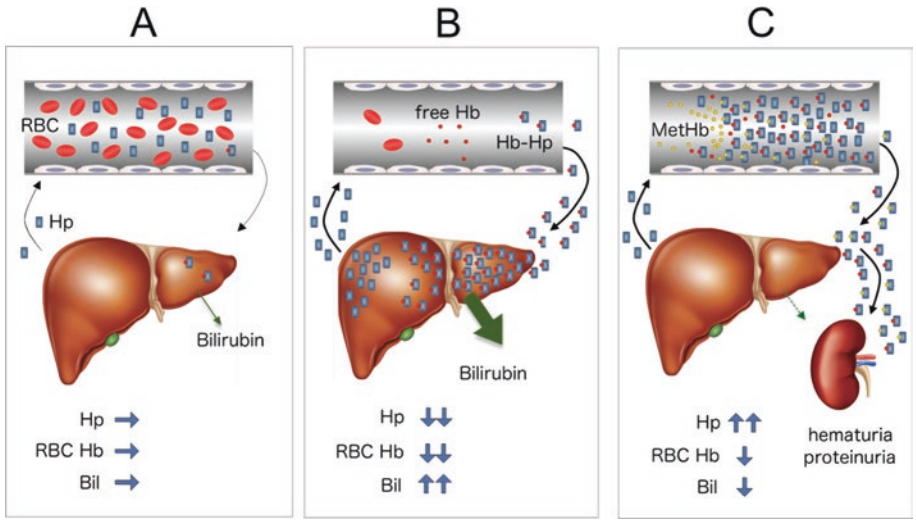

Fig. 7.3 Hepatic uptake of Hb-Hp complex and heme degradation into bilirubin. In normal condition, small amount of Hb derived from hemolysis is rapidly bound by circulating Hp, forming Hb-Hp complex, which is rapidly uptaken by the liver through specific receptors (**a**). When massive hemolysis occurs, as in the case of hemolytic anemia, large amount of free Hb is bound by serum Hp and transferred to the liver (**b**). The results are significant reduction of serum Hp and increased of indirect bilirubin derived from the heme degradation pathway. In the HO-1-deficient patient, there was accumulation of massive levels of both OxyHb and MetHb bound to Hp within the serum. Despite the apparent intravascular hemolysis, total Hp content was significantly increased, serum bilirubin level remained low, and there was overflow of Hb-Hp complex within the urine (**c**)

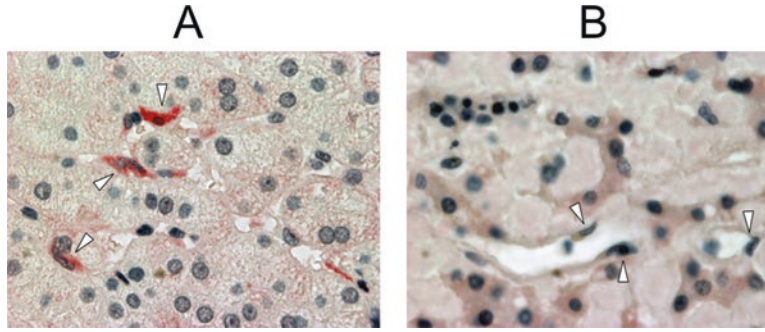

Fig. 7.4 HO-1 production by hepatic Kupffer cells. HO-1 immunostaining was performed using anti-HO-1 rabbit antiserum and alkaline phosphatase-conjugated goat anti-rabbit antibody. Alkaline phosphatase activity was visualized using Fast Red TR salt. Kupffer cells of the control liver produced significant levels of HO-1 (A, arrow heads), whereas HO-1 was not detectable within Kupffer cells of the patient liver (B, arrow heads)

Heme oxygenase (HO) is an enzyme which plays a key role in heme degradation to biliverdin. Three isoforms of HO are known, including HO-1, HO-2, and HO-3 (Maines 1988). Among these isoforms, HO-1 is peculiar in that it is rapidly induced in response to various oxidative stresses (Nath 2006). In contrast, HO-2 is a constitutive isoform that is expressed under homeostatic conditions. HO-3 is not catalytically active, but is thought to work in oxygen sensing. Immunohistochemical analysis of the liver biopsy specimen showed that Kupffer cells did not produce HO-1 in the patient's liver (Fig. 7.4). Exposure of Epstein-Barr virus-transformed lymphoblastoid cell line (LCL) derived from the patient to oxidative stress such as hemin, cad-

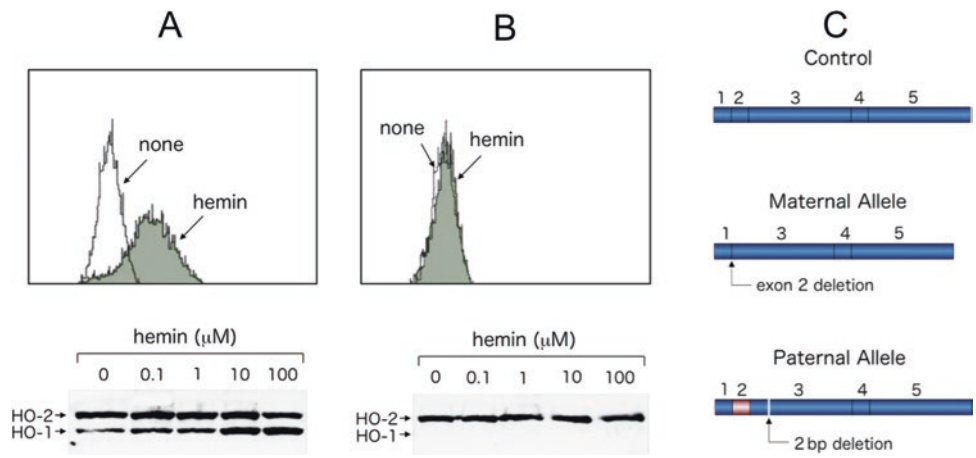

Fig. 7.5 Hemin-induced HO-1 production by LCL. LCL from control and the patient were stimulated with hemin and HO-1 production was compared by flow cytometry and immunoblotting. Large amount of HO-1 was produced dose-dependently upon hemin exposure of normal control LCL (**a**), whereas it was not induced in the patient LCL (**b**). HO-2 was constitutively produced in both control and the patient LCLs. Mutation analysis of the patient and the parents revealed that the patient had compound heterozygote mutations of HO-1 gene. Exon 2 deletion in the maternal allele and 2 bp deletion within exon 3 of the paternal allele were detected (**c**)

mium, or sodium arsenite did not induce HO-1 protein (Fig. 7.5a and b). HO-2 was constitutively expressed in both controls and the patient LCL. HO-1 gene analysis revealed that the patient had compound heterozygotes of HO-1 gene mutations (Yachie et al. 1999). The maternal allele lacked the second exon, and the paternal allele showed two base pair deletions within the third exon (Fig. 7.5c).

Pathological examination of the first case of HO-1 deficiency revealed characteristic tissue injury. Notably, cellular injury was confined to selected organs and cell types, including the kidney, the liver, circulating monocytes, and vascular endothelial cells. In the kidney, mild mesangial proliferation and thickening of the capillary loop were observed within the glomeruli (Fig. 7.6a). Electron microscopy revealed marked swelling of the endothelial cells and their detachment throughout the glomerular capillary (Fig. 7.6b). In addition to the glomerular damage, tubulointerstitial injury with tubular atrophy was significant. The liver was massively enlarged, and there was a significant amyloid accumulation resulting in marked atrophy of hepatocytes (Fig. 7.6c, d). Scattered foci of iron deposits were observed in both the kidney and the liver (Kawashima et al. 2002). Cytoplasm of the circulating monocytes was vacuolated, and monocyte surface antigens were significantly different from normal profiles, as described later.

After we reported the first case of human HO-1 deficiency, only five additional cases have been confirmed by HO-1 gene analysis (Radhakrishnan 2011a, b, and personal communication). Fever, absence of the spleen, hemolytic anemia, hematuria/proteinuria, and the absence of jaundice seem to be the common denominators of the disease. The common clinical and laboratory features are summarized in Tables 7.1 and 7.2, respectively. All cases from India (from the second to the sixth cases) had identical homozygous mutation. However, the disease onset and durations vary significantly depending on the case. Laboratory findings were characterized by marked increase of platelet. Levels of hepatic enzymes, such as LDH, AST, and ALT, were significantly elevated. Low to normal serum bilirubin and high Hp concentration in the presence of hemolysis seems to be the hallmark of the illness.

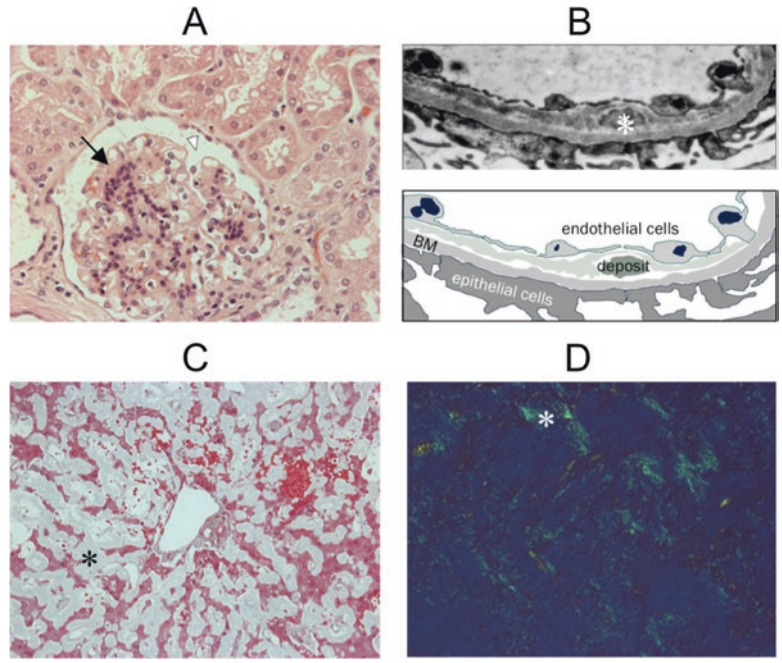

Fig. 7.6 Renal and hepatic pathology of the HO-1-deficient patient. Hematoxylin and eosin stain of the renal biopsy specimen showed mild mesangial proliferation and thickening of the capillary loop were observed within the glomeruli (**a**). Electron microscopy revealed marked swelling of the endothelial cells and their detachment throughout the glomerular capillary (**b**). The liver was massively enlarged, and there was a significant amyloid accumulation. Azan staining shows marked atrophy of hepatocytes, but fibrosis was minimum (**c**). Congo red stain shows apple-green birefringence under polarized light (**d**)

7.2 Diagnosis

Because only limited numbers of patients with HO-1 deficiency (OMIM#614034) are known, there are no established diagnostic criteria for this very rare disease. Combinations of (1) absence or hypoplasia of the spleen, (2) extremely elevated LDH and ferritin, (3) leukocytosis and thrombocytosis, and (4) hemolytic anemia without jaundice are highly suggestive of this disease. Although the age of onset among the six HO-1 deficiency cases varied, ranging from infancy to 15 years of age, laboratory data and clinical profiles were surprisingly uniform. Fever, hemolytic anemia, and hematuria/proteinuria were consistent findings. In all cases, bilirubin remained within normal range, while serum ferritin and LDH values were invariably high. Although the absence or hypoplasia of the spleen seems to be the hallmark of the illness, its functional significance in patients with HO-1 deficiency has not been determined.

Differential diagnosis includes various childhood inflammatory illnesses including sJIA and other autoinflammatory diseases such as CINCA or NOMID.

7.3 Biochemical Perspectives or Molecular Perspectives

Heme is a major component of Hb, the product of erythrocyte destruction. Heme is constantly produced in vivo and it is extremely toxic to cells (Balla et al. 2007). Therefore, constitutive mechanisms exist to cancel the toxic effect of heme. Serum Hp binds free Hb efficiently, and the Hb-Hp complex is promptly taken up by phagocytes and hepatocytes which express the recep-

Table 7.1 Clinical characteristics of 6 cases

Findings	1st case (our patient)	2nd case	3nd case	4th case	5th case	6th case
Sex	Male	Female	Male	Male	Female	Male
Onset/diagnosis	2 year/5 year	15 year/16 year	6 month/2 year	10 year/10 year	7 year/9 year	13 month/20 month
Death at	6 year	16 year	3 year	Alive	Alive	2 year
Fever	+	+	+	+	+	+
Arthralgia	+	−	−	−	−	−
Hemolytic anemia	+++	++	++	++	++	++
Jaundice	−	−	−	−	−	−
Hematuria/proteinuria	+/+	+/+	+/+	+/+	+/+	+/+
Asplenia	+	+	+	+	+	+
Prominent forehead	+	+	+	+	+	+
Hypertension	+	+	+	+	+	−
Cerebral bleeding	+	+	+	−	−	−
Growth delay	+	−	+	+	+	±

Table 7.2 Mutations and laboratory data

Findings	1st case (our patient)	2nd case	3rd case	4th case	5th case	6th case
Mutation (s)	exon2 del	R44X	R44X	R44X	R44X	R44X
HO-1 gene	2bp del (exon3)	(homo)	(homo)	(homo)	(homo)	(homo)
Mother	exon2 del	Not done	Not done	R44X/wild	R44X/wild	R44X/wild
Father	exon3;2bp del	Not done	Not done	R44X/wild	R44X/wild	R44X/wild
CRP (mg/dL; <0.4)	6.7	30.8	5.3	Normal	24.0	4.8
FDP-DD (mg/mL; <2.5)	186.1	>8	Not done	Normal	Not done	Not done
WBC ($\times 10^3/\mu L$; 3.3–8.8)	51.6	18.5	38.0	39.6	Not available	43.2
Plt ($\times 10^4/\mu L$; 13–35)	226	137	109	117	100	123
Ferritin (ng/mL; <280)	780	4912	15,530	15,358	2500	>2000
LDH (IU/L; <229)	17,470	9462	12,858	16,000	4000	21,400
AST/ALT (IU/L; <33/27)	448/74	982/149	1080/283	689/68	300/80	652/133
Bilirubin (mg/dL; <1.3)	0.2	0.64	1.2	0.3	0.4	Low

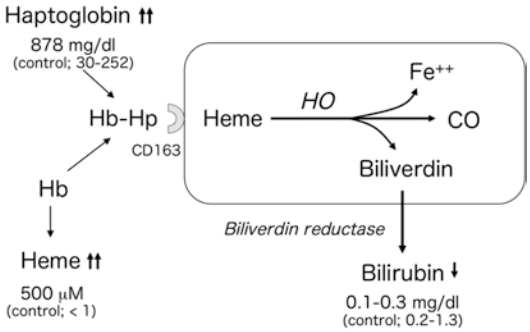

Fig. 7.7 Catabolic pathway of heme within the liver. Hb-Hp complex is uptaken by hepatocytes via its receptor, CD163. Heme is then catabolized to Fe^{++}, CO, and biliverdin by HO. All three molecules are known to exert potent antioxidative action

tors for the complex, now known as CD163 (Madsen et al. 2004).

Heme catabolic pathway within the liver is shown in Fig. 7.7. Of particular importance is the fact that HO-1 not only plays role as a catabolic enzyme of heme degradation pathway; it also induces molecules, including carbon monoxide (CO), which exerts potent antioxidative functions (Nakahira et al. 2006). HO-1 deficiency will lead to significant reduction of these molecules and subsequent cellular and tissue injury. However, there are numerous questions to be answered regarding the pathogenesis and the biochemical and molecular perspectives of HO-1 deficiency. Some of these questions are shown below.

7.3.1 Question 1: Why Only Certain Organs and Cell Types Are Damaged in HO-1 Deficiency?

Of particular interest is the fact that only selected cells or organs are damaged in HO-1 deficiency. Several mechanisms may explain this. First of all, these susceptible cells seem to be the targets of constant oxidative stress exposure, both for anatomical and functional reasons. Vascular endothelial cells are the target of shear stress and are exposed to multiple oxidative stresses, including hemolysis, pH changes, and hypoxemia for obvious reasons. In the HO-1-deficient patient, secondary accumulation of heme proteins, cholesterol, and fragmented erythrocytes further aggravates the oxidative stresses. Renal tubular cells are constantly exposed to hematuria, proteinuria, and various other excretory substances in urine. Tissue macrophages and circulating monocytes are frequently turned on to exert their scavenger functions. In addition, they function as one of the central players of innate and acquired immunity. Second, while these cells may be particularly sensitive to oxidative injury, they may serve as a high-quality sensor of oxidative stress for the susceptible organs. Upon exposure to oxi-

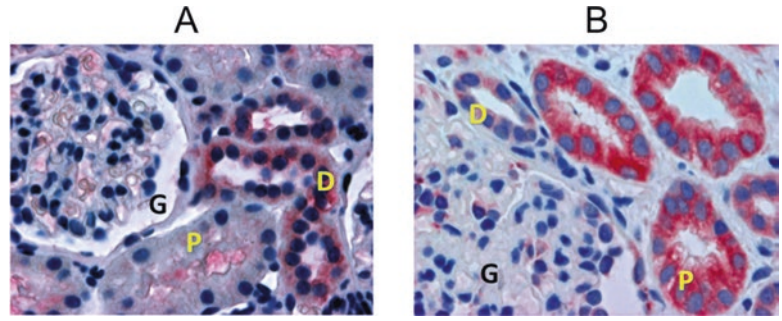

Fig. 7.8 HO-1 production by renal tubular epithelium. In control kidney (**a**), HO-1 is not detectable within the glomerulus (g) or proximal tubular epithelial cells (p). In contrast, distal tubular epithelial cells (d) constantly express significant level of HO-1. In patients with hemolytic uremic syndrome (**b**), hematuria induced high levels of HO-1 within proximal tubular epithelial cells (p), whereas glomerulus (g) produced little HO-1, and distal tubular epithelial cells (d) produced only modest level of HO-1

dative insults, these sensor cells rapidly induce antioxidative molecules. As a result, the organs are saved from critical damage. HO-1 may participate as a central molecule to neutralize the oxidative stress.

In normal rat and human kidneys, renal tubular epithelial cells express HO-1 selectively. Distal tubules express significantly more HO-1 than the proximal tubules in a normal kidney (Fig. 7.8a). However, significant level of HO-1 expression is induced within proximal tubules in patients with hematuria or proteinuria (Fig. 7.8b). Proximal tubular epithelial cells also produce significant levels of HO-1 upon various oxidative stimuli in vitro, indicating that these cells are inherently capable of responding to exogenous noxious insults (Yang et al. 2003).

7.3.2 Question 2: How Does the Hypoplasia of the Spleen Occur and What Is Its Functional Significance?

We do not know exactly why all the patients with HO-1 deficiency lacked functional spleen. One hypothesis is that HO-1 deficiency and subsequent vascular endothelial injury results in early (most likely in utero) vascular damage involving the splenic artery. Due to accumulating injured blood cells, the patients' spleens are overworked. The continuing splenic overwork may lead to the enhanced oxidative damage to the splenic vasculatures which are already vulnerable to oxidative stress. It is intriguing in this respect that hypomorphic mutation of HO-1 in mice resulted in the progressive atrophy of the spleen due to fibrotic changes in the splenic artery (Kovtunovych et al. 2010). These assumptions will remain as such until cases of HO-1 deficiency with intact splenic function are discovered.

Regardless of the mechanism leading to splenic hypoplasia, splenic dysfunction helps the survival of damaged, but still functioning, blood cells and prevents the fatal outcome. In contrast, the presence of functionally intact spleen may accelerate the destruction of injured blood cells thereby leading to fatal hemolysis and thrombocytopenia.

7.3.3 Question 3: What Is the Mechanism of Accelerated Cell Injury Seen in HO-1 Deficiency?

Direct consequences of HO-1 deficiency include extensive cell injury due to lack of the enzyme and induction of cell dysfunction, in particular, scavenger functions of macrophages. As we cannot extrapolate that these are the universal features observed in every HO-1-deficient patient, we analyzed the functional significance of HO-1

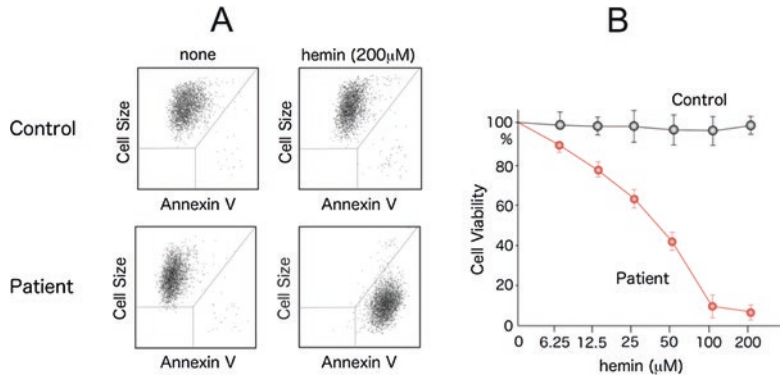

Fig. 7.9 Hemin-induced cell injury in HO-1 deficiency. LCLs from control and the patients were induced with different concentrations of heme. Apoptosis or injury of LCL was evaluated by morphological examination and flow cytometry. Apoptotic cells are identified by low cell size and high annexin V binding. Few control LCL, but most of the patient LCL underwent apoptosis with 200 μM hemin (**a**). Hemin induced rapid apoptosis or cell injury in HO-1-deficient patient in a dose-dependent fashion, whereas the cells remained intact in controls (**b**)

deficiency using the LCL derived from the patient.

LCL of the HO-1-deficient patient (HO-1-deficient LCL) was extremely sensitive to hemin-induced cellular injury (Fig.7.9). Extreme sensitivity of HO-1-deficient LCL to hemin stimulation could not be reversed by the addition of apoferritin or bilirubin. Furthermore, ferritin production by HO-1-deficient LCL was comparable with that by control LCL with or without the addition of hemin. Although ferritin and bilirubin may act as antioxidant in certain situations, they do not contribute much to the protection of cells from hemin-induced cell injury, at high hemin concentrations. LCL transfected with HO-1 gene was significantly less sensitive to hemin-induced cellular injury (Yachie et al. 1999). These results support the notion that degradation of heme by HO-1 is directly responsible for the reversal of cellular injury.

7.3.4 Question 4: What Are the Functional Defects of Macrophages?

In addition to cellular injury to the resident macrophages, the patient exhibited peculiar findings indicating a disturbance of macrophage scavenger function. Asplenia was certainly a significant contribution to the reduced scavenging function in the patient, resulting in the overload by other reticuloendothelial systems, including circulating macrophages and the hepatic Kupffer cells. The patient's peripheral blood monocytes exhibited some morphological characteristics, including prominent vacuolation and basophilic cytoplasm (Fig. 7.1b). These changes may reflect a persistent, systemic inflammatory reaction. In addition to morphological changes, the surface antigen expression by these monocytes was abnormal, i.e., expressions of HLA-DR and CD36 were significantly reduced as compared with normal monocytes (Yachie et al. 2002). CD14 expression was comparable to that in the control. The reduction in the antigen expression was constantly observed after repeated examinations, indicating that these changes reflect abnormal monocyte functions in the patient.

Consistent with the changes in the surface molecules, monocyte phagocytic functions were also impaired dramatically. Although phagocytosis of fluorescence-labeled latex beads did not change significantly, that of opsonized erythrocytes was almost completely abolished. These results indicate that reduction of surface molecules are directly related to the abolishment of receptor-mediated phagocytosis of opsonized erythrocytes by monocytes. Various hematological and biochemical abnormalities seen in the

HO-1-deficient patient, including increased Hp concentration, abundance of fragmented erythrocytes and thrombocytosis, and hyperlipidemia, may all be explained by the reduced scavenging functions of phagocytes.

7.3.5 Question 5: What Is the Role of Damaged Endothelial Cells in the Regulation of the Coagulation/Fibrinolytic System?

Another distinct feature of the first human HO-1 deficiency case was the defective endothelial function, as represented by extremely abnormal parameters of coagulation/fibrinolysis. Unlike in cases of other hematological illnesses associated with disseminated intravascular coagulation, our patient exhibited extraordinarily elevated values for thrombin-antithrombin complex, FDP, and plasmin-α_2 plasmin inhibitor complex. Paradoxically, the platelet numbers constantly increased. The data indicated that HO-1 or HO-1 products, such as CO, may be associated with the regulation of the coagulation/fibrinolytic system.

We recently demonstrated in in vitro cultures that a CO-releasing molecule suppressed TNF-α-induced upregulation of tissue factor and plasminogen activator inhibitor type 1 by human umbilical vein endothelial cells. It also suppressed mitogen-activated protein kinases and NK-κB signaling pathway activation by TNF-α. Lipopolysaccharide (LPS)-induced TNF-α production by circulating mononuclear cells was also significantly inhibited by the CO-releasing molecules (Maruyama et al. 2012). These results may explain the characteristic findings seen in the HO-1-deficient patient. At the same time, the data support the view that CO-releasing molecules may constitute a novel anticoagulative and anti-inflammatory therapy.

A summary of the macrophage activation and endothelial cell dysfunction is shown in Fig. 7.10. Lack of HO-1 resulted in unregulated activation of macrophages with excess inflammatory cytokine release. At the same time, HO-1 deficiency resulted in overproduction of tissue factor by endothelial cells and macrophages, leading to the abnormal activation of the coagulation/fibrinolysis system. The figure illustrates the role of HO-1 as an inhibitor of cytokine overproduction and endothelial cell dysfunction. It was intriguing that the HO-1-deficient patient did not run an acute catastrophic course. On the contrary, prolonged and sustained activation of macrophages, platelets, and endothelial cells led to exhaustion and dysfunction of these cells.

7.3.6 Question 6: What Is the Impact of HO-1 Deficiency on Macrophage Function?

Monocytes/macrophages are composed of at least two functionally distinct subsets, M1 and M2 (Geissmann et al. 2003). The different subsets of the monocyte/macrophage lineage differentiate in response to environmental stimuli. M1 macrophages are the "classical" macrophages, and they comprise the pro-inflammatory subset, whereas M2 macrophages are "alternatively" activated macrophages. They resolve inflammatory responses, perform scavenger functions, and promote tissue remodeling and repair. Interferon (IFN)-γ is the key cytokine driving the M1 pathway, whereas IL-4, IL-10, and steroids promote monocyte differentiation into M2 macrophages.

We reported previously that circulating monocytes produce significant levels of HO-1 during Kawasaki disease and infectious diseases, suggesting its anti-inflammatory role during these illnesses (Yachie et al. 2003). Furthermore, we investigated the profiles of cytokine mRNA expression in two subsets of circulating monocytes (Mizuno et al. 2005). In this study, freshly isolated $CD16^{high}/CCR2^{negative}$ monocytes expressed significant levels of HO-1 mRNA in vivo. They produced little IL-10 upon stimulation with LPS. In contrast, the major subset of $CD16^{low}/CCR2^{positive}$ monocytes did not express HO-1 mRNA in vivo, whereas they responded significantly to LPS and produced IL-10. The fractions of $CD16^{high}/CCR2^{negative}$ monocytes

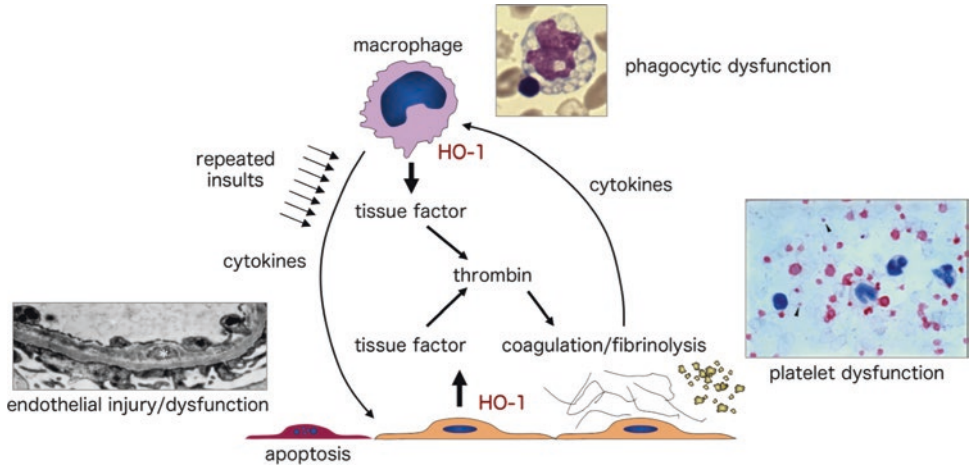

Fig. 7.10 Summary of the macrophage activation and endothelial cell dysfunction. Lack of HO-1 resulted in unregulated activation of macrophages with excess inflammatory cytokine release. At the same time, HO-1 deficiency resulted in overproduction of tissue factor by endothelial cells, leading to the abnormal activation of the coagulation/fibrinolysis system. Prolonged and sustained activation of monocytes, platelets, and endothelial cells leads to exhaustion and dysfunction of these cells.

increased during various acute inflammatory diseases, such as Kawasaki disease and influenza virus infection, suggesting that monocytes play anti-inflammatory roles through HO-1 production.

In macrophages and dendritic cells, CO reduces pro-inflammatory and increases anti-inflammatory cytokine secretion in response to LPS (Lee and Chau 2002). HO-1-mediated anti-inflammatory effects may therefore be closely linked to anti-inflammatory mechanisms, such as the suppression of the immune and inflammatory responses in macrophages via diminished antigen-presenting capacity and cytokine synthesis (Listopad et al. 2007). This is consistent with our finding in the HO-1-deficient patient, in whom the lack of HO-1 resulted in a marked rise in circulating heme and subsequent oxidative vascular and tissue injury, anemia, and chronic inflammation. Schaer et al. reported that macrophages express upregulated levels of CD163 in sepsis-induced hemophagocytic syndrome (Schaer et al. 2006). HO-1 is induced by CD163-mediated Hb-Hp complex uptake (Fig. 7.11) (Yamazaki et al. 2007). These macrophages expressed significant levels of HO-1, suggesting their role as a negative regulator of inflammation.

7.4 Therapy

No effective therapy is known. However, careful avoidance of various types of external stress, including infections, physical stresses, and medical interventions, may at least prolong the onset of the catastrophic episodes. There is no evidence to support the roles of anti-inflammatory drugs or immunosuppressive agents. Experiences with only limited numbers of HO-1-deficient patients indicate that once the onset of the inflammatory processes is triggered, it is extremely difficult to prevent the progression of the subsequent organ damage.

End-of-Chapter Questions

1. What are the common clinical findings of human HO-1 deficiency?
2. What are the common laboratory features of human HO-1 deficiency?
3. What could be the differential diagnosis of patients with HO-1 deficiency?
4. The ages of onset for each HO-1-deficient patient are significantly different. What are the possible reasons for this?

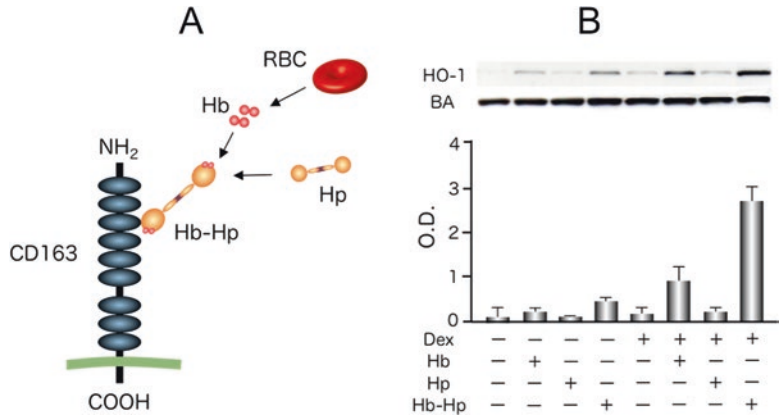

Fig. 7.11 HO-1 induction by CD163-mediated Hb-Hp complex uptake by monocytes. Hb-Hp complex binds to CD163 on monocyte surface (**a**). Binding of Hb-Hp complex efficiently induces HO-1 production by monocytes. Peripheral blood mononuclear cells were pretreated with or without dexamethasone (Dex) for 24 h. Cells were then treated with Hb, Hp, or Hb-Hp complex for 24 h. In controls, cells were cultured with growth medium for the same period. Cell lysates were prepared, and HO-1 and β-actin (BA) expression were examined by Western blotting. Intensity of each band was quantified by densitometry, and optical density (O.D.) is shown (**b**). $*p < 0.1$. $**p < 0.01$. Bars indicate standard deviations

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