EDITORIAL

ISSN 1230-025X

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Haptoglobin in the New Millenium
Haptoglobina w nowym tysiącleciu

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Abstract

Haptoglobin structure and genetics indicate its significance not only as an inflammatory biomarker, but also as an immunomodulator and important element in human physiology (reproduction) and pathology (cardiovascular disease). Haptoglobin phenotyping finds numerous applications in practical medicine. Controversy on a role of haptoglobin as a trypanosome factor, is also described (Adv. Clin. Exp. Med. 2004, 13, 1, 3–18).

Key words: haptoglobin, phenotyping, haptoglobin–hemoglobin complex, reproduction, cardiovascular disease, trypanosome factor.

Introduction

Haptoglobin (Hp) is a genetically determined \( \alpha \)-acidic glycoprotein with hemoglobin-binding capacity, present in most body fluids (serum, urine, saliva, amniotic fluid, ascites etc.) and some tissues (e.g. liver, lung, spleen) of humans and other mammals. Genetic polymorphism of Hp (three major phenotypes: 1–1, 2–1, and 2–2) has been associated with different biological functions of this protein. Profound changes in serum Hp level occur in a variety of disease states. Hp should be considered as a protein undergoing two pathophysiological phenomena: an increase in inflammation, infections of different etiology, trauma, tissue damage and malignant proliferation, while a decrease in production during severe hepatocellular deficiency (either a failure in biosynthesis or in the secretion by hepatocytes) or a decrease (as a secondary phenomenon) under hemolytic conditions. Thus changes in the measured concentrations of Hp have been used in the diagnosis and evaluation of treatment response under various pathological conditions [for reviews see 1–3].

Despite years of investigations Hp has remained a subject of interest. In accordance with the title of this article literature on Hp appearing first of all from 2000 to 2003, has been presented.

Haptoglobin Structure and Genetics

Hp consists of \( \alpha \)- and \( \beta \)-chains which in man are encoded by a single gene on chromosome 16 (16q22.3), a region extremely sensitive to genomic DNA rearrangement. Two common alleles Hp1 and Hp2 exist. The Hp gene is organized into five exons. Hp is presumably synthesized as single precursor protein that is proteolytically processed.
after translation to form the α and β subunits. The first four exons encode for the α subunit while the last exon encodes for the β subunit. The Hp2 allele arises from an internal duplication of a 7 kb DNA fragment that includes exons 3 and 4 of the Hp1 gene, resulting in the Hp2 allele having seven exons. Most likely, formation of the Hp2 allele is the result of a breakage and reunion event at non-homologous positions within the fourth and second introns of two Hp1 genes. As a consequence of this crossing-over event, exons 5 and 6 of Hp2 allele originate from exons 3 and 4, respectively, of one of these Hp1 genes. The β heavy chain has 425 amino acid residues. Two β chains linked by disulphide bridges, containing carbohydrate are identical in Hp types. Light chain disulphide bridges, containing carbohydrate are 425 amino acid residues. Two of one of these Hp1 genes. The allele originate from exons 3 and 4, respectively, of this crossing−over event, exons 5 and 6 of Hp2 homologous positions within the fourth and se−exons. Most likely, formation of the Hp2 allele is gene, resulting in the Hp2 allele having seven fragment that includes exons 3 and 4 of the Hp1 gene, arises from an internal duplication of a 7 kb DNA shows three variant polypeptides: α1F, α1S, and α2 (152 amino acids). Hp α2F and α2S differ by the Lys position 54 substituted by Glu [4, 5].

In humans, a common polymorphism of the Hp gene, characterized by alleles Hp1 and Hp2, gives rise to structurally and functionally distinct Hp phenotypes: Hp 1–1, Hp 2–1 and Hp 2–2. Moreover Hp genotyping is potentially able to discriminate among alleles Hp 1S, Hp 1F, subtype Hp 2FS (almost exclusively in Japanese populations), Hp 2SF, Hp 2SS and Hp 2FF. For Hp 1–1 and Hp 2–2 phenotypes there is one tetramer consisting of (α1−1)β2, or (α2−2)β2. However, for the Hp 2–1 phenotype there are 3 permutations, namely (α1−1)β2, (α2−2)β2 and (α2−1)α1β2 [5, 6].

Human Hp2 allele contains a 1.7-kilobase intragenic duplication that arose after a unique non-homologous duplication between the prototype Hp1 alleles. During a genetic screening of 13 000 children of survivors exposed to the atomic bomb radiation and 10 000 of control, two children suspected of carrying de novo mutations at the Hp locus were identified as mosaics comprising Hp 2–2 and Hp 2–1 cells at a ratio of 3 : 1. This Hp2 to Hp1 reversion must have occurred in early embryogenesis in somatic as well as male germ cells [7].

5’ breakpoint of the Hp0 allele was described as being located 5.2 kilobase (kb) upstream of exon 1 of the Hp and the 3’ breakpoint was positioned between 52 and 53 base pair upstream of exon 5 of the Hp related gene. Hp0 alleles 8, 16 and 17 were found in Koreans (1/1500), Japanese (1/4000) and Chinese (1/1000) but not in Africans and European-Africans. Hp0 allele occurs more often than that of IgA deficiency in Japanese. More attention should be paid on Hp deficiency and anti Hp antibody as the cause of transfusion-related anaphylactic reactions in Asian population. The risk to produce anti Hp antibody by blood transfusion exists [8]. A case of a patient suffering from anaphylactic shock after transfusion, was described. Anhaptoglobinemia owing to homozygosity of the Hp0 allele was diagnosed. Patients with haptoglobin deficiency associated with haptoglobin IgG antibodies, who experienced severe anaphylactic nonhemolytic transfusion reactions, have been identified in Japan [9]. Washed platelet concentrates and washed red blood cells were useful in preventing transfusion-related anaphylactic reactions [10].

The mature Hp is a glycoprotein that is 16% carbohydrate. The carbohydrate component is N-linked complex consisting of N-acetylgalcosamine, mannose, galactose, fucose and sialic acid on the β chains at positions 23, 46, 50 and 80 [5]. About 75% of charged Hp glycans are of biantennary complex structure (some of them lack one terminal sialic acid molecule). Biantennary structures make up almost 25% and highly branched tetrasialylated do not exceed 1%. In Hp 2–2 difference in trisialylated oligosaccharide with one 2→3 linked sialic acid residue was found. In the congenital disorder of glycosylation some alterations were found in the relative content of monosialo-, bisialylated glycans [11]. Hp is known to be abnormally glycosylated in different diseases. For example in streptozotocin-induced diabetic rats a significant increase in β chain of Hp and a decrease of α1-acid glycoprotein were observed both in concentration and glycosylation [12].

Out of four potential sites of N-linked oligosaccharides in Hp β chain two of them exist near the binding sites to free heme and the site for inhibition of lipid peroxidation. Aberrant glycosylation of Hp might decrease the capacity of radical scavenger, leading to accumulation of free radicals. A relationship between the glycosylation of Hp and the suppression of hepatoma development in transgenic mouse, was suggested [13].

Heterogeneity of Hp gene was discovered with a novel insertion/deletion (I/D) polymorphism of 7 base pairs in intron 4 of the Hp1 allele and introns 4 and 6 of the Hp2 allele. Low-density lipoprotein-cholesterol and C-reactive protein concentrations and the apolipoprotein A1/A2 ratios differed significantly between Hp D/1 genotypes [4].

Analysis of human genomic clones led to the isolation of an haptoglobin-related protein (Hpr) sequence and to a demonstration of its structural homology to the Hp gene. The two genes are closely linked, with Hpr being 2.2 kb downstream of Hp. The sequence of Hp and Hpr are highly homologous, although the colinearity is interrupted by an Alu sequence inserted in the 5′ flanking region and by a retrovirus-like sequence inserted in the first intron of Hpr [14].

Transcription of the rat gene encoding Hp is highly induced during acute phase response, which
has been mediated by inducible STAT3 member of the Signal Transducer and Activators of Transcription (STATs) family proteins. Under normal conditions a member of these proteins STAT5b is expressed and binds to the hormone regulatory element of the rat Hp gene. Nuclear accumulation and binding of inducible STAT3 proteins to the regulatory element following turpentine treatment implicates that STAT5b negatively regulates Hp gene expression during normal conditions [15].

The nuclear matrix is an extensive proteinaceous structural foundation of the nucleus, participating in many nuclear activities e.g. nuclear signalling and compartmentalization and in genome organization. Thus it serves as a base to which chromatin loops are anchored through specific matrix attachment regions on the DNA in the course of tissue-specific activation of genes. At these points the DNA is presumably organized by transcription factors and nuclear matrix proteins. Two major DNA-binding nuclear matrix proteins were detected: a DNA sequence-specific 32 kD isoform of transcription factor C/EBPβ, that bind the Hp rat gene hormone-responsive cis-element and p55 and lamins A and C that bound to DNA non-specifically [16].

Matrix assisted laser desorption/ionization-quadrupole ion trap-time of flight mass spectrometry sequencing resolved structures of unidentified peptides obtained by in-gel tryptic digestion of Hp α, chain from human plasma proteomes. Previously unassigned sequences (amino acids 65–87, 40–54, 99–113) were determined [17].

IL-6 is considered to be the most important regulator of the Hp gene in human hepatocytes. Within 180 bp upstream from the transcriptional start site in the 5-flanking region of the Hp gene, there exist three IL-6-responsive regions (HaptoA, HaptoB, HaptoC) that interact with the CCAAT/enhancer-binding protein (C/EBP), a transcription factor. Among the members of the C/EBP family, the expression of both β and δ is dramatically increased in IL-6 treated hepatocytes and they strongly bind the A and C regions. Hp is up-regulated by all-trans-retinoic acid (ATRA) in a human monocytic cell line. Treatment with ATRA increased C/EBPα and β expression, but decreased that of δ. C/EBPα is involved in the activation of Hp gene expression by ATRA in human monocytic cells [18]. ATRA is a potent Hp-inducer in these cells through a signal pathway different from hepatocytes [19].

Intestinal epithelial cells are an integral component of the mucosal immune system and participate in an acute phase response. Sodium butyrate and Trichostatin A attenuated the IL-1-dependent induction of Hp gene as well as C/EBPβ and δ transcription factors, containing complexes binding to the HaptoA. Deacetylase inhibitors may down-regulate IL-1-dependent induction of Hp and C/EBP isoforms that represent a target for the action of butyrate in the control of acute phase response in intestinal epithelial cells [20].

Molecular chaperones are defined as proteins that bind and stabilize an otherwise unstable conformer of another protein and by doing so facilitate the correct folding of this protein in vivo. A key activity is its ability to protect proteins from aggregation under stress conditions. Human Hp is known to have chaperone-like activity in preventing thermally-induced aggregation of proteins. The presence of at least two different chaperone-binding sites on each Hp β chain was found. The chaperone-like activity decreases until a 1:1 molar ratio of Hp to hemoglobin (Hb) is reached (one chaperone-binding site is blocked). Hp prevents aggregation of non-native structures by providing appropriately placed hydrophobic surfaces [21].

Histamine deficiency suppresses murine Hp production and modifies hepatic protein tyrosine phosphorylation. This reaction was significantly decreased in histidine decarboxylase deficient mice, but was largely diminished if the animals were kept on a histamine-rich diet. It looks like lower Hp production is diet inducible [22].

Increase in adipose mass results in obesity and modulation of several factors in white adipose tissue (WAT). Tumor necrosis factor (TNF) α and leptin are upregulated in adipose tissue in obesity. Hp expression in WAT is increased in obesity in rodents and TNF α is an important signal for this regulation [23].

**Haptoglobin-Hemoglobin Complex**

Hp combines rapidly and irreversibly with hemoglobin (Hb). The binding is one of the strongest known non-covalent interactions in biology, the association constant being greater than 10^{21} mol/l. The Hp-Hb complex in plasma is rapidly cleared by the reticuloendothelial system in the liver. Hb binding is apparently not an essential function of Hp, since the condition of ahaptoglobinemia is clinically silent. The high affinity of Hp for Hb and the toxicity of Hb suggest important physiological functions of Hp during pathological conditions where free Hb is produced. Presence of specific receptors on liver parenchymal cells, that endocytose the Hp-Hb complex had led to conclusions that a major function of the complex formation is the hepatic clearance and degradation of free Hb. During severe intravascular hemolysis or transfusion of Hb solution there is Hb precipitation in the renal tissues associated with acute renal failure and
a concomittant depletion of Hp. Thus Hp-retards the passage of Hb through the glomeruli into the renal tubular cells [5].

Formation of the Hp-Hb complex in vivo can play a role in preventing the Hb-driven generation of hydroxyl radical and lipid peroxidation in areas of inflammation. On the other hand, Hp may bind analogous iron-binding proteins of microbial origin and thus provide a microbicidal mechanism to augment the destruction of intracellular pathogens. The complex was found to display an inhibitory effect on the endothelium-derived relaxing factor – nitric oxide.

Hp and the complex Hp-Hb being too large to be filtered in the glomeruli, is catabolized by the mononuclear phagocyte system in liver and spleen. A molecular mechanism of Hb for uptake in the renal proximal tubule involves the endocytic receptors megalin and cubulin [24]. Beside Hp, hemopexin binds with high affinity Hb and heme. Both the proteins are important factors modulating renal excretion of Hb and its targeting to the reticuloendothelial system. Thus plasma levels of Hp and hemopexin can affect the sensitivity of the kidney to Hb overload [25].

During intravascular hemolysis Hb binds to Hp leading to endocytosis of the complex by the macrophage receptor CD163 [101].

The toxicity of free Hb and heme is due to their ability to mediate hydroxyl-radical generation through the release of iron. In the absence of superoxide dismutase and catalase ferrous (Fe^{2+}), ferric (Fe^{3+}), and ferryl (Fe^{4+}) hemoglobin are formed with further formation of prostaglandin-like compounds (F_{2}-isoprostanes) potent vasoconstrictors. Hp-Hb complex reduces tissue oxidative damage by Hb [5].

The vasoconstrictive activity of Hb is a major problem in the development of Hb-based artificial blood. Hp may be important in reducing this activity, probably through its antioxidant effects on low-density lipoprotein oxidation. Association of Hp and hemolytic diseases such as sickle cell anemia and malaria strongly suggest that Hp-Hb interactions are physiologically important.

Iron as a cofactor for a vast number of enzymes is an essential, growth-limiting nutrient for bacteria. *Staphylococcus aureus* is an extremely adaptable pathogen causing a wide variety of infections. Staphylococcal surface proteins that directly interact with host extracellular proteins greatly contribute to virulence. A novel member of the family of Gram-positive anchor protein – staphylococcal receptor A was found to bind Hp and Hp-Hb complex. Expression of this receptor was strictly controlled by iron through the iron-dependent transcriptional regulator. This receptor belongs to virulence factors with a function related to iron acquisition [26].

Hb induces expression of a receptor on *Candida albicans* for fibronectin and other extracellular matrix proteins by ferric, ferrous and cobalt-protoporphyrin derivatives. Hp specifically abrogates this response, indicating that interaction of Hb with the fungal cell surface initiates a signal transduction pathway that leads to receptor expression [27, 28].

Heme compounds are an important source of iron for pathogenic neisseriae. The product of a gene *hemO* for *Neisseria meningitidis* is essential for heme, hemoglobin and Hp-Hb util-ization [29].

During surgery for major trauma serum Hp concentration decreased after blood transfusion of 1000 ml, or more whole blood with a mean storage time of 12.2 days. Free Hb was detected after transfusion of 2 l, when total Hp concentration decreased to 1000 mg l^{-1}. Serum Hp concentration correlated negatively with storage time of transfused blood. Transfusion of old blood might decrease serum Hp, which increases free Hb [30].

Patients with postrenal transplant erythrocytosis (frequent complication) were treated with angiotensin-converting enzyme inhibitors. Progressive and significant fall in Hb and hematocrit, erythropoietin was observed. Indices of red blood destruction including Hp and bilirubin were unaffected by the inhibitors as was creatininemia and kalemia [31].

### Haptoglobin as a Ligand of Immune Cells

Hp has an important biological function in host defence against infections and inflammation, acting as a natural antagonist for receptor-ligand activation of the immune system.

There is evidence for immunomodulatory potential of Hp. It was shown to dampen neutrophil metabolism, monocyte and macrophage functions and to interfere with T- and B-cell proliferation. Hp binds to monocytes, granulocytes, natural killer cells, and subsets of T and B lymphocytes that involves the CD11b/CD18 receptor. Specific interaction of Hp with human mast cells line HMC-1 via a receptor different from CD11b/CD18 and CD22, may play a role in the modulation of mast cell functions [32].

The receptor for the Hp-Hb complex, leading to endocytosis has been identified as the acute phase-regulated and signal-inducing monocyte/macrophage protein CD163 [27]. Fab antibody specifically inhibited the binding of Hp-Hb complexes to CD163 (no binding for Hp or Hb) and blocked...
their uptake in CD163-transfected cells. This provides a new potential tool for measuring and removal of Hp-Hb complexes from plasma/serum [33].

Different subsets of T helper lymphocytes (Th1, Th2) are responsible for induction and regulation of cellular and humoral responses. IL-2 and interferon-γ (IFN-γ) are produced by Th1 cells and favour cell-mediated responses, whereas IL-4, -5, -10, -13 are produced by Th2 cells and mediate predominantly humoral and eosinophilic responses. Hp has been reported to be potent immunosuppressor of lymphocyte function. Hp inhibits phytohemagglutinin-induced blastogenesis of lymphocytes and suppresses T-lymphocyte response to lipopolysaccharide-induced acute phase. Hp specifically interacts with both resting and activated CD4+ and CD8+ T cells. This specific binding results in a strong suppression of induced T-cell proliferation. Hp exhibits a strong in vivo inhibitory effect on Th2 cytokine release. The presence of Hp promotes a dominant Th1 cellular response over Th2 activation. This points to the role of Hp in balancing immune responses [34].

Alterations in the immune system of patients suffering from major depression, particularly in the acute one (inflammatory reactions) have been reported. The monocyte-macrophage system has received the most attention. In spite of differences in immune pattern between patients with melancholic depression compared to non-melancholic patients, Hp serum levels and IL-1β production were unchanged in the both groups [35].

Reproduction

Latter 5 years attention has been concentrated on the role of Hp in the reproductive process, when immune system, inflammation and angiogenesis are significantly involved in ovulation and implantation. Moreover, gynecological disorders, especially endometriosis have been studied.

Hp by virtue of its immunomodulatory properties, could be a regulatory factor during reproduction. The intensity of the β chain of Hp from decidual graviditatis was significantly higher than from non-pregnant endometrium in the proliferative phase and in the secretory phase. Hp in the uterus may exert different functions such as the binding of Hb, but could also be involved in the multi-factorial mechanism protecting the fetus from a maternal allograft-like immune response [36]. Involvement of Hp in reproduction was described in experiments in the outcome of in vitro fertilization and embryo transfer [37].

“Haptoglobin-like” protein (40 kD) in bovine oviductal fluid (N-terminal region sharing 81% identity with the β subunit of bovine Hp) was localized in the lumina of blood vessels and in the extracellular matrix of ovarian and oviductal lumen. Within the ovary this protein was in the vascular granulosa cells and follicular fluid of antral follicles but not in the theca cells or preantral follicles. “Hp-like” protein contributes to ovarian follicular development and oviductal function [38].

Endometriosis is a gynecological disorder defined as ectopic growth of endometrial glands and stroma. The disease process initiates with endometrial sloughing at menses, with subsequent retrograde flow of endometrial fragments through the fallopian tubes and into the peritoneal cavity. Retrograde menstruation occurs in most women with patent fallopian tubes. Menstrual debris contains normal, apoptotic and necrotic cells, increased expression of metalloproteinases, disorder expression of filamentous action from cell borders. Macrophage activation causes synthesis and release of cytokines and growth factors that may regulate immune response [39].

Through 1998–1999 it has been shown that an acidic glycoprotein synthesized and secreted by endometriotic lesions (called ENDO-1) shared significant homology with the β chain of rat, mouse, and human Hp (99.4% homology) [40, 41]. During the secretory stage ENDO-1 mRNA expression by endometriotic lesions and ectopic endometrium from women with disease was 19-fold greater than by peritoneum, 28 than endometrium, 37 than ectopic endometrium regardless of cycle stage [42]. ENDO-1 appeared as possibly associated with localized angiogenesis and altered immune response involved with the etiology/pathophysiology of endometriosis.

Peritoneal endometriotic tissues synthesize and secrete Hp, which has an analogous nucleotide sequence to hepatic haptoglobin found in serum. Apparent molecular weight of peritoneal Hp (pHp) was 3 kD smaller than serum Hp. 3 kD variance was due to carbohydrate content – one N-glycan chain – variation in the ratios of α(2–3) to α(2–6) sialic acid and fucose residues. Recombinant pHp was 100-fold over-expressed with a similar glycosylation pattern to pHp albeit in an unprocessed α-β Hp polypeptide form. Differences between pHp and serum Hp glycosylation which likely impart pHp with known immunomodulatory functions may be used as epitopes for development of immune based therapeutics for novel, nonsurgical management of endometriosis [43].

Treatment with human recombinant IL-6 and dexamethasone significantly increased Hp production by peritoneal and peritoneal endometriotic cells in a dose- and time-dependent manner. Endometriotic lesions synthesize and secrete a unique
form of Hp that is up-regulated by IL-6. Hp adheres to peritoneal macrophages, decreases adhesion, which may influence phagocytic function; and up-regulates IL-6 production. Hence a feed-forward loop is proposed which in turn increases endometriotic Hp and promotes establishment of endometriosis [39, 44].

Oxidative stress is a component of the inflammatory reaction associated with endometriosis. Retrograde menstruation is likely to carry highly pro-oxidant factors like hem and iron, into peritoneal cavity, as well as apoptotic endometrial cells which are well known inducers of oxidative stress. Reactive oxygen species may be involved in endometriosis-associated infertility and may play a role in the regulation of the expression of genes encoding immunoregulators, cytokines and cell adhesion molecules implicated in the pathogenesis of endometriosis [45].

Heme may be involved in the pathogenesis and/or development of endometriosis. However, the heme oxygenase (HO) system, might be insufficient to detoxify heme in endometriosis. HO-1 and -2 were strongly expressed in ectopic endometrium, especially in red lesions [46].

The activity of lecithin-cholesterol acyltransferase (LCAT; E.C.2.3.1.43) is involved in the removal of cholesterol excess from peripheral cells. This activity is stimulated by the high density apolipoproteins A1 (ApoA1). Hp was previously found to be associated with ApoA1 in ovarian follicular fluid. When isolated LCAT was incubated with fractions containing different Hp/ApoA1 ratios, the enzyme activity was negatively correlated with the ratio. This suggests that Hp inhibits the reverse transport of cholesterol by preventing ApoA1 stimulation of LCAT activity [47].

In the ovarian follicle the granulosa cells play an important steroidogenic role. Increased Hp penetration into the preovulatory follicle, such as that triggered by enhanced concentration in plasma or altered permeability of the blood-follicle barrier, might interfere with the release of estrogen esters and the elimination of cholesterol excess from the granulosa-lutein cells. It is possible that, should Hp severely inhibit the reverse cholesterol transport, free cholesterol would accumulate at levels that might be toxic for the granulosa cell. This may be of interest in the study of human fertilization and assisted reproduction technologies.

11β-aryl substituted 17α-acetoxy progesterone analogue (CDB-2914) antagonized exogenous and endogenous progesterone-stimulated uterine Hp synthesis and secretion in immature and adult mated rabbits, respectively. CDB-2914 is a potent, orally active anti-progestin with weak anti-glucocorticoid activity. Inhibited implantation in adult rats and rabbits demonstrated its potential as a post-coital anti-fertility contraceptive drug [48].

Cardiovascular Disease

A characteristic function of Hp is to bind Hb to form a stable complex Hp-Hb and thereby prevent Hb-induced oxidative tissue damage. Clearance of the Hp-Hb complex (but not free Hp or Hb) is mediated by the monocyte-macrophage scavenger receptor CD163. CD163 expression is highly increased by IL-6, -10 and glucocorticoids, whereas lipopolysaccharide and IFN-γ down regulate the expression. This specific receptor-ligand interaction explains the depletion of circulating Hp in individuals with increased intravascular hemolysis. Moreover, the CD163-mediated endocytosis may represent a major pathway for uptake of iron in the tissue macrophages. The biological role of CD163 might be related: the clearance of Hb and a potential immunoregulatory (anti-inflammatory) function. It might be possible to use CD163 Hb-receptor as a gateway for gene-drug targeting of macrophages in regions with tissue damage e.g. atherosclerotic plaques [49, 50]. Soluble plasma form of CD163 is potentially valuable in monitoring patients with infections and myelomonocytic leukemia [51].

Severe impairment exists in the ability of Hp to prevent oxidation mediated by glycosylated Hb. Specific interaction between diabetes, cardiovascular disease, and Hp genotype is the result of the heightened urgency of rapidly clearing glycosylated Hp-Hb complexes from the subendothelial space before they can oxidatively modify low-density lipoprotein (LDL) to those atherogenic oxidized [52]. Lipoprotein A an atherogenic particle resembles a LDL in which apolipoprotein A is linked to apolipoprotein B-100 by a disulphide bond. Its synthesis concurrently increases with acute phase proteins, especially those with a high sialic acid content [53].

The altered levels of von Willebrand factor, plasminogen activator inhibitor 1, tissue plasminogen activator in rheumatoid arthritis (RA) patients with cardiovascular disease progression indicates a status of hypofibrinolysis. Higher levels of erythrocyte sedimentation rate and Hp may reflect the importance of inflammatory process for the development of cardiovascular disease in RA [54].

During cardiac bypass surgery in fractions antegradely flushed coronary cardioplegia solution by balloon-cuffed catheter from coronary sinus in angiographically extensive or minor coronary disease was found 40 kD protein which demonstrated complete homology with β chain of Hp. Its
concentration was markedly increased in perfusates from atherosclerotic coronary arteries. Results of this study may permit identification of of diverse plasma-detectable markers of atherosclerosis, and the subsequent non-invasive evaluation of in vivo arterial pathology [56].

Chronic changes in blood flow induce structural remodeling of the arterial wall to normalize shear stress. Structural remodeling is an important determinant of luminal narrowing after balloon angioplasty and in de novo atherosclerosis. Arterial expression of Hp is increased during arterial remodeling after sustained flow changes and plays an important role in cell migration and arterial restructuring. In the liver Hp expression is mostly regulated by IL-6. In the artery, shear stress and nitric oxide (NO) inflammation IL-6 expression. NO synthesis is involved in the regulation of arterial wall Hp expression after sustained flow changes [56].

Collagen turnover and cell migration are fundamental aspects of arterial restructuring. Expression of Hp mRNA was found in adventitial fibroblasts of rabbit arteries. Stimulation of Hp expression in mice fibroblasts stimulated migration of wild-type fibroblasts. This new function of Hp may be explained by facilitating cell migration through accumulation of a temporary gelatin matrix. Hp is highly expressed in arterial tissue and is involved in arterial restructuring. This new Hp function may also apply to other functional and pathological restructuring processes such as angiogenesis, tissue repair and tumor cell invasion. Hp in experiments with rats was locally expressed in arthritic and oncological tissues. Hp was also increased in the arthritic Achilles tendon, in the arthritic ankle, in tumours of kidney, tumour and stromal cells that are recognized for enhanced cell migration and matrix turnover. Arterial restructuring determines the degree of lumen loss in pathological arterial processes like restenosis and atherosclerosis and is the major determinant of arterial shrinkage after balloon dilatation. In experiments with rabbits arterial Hp expression was increased early after balloon dilatation whereas liver Hp did not change. Arterial Hp consists of an unique set of glycoforms (the reactivities to the specific lectins as compared to liver Hp) [57–59].

Restenosis occurs in 16 to 50% patients who undergo percutaneous transluminal coronary angioplasty (PTCA), thereby severely limiting its longterm benefits. Oxidative stress plays a major role in the pathogenesis of restenosis after PTCA. Pathophysiological implications with respect to the role of Hb-mediated oxidant injury exist Antioxidant therapy designed to prevent Hb driven oxidative tissue damage may be of benefit in reducing the incidence of restenosis after PTCA [60].

Hp phenotype was determined in the development of restenosis who underwent stent implantation followed by repeat angiography with quantitative coronary angiography. No significant differences were found between patients (diabetic and non diabetic) segregated by phenotype with respect to clinical, procedural and angiographic factors. However, the risk of developing restenosis was greater in subjects with Hp 2–2 phenotype. This may help in managing diabetic patients with coronary artery disease [61].

Haptoglobin Phenotyping – Applications in Practical Medicine

The occurrence of polymorphism of the haptoglobin locus motivated many investigations directed at the determination of possible associations between Hp phenotype and different disorders. Identification of persons with high risk for a disease on the basis of Hp phenotype could have been a rather stimulating goal of preventive medicine. Several functional differences between Hp phenotypes have been demonstrated, which suggest to have important biological and clinical consequences. Criticism of this type of associations are directed at the selection and validity of the appropriate control group (ethnicity, fertility, geographic origin etc.) [2, 62–65]. Some examples, especially those from 2000–2003 follow.

Involvement of Hp phenotypes in spontaneous human reproduction (Hp 1–1 phenotype individuals would reproduce at earlier age and have higher natural fertility potential than subjects with other phenotypes) was indicated [62]. In the experiment in vitro with fertilization and embryo transfer it was shown higher pregnancy rate in the patients with the Hp 2–2 phenotype (38%) than in those with Hp 2–1 phenotype (16%). Serum Hp α2 subunit levels in the patients who achieved pregnancy within four attempts at the experiment were higher than in the others [37].

There exists a growing body of evidence that diabetic vascular disease develops only in those patients who are genetically susceptible. Diabetic individuals homozygous for the Hp2 allele were at 5-fold greater risk of cardiovascular disease compared with diabetic individuals homozygous for the Hp1 allele. The rate of clearance of Hp 1–1 complex with Hb by CD163 (monocyte-macrophage scavenger receptor) was markedly greater than that of Hb-Hp 2–2 [52].

The coronary artery collateral circulation is the chief determinant of the size of a myocardial
infarction. Functional allelic polymorphism in the Hp gene is correlated with a number of diabetic vascular complications. Hp phenotype appears to be associated with the development of the coronary collateral circulation in diabetic patients with coronary artery disease. Hp 2–2 may predispose to less compensation for coronary artery stenosis in diabetic patients, and thereby portended a worse prognosis [66].

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Functional allelic polymorphism in Hp predicts which patients with diabetes mellitus develop vascular complications such as diabetic nephropathy, and an increased incidence of restenosis after percutaneous transluminal coronary angioplasty (PTCA). Patients who were homozygous for the 2–2 phenotype were more likely to develop restenosis, than patients with 2–1 phenotype. The latter were more likely to develop restenosis than patients with 1–1 phenotype. Hp phenotype was found to be a significant predictor of restenosis after angioplasty [60]. Hp 2–2 in diabetic patients was highly predictive of adverse cardiac events, particularly myocardial infarction and death in the 1-year period after PTCA. This may be useful in the evaluation of new therapies to reduce cardiovascular risk after PTCA [67].

In the longitudinal study of coronary heart disease mortality it was found rather surprisingly that Hp 1–1 subjects were at doubled risk for the coronary heart disease mortality [67].

Hp 1–1 phenotype was reported to have a reduced risk for the development of diabetic microangiopathies in the Israeli study [69]. Contrary, in Japanese study Hp1 allele had not a protective effect in the development of retinopathy and nephropathy, and was not directly associated with an increased risk for the development of microangiopathy [70].

Obstructive sleep apnea syndrome is associated with a marked increase in the risk for cardiovascular disease. Significant interaction effect of Hp 2–2 and the presence of sleep apnea patients (younger than 55 years) on the cardiovascular disease was 2.32-fold higher than with Hp 2–1 [71].

Smokers with Hp 2–2 phenotype had significantly higher levels of total- and LDL-cholesterol while levels of HDL-cholesterol and ferroxidase activity were significantly lower than in nonsmokers. Therefore inheritance of Hp 2–2 predispose to more oxidative stress and cardiovascular disease [72].

Kawasaki disease (KD) is an acute febrile illness characterized by multiple clinical and biochemical features of inflammation and the most common complications of coronary artery abnormality. May cause sudden death from cardiac disease during the convalescent stage. Patients with Hp 2–1 have patterns of delayed or incomplete presentation of clinical symptoms, The late diagnosis of KD is associated with Hp phenotype. Confirming Hp phenotype of patients with suspected KD may assist physicians in the early diagnosis and more effective treatment to prevent cardiac complications [73].

In humans the iron status is influenced by environmental and genetic factors. Among them Hp has been shown to affect iron turnover. In males the Hp 2–2 phenotype was associated with higher serum iron, transferrin saturation and ferritin concentrations than Hp 1–1 and 2–1, whereas soluble transferrin receptor concentrations were lower. Serum ferritin correlated with monocyte L-ferritin content, which was also highest in the male Hp 2–2 subgroup. In vitro monocyte-macrophages took up a small fraction of 125I-labeled Hb complexes to Hp 2–2 but not to Hp 1–1 or 2–1, probably because of an yet undescribed iron delocalization pathway, selectively occurring in Hp 2–2 subjects [74, 75]. However, iron status in black persons was not influenced by Hp polymorphism [76].

Hereditary hemochromatosis is an autosomal recessive genetic disorder of iron metabolism. The disease is usually due to a mutation in the HLA-linked hemochromatosis gene (HFE) on chromosome 6, that leads to a change from cysteine to tyrosine at position 282 in the HFE protein. In 2001 was described overrepresentation of Hp 2–2 type among C282Y homozygous hemochromatic patients. Iron overload was more pronounced in male patients carrying Hp 2–2. It was suggested the Hp polymorphism affects iron metabolism in hereditary hemochromatosis [77, 78].

However, results of the above cited papers of 2000–2001 [74, 75] were impaired in 2002–2003 with conclusion that the effect of Hp type on iron homeostasis could not account for the marked phenotypic variation in patients homozygous for the HFE C282Y mutation [79]. Moreover, the study of 265 control subjects and 173 subjects homozygous for HFE C282Y, showed that Hp 2–2 frequencies did not differ in control subjects and C282Y homozygotes. Thus, Hp 2–2 was not a risk factor for disease development in hereditary hemochromatosis. Also transferrin saturation and
serum ferritin concentrations did not vary with Hp type [80].

The CD4 cell counts, determined by flow cytometry from peripheral blood mononuclear cells were severely diminished in seropositive patients with the Hp2–2 phenotype. In contrast, the CD4 cell counts for patients with the Hp0 phenotype remained relatively high. The Hp2–2 phenotype is associated with poor outcome in HIV-1 infection, whereas the Hp0 phenotype is associated with a better prognosis once the patient is infected with HIV-1. Haptoglobin polymorphism plays a significant role in HIV-1 infection and transmission [81]. Hp0 may have a protective effect in HIV-1 infection. Hp0 individuals in Ghana showed a highly significant reduced risk for HIV-1 infection [82].

One of the major side effects of the combination therapy for chronic hepatitis C is ribavirin induced hemolytic anemia. This disease is influenced by the pretreatment platelet level, the administered amount of interferon α and the Hp phenotype. The drop in hemoglobin level was higher in the patients carrying the Hp 1–1 phenotype as compared with Hp 2–1 and 2–2. Before starting high-dose interferon treatment in patients with low platelet counts the test should be performed. If the Hp phenotype demonstrates an additional risk (e.g. Hp 1–1) to develop anemia, the clinician can decide to give a lower interferon dose. Careful search for the minimal dose of ribavirin is suggested [83].

A correlation exists between iron metabolism and calcium, phosphate, and magnesium turnover. In particular, iron availability can play a fundamental role in bone metabolism and iron depletion can lead to bone demineralization, especially that advancing age of women, a prolonged period of amenorhoea and BMI are risk factors for osteoporosis. In search for a genetic marker of iron disposal as a risk factor for post-menopausal osteoporosis Hp was chosen because of its role in haptoglobin metabolism. Only about 5% of daily iron turnover comes from intestinal absorption, most of it coming from Hb turnover, which requires hemoxygenase, Hp, and hemoglobin. It was shown that presence of Hp genotype 1–1 is an important risk factor for postmenopausal osteoporosis. This may be useful in clinical practice to identify in advance the women who will probably develop osteoporosis and so allow primary prevention of the disease and reduce the social cost of its consequences [84].

The increase in plasma Hp level during dietary Mg deficiency depends on the increase in Hp mRNA level in the liver. This increase in mRNA is not only directly correlated with a low Mg level in plasma, but also indirectly correlated with cytokines (IL-1β and IL-6) [85].

Hp 1–1 was significantly more prevalent among the Plasmodium falciparum malaria patients than among healthy controls, whereas Hp 2–2 and 2–1 were underrepresented. It is suggested that Hp 1–1 is associated with susceptibility to P. falciparum malaria in general, and to the development of severe disease in particular [86].

Some of the above investigations clearly show a significant association between Hp phenotypes and human physiopathology, while others provide contradictory results. For the time being, such inconclusive reports indicate that the Hp phenotype has little if any practical use in clinical science.

Haptoglobin as an Inflammatory Marker

Overall reference range for serum Hp was 0.12–2.0 g/l as proposed by the IFCC Committee on Plasma Proteins [87, 88]. Hp belongs to acute phase proteins (APP). Its level is increased up to 3–8 fold in response to injury, i.e. surgery and burns, bacterial or parasitic infections, ischaemic necrosis, connective tissue diseases, chemical irritants, malignancy [1, 2].

Transcriptional process of the rat Hp gene during acute phase response was mediated by the liver nucleoprotein p29 homologous to the HMG-1 chromatin-associated protein. HMG-1 binding sites in the rat Hp gene cis-regulatory subelements A and C revealed an increase in its DNA-binding. This increase could be consequence of release of HMG-1 from the chromatin and subsequent increase in its nuclear amount [89].

Acute inflammatory response under the stress of thermal injury in rats assessed increased serum levels of IL-1, IL-6, TNF, Hp, and α2-macroglobulin. However, appearance and time-course of particular mediators were different e.g. IL-6 one hour after thermal injury up to day 1, Hp and TNF after 12 h rising further on days 1 and 3 [90].

The acute inflammatory response that is observed after infection is characterized by vasodilation, increased vascular permeability, neutrophil recruitment and activation and fever. The thermal component of fever is poorly understood aspect of inflammation. BALB/c and C57BL/6 mice were exposed to mild, long-duration whole-body hyperthermia (WBH). WBH alone did not affect serum concentrations of TNF-α, IL-6, IL-1β, α2-acid glycoprotein and Hp. However, when WBH was applied after administration of lipopolysaccharide cytokines were increased in both strains whereas acute phase proteins only in BALB/c on. In experiments with LPS-treated peritoneal cells decreased cytokine production was observed [91].

In a multivariate analysis model the value of
57 different proteins in serum and malignant and nonmalignant ascitic fluids were determined. Discriminant analysis showed that 5 ascitic fluid measurements (total protein, lactate dehydrogenase, TNF-α, C4, Hp) were sufficient for a model to correctly classify 89% of cases. These parameters could be extremely useful in the clinical setting [92].

Markers of experimental acute inflammation (Hp, C-reactive protein – CRP, fibrinogen) were evaluated in serum/plasma of the Wistar Han rats. CRP levels peaked at 25–40 h to approximately 120% of control, Hp levels increased maximally at 48 h (426% of control). Serum Hp was shown to be the most sensitive and useful marker of acute inflammation [93].

Acute phase response (Hp, IL-6 and serum amyloid A – SAA) elicited by Actinobacillus pleuropneumoniae its reduction on treatment with various antibiotics was studied in serum from specific pathogen free pigs. These effects can be applied to monitor therapeutic effects of antimicrobial drugs in pigs and could add valuable information about the stage of infection during a disease outbreak [94]. Bovine model with respiratory syncytial virus, analysing the induction of two most dominant bovine acute phase proteins Hp and SAA may find application in veterinary. The magnitude and the duration of the Hp response was found to correlate well with the severity of clinical signs (fever) and with the extent of lung consolidation while SAA responded most rapidly to infection [95]. The parallel development of inflammation and increased concentrations of serum amyloid A and Hp in milk from lactating cows inoculated with Streptococcus uberis points these proteins as potential diagnostic factors for the early detection of mastitis [96].

Mg deficit is related to various diseases, including heart disease and diabetes mellitus. The increase in plasma Hp level during dietary Mg deficiency depends on the increase in Hp mRNA level in the liver. This increase is directly correlated with a low Mg level in plasma and indirectly with IL-1β and IL-6 [85].

Over 100 structurally diverse chemicals (hyperlipidemic drugs, plasticizers, fungicides, herbicides, industrial solvents) induce acute increases in peroxisomes as well as increase synthesis of fatty acid oxidation enzymes, in rodent hepatocytes – peroxisome proliferators (PP). Activation of receptor by different PPs leads to dysregulation of hepatic APP gene expression in rats and mice (alterations in cytokine signalling networks). APP genes including Hp, ceruloplasmin, β-fibrinogen, α1-acid glycoprotein were upregulated in WY-14643 induced tumors but downregulated in the livers of rats [97].

Hyporetinemia (serum retinol 0.70 mmol/l) may be a consequence of chronic inflammation during malarial infections. In the study of children in Papua New Guinea with Plasmodium falciparum (PF) malaria acute phase proteins (transhyretin, α-cid glycoprotein, C-reactive protein, Hp) were found to be significantly correlated with splenomegalia. Hp concentration was reduced because of clearing free Hb from recent hemolysis. The rate of hypohaptoglobinemia was mild in comparison with studies on acute malarial infections [98]. The severity of anaemia associated with acute (PF) malaria (haemolysis, bone-marrow suppression, iron deficiency). Haemolysis with higher plasma concentrations of unconjugated bilirubin, lower Hp and Hb is the prime cause of the anaemia but pre-existent iron deficiency aggravates the severity of the anaemia [99]. Extent of oxidative stress in erythrocytes of patients with acute (PF) malaria erythrocyte thiobarbituric acid-reactive (ETBAR) substance (the indicator of lipid peroxidation), and intracellular, membrane and extracellular antioxidants were estimated. A significant correlation existed between ETBAR and the haemolytic indices such as Hb plasma unconjugated bilirubin and Hp concentrations. Hb and Hp correlated inversely, bilirubin directly with ETBAR. Erythrocyte membrane lipid peroxidation may be a major contributor for haemolysis and anaemia [100].

**Trypanosome Factor**

The African trypanosome Trypanosoma brucei Rhodesiense (TbR) causes human sleeping sickness, whereas the morphologically indistinguishable Trypanosoma brucei brucei (Tbb) cannot infect humans but causes the bovine disease called Nagana.

Trypanosome lytic factor (TLF) provides innate protection for humans against infection by Tbb but not against TbR. TLF exists in two forms: TLF-1 (a 500 kD lipoprotein which contains apolipoprotein A-I (apo A-I), haptoglobin-related protein (Hpr), trace amounts of paraoxygenase, apo A-II and Hp; TLF-2 – an approximately 1000 kD protein complex, contains IgM, apo A-I, other proteins and Hpr, no lipid. Numerous studies identified the lytic factor of human serum as Hpr that might be necessary to allow receptor mediated uptake of the lytic particle into trypanosome. Hpr was thought to be responsible for TLF-mediated toxicity of Tbb.

There were two models of TLF-1 mediated lysis:

1. Hpr causes lysosomal disruption by inducing lipid peroxidation of lysosomal membranes
(TLF-1 killing requires high intracellular H₂O₂ concentrations, polyunsaturated lipid and an iron source). Low temperature binding studies reveal two receptors for TLF-1. Hpr mediates the high affinity binding of TLF-1 to Tbb through a haptoglobin-like receptor.

2. Hpr is conformationally altered by an intracellular disulphide isomerase activity following uptake and prior to delivery to the lysosome to become directly membranolytic.

Hpr and Apo A-I are largely resistant to intracellular degradation. Glycosylation of TLF-1 lipoproteins could also provide protection as glycosylation is thought to protect mammalian lysosomal-associated membrane proteins from lysosomal proteolysis. Comparison with TLF-1 reduced in vitro into its α- and β-subunits suggests that the 36 kD band represents the β-subunit of Hpr. Significance of Hpr reduction for TLF toxicity may possibly play a direct role in TLF-1 mediated lysis. TLF-1 is a powerful toxin against Tbb, yet circulates as a normal human blood component without detriment to humans [101–104]. Recent studies showed the existence of a trypanosome lipoprotein scavenger receptor which facilitates the endocytosis of lipoproteins exhibiting selective lipid uptake including TLF-1. This receptor may constitute the major pathway of the parasite mediating the uptake of essential lipids [105].

There is no detectable trypanolytic activity in wild-type mouse serum and expression of the Hpr protein in the transgenic mice did not generate any trypanolytic activity in the serum. Human Hpr protein in mice is mainly associated with lipoproteins, but its expression is insufficient to reproduce this activity in mice. Differences in the assembly of Hpr into HDL could abolish trypanosome killing by altering either the interaction of Hpr with the trypanosome cell surface receptor or directly destroying the trypanolytic activity of the protein [106].

The resistance of Tbr to lysis by normal human serum is conferred by a gene that encodes a truncated form of the variant surface glycoprotein in lysosome-termed serum resistance associated protein (SRA) with amino-terminal α-helix. Probably apolipoprotein L-I (apo L-I) is the trypanosome lytic factor, and SRA confers resistance to lysis by interaction with apo L-I in the lysosome, presumably through a coiled-coil protein-protein interaction [107].

Recent advances in molecular biology and development of modern spheres of science (e.g. proteomics and genomics) and new biotechnologies (e.g. cDNA microarray technology) have made it possible to identify low picomoles levels of Hp present in biological materials by such methods as sequence homology search, peptide mass fingerprinting techniques, capillary zone electrophoresis, agarose gel isoelectric focusing [108, 109]. Currently, the most common procedures involved for the purification of human Hp are: two-dimensional electrophoresis, hemoglobin- or a monoclonal antibody-affinity column chromatography and multiple high performance liquid chromatography [110–112].

Numerous associations between Hp phenotypes and different disorders described for dozens of times suggest broad significance in clinical medicine in spite of negative results obtained in some studies. Hp biological activities discovered through more than 50 years have represented delusive richness. Hp seems to function in association with the immune system i.e. to protect the host against all the dangers of an acute phase reaction. Is this the general purpose or design that Hp is serving in living organisms? “That is the (teleological) question”. Future studies on the response will be continued.

References


Haptoglobin in the New Millenium


Haptoglobin in the New Millennium


