Haptoglobin, inflammation and disease

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Summary Haptoglobin is an acute phase protein that scavenges haemoglobin in the event of intravascular or extravascular haemolysis. The protein exists in humans as three main phenotypes, Hp1-1, Hp2-2 and Hp2-1. Accumulated data on the protein’s function has established its strong association with diseases that have inflammatory causes. These include parasitic (malaria), infectious (HIV, tuberculosis) and non-infectious diseases (diabetes, cardiovascular disease and obesity) among others. Phenotype-dependent poor disease outcomes have been linked with the Hp2-2 phenotype. The present review brings this association into perspective by looking at the functions of the protein and how defects in these functions associated with the Hp2 allele affect disease outcome. A model is provided to explain the mechanism, which appears to be largely immunomodulatory.

1. Introduction

Haptoglobin (Hp) is a unique acute phase protein that primarily scavenges haemoglobin (Hb) released into the circulation by haemolysis or normal red blood cell (RBC) turnover. The protein was first discovered by Polonovski and Jayle (1938) while Smithies determined the polymorphism in the protein (Smithies and Walker, 1955). The plasma concentration of Hp increases several fold in the event of an inflammatory stimulus such as infection, injury or malignancy, whether local (vascular) or systemic (extravascular). IL-6, produced through the activities of the primary cytokines TNF-α and IL-1, is the major inducer of the expression of the protein (Oliviero et al., 1987).

In human populations three common phenotypes are represented: Hp1-1, Hp2-2 and the heterozygous Hp2-1, which is determined by two alleles HP1 and HP2 (Smithies and Walker, 1955). The HP2 allele contains a partial duplication of approximately 1.7 kb from an unequal crossing over event between HP1 alleles (Maeda et al., 1984). The Hp gene transcript yields α and β protein subunits interconnected by disulfide bridges after post-translational processing. Inherited variations in the α subunit give rise to the alleles that define the common phenotypes (Bowman and Kurosky, 1982). A less common phenotype is Hp2-1 modified (Hp2-1M), which has been observed in Africans and African-Americans and results from an A-61C mutation in the promoter region of the Hp gene (Maeda, 1991). This mutation causes decreased gene expression in subjects with the Hp2-1M phenotype. The mutation is also present in the Hp0 phenotype, characterized by significantly reduced (hypohaptoglobinemia) or null expression (ahaptoglobinemia) of the protein (Grant and Maeda, 1993; Teye et al., 2003).
Southeast Asian populations, the Hp0 phenotype arises from a deletion in the promoter region of the Hp gene that gives rise to the HPdel allele (Koda et al., 1998). Subjects homozygous for the HPdel allele are ahaptoglobinaemic whilst those heterozygous are hypohaptoglobinaemic.

Hp has been reported to be involved in modulating the immune response, autoimmune diseases, and major inflammatory disorders (Delanghe et al., 1999; Eaton et al., 1982; Levy, 2003; Oh et al., 1990; Quaye et al., 2006). These observations appear to stem from its inherent antioxidant property, its unique role in binding Hb and its ability to stimulate cells of the monocyte/macrophage lineage. This review summarizes current knowledge of the role of Hp in relation to inflammation and major diseases with inflammatory causes. An attempt is made to examine these relationships in terms of cause (the biochemical activities of the protein) and effect (the disease that results from dysregulation of these activities).

2. Hp tissue expression

Human Hp cDNA was originally cloned from hepatocytes, where Hp gene expression is most abundant (Yang et al., 1983). Currently, the transcript has been detected in many tissues, including white adipose tissue, brown adipose tissue, ovary, lungs, testis, arteries and placenta (Friedrichs et al., 1995; Kalmodvarin et al., 1991; Yang et al., 1995).

In humans, the induction of Hp gene expression is mediated primarily by IL-6, which is the common cytokine mediator for Hp gene expression in the liver of all species studied. Three IL-6-responsive regulatory regions are present on the Hp gene promoter, A (-157), B (-111) and C (-61), which together constitute the binding site for hormones released during the acute phase response (Baumann et al., 1999). The binding of hormones and cytokines to the regulatory regions promotes the transcription of the Hp gene and increases the synthesis of the protein.

3. Hp function

3.1. Antioxidant/anti-inflammatory role

Under conditions of compromised oxygen supply, such as occurs in injury, infection or malignancy, oxygen species with free unpaired electrons are generated during mitochondrial electron transport. Referred to as highly reactive oxygen species (ROS), their production causes damage to cell membranes and macromolecules (lipids, proteins and DNA) (Valko et al., 2007). In addition, cells of the immune system generating ROS also produce reactive nitrogen species (RNS), for example peroxynitrite (ONOO-) by the interaction between nitric oxide and superoxide, which are equally damaging to cell membranes and DNA.

Cellular non-enzymatic antioxidants, which include vitamin C, vitamin E, glutathione and others, maintain cellular redox homeostasis in order to prevent or control cellular damage in the event of an inflammatory response. Hp possesses an innate phenotype-dependent antioxidant activity that exceeds by far that of vitamin C (ascorbic acid) (Tseng et al., 2004) and contributes significantly to this process, particularly at extravascular sites. Both in vitro and in vivo studies have established that subjects with the Hp1-1 phenotype are more likely to resist cellular oxidative stress than those with the Hp2-2 phenotype, with Hp2-1 being intermediate (Tseng et al., 2004). The specific antioxidant functions of the protein at extravascular sites are: activation of neutrophils; maintenance of reverse cholesterol transport; inhibition of cyclooxygenase (COX) and lipoxygenase (LOX); and other antioxidant activities, and are described below.

3.1.1. Activation of neutrophils

During exercise, injury, trauma or infection, the target cells secrete the primary pro-inflammatory cytokines IL-1β and TNF-α, which send signals to adjacent vascular cells to initiate activation of endothelial cells and recruitment of neutrophils. The activated neutrophils, which are first in the line of defence, then aid in the recruitment of other inflammatory cells following extravasation (Kushner, 1993). Neutrophils engage in generating ROS as well as recruiting other defence cells, particularly monocytes and macrophages. Hp is synthesized during granulocyte differentiation and stored for release when neutrophils are activated (Theilgaard-Monch et al., 2006). Neutrophil activation can occur in the absence of patent injury (during exercise) (Quindry et al., 2003). By quenching the respiratory burst, Hp assists in mitigating potential damage to the surrounding tissues. In a recent study on ischaemia/reperfusion injury and myocardial infarct size in mice, it was shown that the Hp2 allele was associated with a higher level of lipid peroxidation products than the Hp1 allele, which also boosted the expression of the antioxidant cytokine IL-10 (Blum et al., 2007). The Hp2-2 phenotype has consistently been observed to be a risk factor in inflammatory diseases (Delanghe et al., 1999; Levy, 2004; Papp et al., 2007), attributed to its compromised antioxidant role compared to the Hp1 allele.

3.1.2. Maintenance of reverse cholesterol transport

Elevated levels of low density lipoproteins (LDL) constitute a major risk for atherosclerosis. Oxidized LDL readily infiltrates into the arterial intima, where it is endocytosed by macrophages and eventually leads to formation of foam cells and fatty streaks (Hovingh et al., 2005). On the other hand, high density lipoproteins (HDL) play a key role in reverse cholesterol transport, together with the enzyme lecithin cholesterol acyl-transferase (LCAT). HDL in the form of Apo A-1 stimulates LCAT and serves as the acceptor for the esterified cholesterol/phospholipid released by the enzyme. In this way, Apo A-1 sustains cholesterol transport for degradation in the liver, preventing its accumulation in macrophages to form foam cells. In a recent report, it was shown that Hp binds to Apo A-1 to protect it from free radical-mediated damage and also prevents HDL from forming adducts with other lipoprotein molecules (Salvatore et al., 2007). This has an important health benefit in cardiovascular disease.

3.1.3. Inhibition of COX and LOX

Arachidonic acid is the substrate for the synthesis of leukotrienes and prostaglandins (PGE) by the enzymes LOX and COX, respectively. COX is made up of two isoforms, COX1 that is constitutively expressed and COX2 that is induced by stimuli including inflammation and hypoxia. Over-expression
of COX2 leads to synthesis of PGE and its metabolites, thromboxane (vasoconstrictor) and prostacyclin (vasodilator). Leukotrienes and prostaglandins participate in immunomodulation, upregulation of apoptotic genes, tissue growth and repair, among others. LOX is also known to participate in oxidizing LDL, which can lead to the generation of lipid peroxides (Palmer and Henrich, 1995). Hp has been shown to inhibit both COX and LOX (using commercial Hp in guinea pigs), which provides a means to modulate responses to inflammation or infection that may be harmful to tissues (Saeed et al., 2007).

3.1.4. Other antioxidant activities
As a molecular chaperone, Hp inhibits the inappropriate self-association of proteins induced by oxidation or heat. This function puts Hp alongside clusterin as major extracellular proteins that protect against protein misfolding, assisting in the preservation of cell function (Yerbury et al., 2005). Hp is persistently elevated in pregnancy, myocardial infarction and obesity (Berkova et al., 2001; Blum et al., 2007; Chieffini et al., 2004). These conditions are characterized by tissue growth and repair that require anti-inflammatory responses. Hp performs this role both as a ligand for Mac-1 (complement receptor type 3, CD11b/CD18) and a required factor for cell migration (de Kleijn et al., 2002) and angiogenesis (Cid et al., 1993). Tissue repair and regeneration are accomplished by collagen turnover and the movement of fibroblasts within the extracellular matrix. Hp inhibits the activities of the matrix metalloproteinases (MMP-2, MMP-9) required for the breakdown of gelatin and directly promotes the migration of fibroblasts needed for tissue regeneration (de Kleijn et al., 2002). At extravascular sites, Hp undertakes significant anti-inflammatory activities that are important for the maintenance of redox homeostasis while augmenting tissue repair (de Kleijn et al., 2002).

3.2. Hb binding
Hp is critical for life, providing cells with oxygen for their energy needs. However, outside of the confines of the erythrocytes (RBCs), Hb is highly toxic (Alayash, 2004). Its prosthetic group, haem, is lipophilic and readily intercalates into cell membranes to disrupt the lipid bilayers. Iron present in haem catalyzes the generation of ROS through the Fenton and Haber-Weiss reactions (Figure 1) (Sadrzadeh et al., 1984). Additionally, Hb avidly binds to nitric oxide, depleting the cell of a major modulator of vascular tone, resulting in changes in vasomotor constriction and endothelial damage (Minneci et al., 2005). The presence of free Hb is therefore a danger signal that is very rapidly attenuated by mechanisms that enable rapid and efficient removal of the protein in circulation or at the site of injury. Hp binds Hb to prevent Hb-related oxidative damage and also limits the release of haem, which exacerbates oxidative stress. Hb may therefore act as a secondary modulator of the anti-inflammatory role of Hp.

In general, Hb removal from the circulation following intravascular and extravascular haemolysis is accomplished by three major mechanisms: high affinity binding of Hb to Hp; high affinity binding of haem to hemopexin (Hpx); and low affinity binding of Hb to CD163. The mechanisms involving Hpx and CD163 appear to function during massive or localized haemolysis, where Hp concentration is overwhelmed or limited, and this review will focus on Hp-Hb interactions.

Both Hp and Hb are made up of α/β-dimers but interaction to form the Hp-Hb complex occurs mostly through the β chain of Hb and the β chain of Hp (Ettrich et al., 2002). Equimolar amounts of Hp bind to Hb, although Hp is normally present in serum in a >400 molar excess compared to free Hb (Bowman and Kurosky, 1982). After forming a Hp-Hb complex, the scavenger receptor CD163 expressed on the surface of monocytes and tissue macrophages enables the endocytosis of the complex, which is followed by degradation in the liver and spleen (Kristiansen et al., 2001). Both the Hp binding capacity (HBC) and affinity of the Hp-Hb complex for CD163 are phenotype dependent (Kristiansen et al., 2001). The HBC measures the capacity of an Hp phenotype to protect against a haemolytic episode (Hp-related cell damage and release of haem). This activity, which is ranked in the order Hp1-1>Hp2-1>Hp2-2, partially explains the ability of the phenotypes to prevent Hb-related oxidative stress (Saeed et al., 2007).

3.3. Immunomodulation
In general, an immune response may be classified as innate or adaptive (Chaplin, 2006). Innate immunity predominantly carries out the post-injury response, while the innate and adaptive arms are integrated in the post-infection response (Medzhitov and Janeway, 2000). In immune modulation, cells of both arms of the immune system, upon contact with danger signals, stimulate the generation of anti-inflammatory and pro-inflammatory factors to repair injured tissue or eliminate an infection, while ensuring that local cellular damage is minimized (Matzinger, 2002; Shi et al., 2003). The effectors of the immune response are cellular (phagocytes and dendritic cells) and humoral (cytokines, complement, acute phase proteins and leukotrienes) (Pillay et al., 2007). Hp has numerous roles in both the cellular and humoral activities of the innate and adaptive systems, including prostaglandin synthesis, leukocyte recruitment and migration, the generation of cytokine patterns following injury and infection, and tissue repair.

In the event of an injury or trauma, the acute inflammatory response leads to the recruitment of neutrophils as the first line of defence, through activated endothelial cells and platelets (Pillay et al., 2007). The endothelial cells activate COX-2, which synthesizes prostaglandin E2, increasing vasodilation and so enabling neutrophil migration. Simultaneously, platelets increase expression of chemokines, platelet derived growth factor (PDGF), TGF-β and cytokines (IL-1, TNF-α) for the recruitment of additional neutrophils as well as monocytes and macrophages in the chronic phase.
move into the lymph nodes to present antigens to naïve cells are subsequently activated to mature by mast cells and (AGEs), membrane components, uric acid and ROS (Valko et al., 2007; Bianchi, 2007) or infectious signals such as cell extravascular sites, immature dendritic cells interact with antigens or danger signals. Danger signals can be biomolecules as monocytes and macrophages are attracted to the site of injury (Kim et al., 1995).

During the repair phase, macrophages express the chemo-attractants PDGF, MCP-1, TNF-α, IL-1 and IL-6. These stimulate the recruitment of more macrophages and fibroblasts to contain the inflammation and begin the wound healing process (Eming et al., 2007). Increased secretion of IL-6 and TNF-α further enhances Hp expression and augments its activities, which also include inhibition of gelatin deposition and a direct effect on fibroblast migration needed for wound healing and repair (Gabay, 2006). Lymphocytes are the final arm of the cellular immune response that contribute to achieving wound repair with limited cellular damage. Specifically, the secretion of cytokines by CD4+ T\(_h\)1 (pro-inflammatory) and T\(_h\)2 (anti-inflammatory) cells is orchestrated to achieve efficient and effective wound healing. A high T\(_h\)1 response could cause cellular damage, while a high T\(_h\)2 response could lead to tissue fibrosis or, in some cases, allergy (Meneghin and Hogaboam, 2007). Thus proper T\(_h\)1/T\(_h\)2 balance is an important mechanism for ensuring effective elimination of injury or an infecting organism while maintaining excellent tissue regeneration.

Hp is an established suppressor of T cell proliferation, exhibiting strong inhibition of T\(_h\)2 cytokine release and weak inhibition of T\(_h\)1 cytokine release (Arredouani et al., 2003). This immunomodulatory role has been found to be phenotype dependent. In a recent publication it was shown that macrophages, activated by phagocytosis of Hp2-2-Hb complex through CD163, shift the T helper cell response towards T\(_h\)1 cytokines, while those activated by Hp1-1-Hb complex generate T\(_h\)2 cytokines with an increased IL-6 and IL-10 cytokine release (Guetta et al., 2007). The balance of the T cell response may be particularly important at extravascular sites, where the local expression of Hp would play a role in reducing damage to the surrounding tissues. At extravascular sites, immature dendritic cells interact with antigens or danger signals. Danger signals can be biomolecular metabolites, including advanced glycation end products (AGEs), membrane components, uric acid and ROS (Valko et al., 2007; Bianchi, 2007) or infectious signals such as cell wall components of bacteria and single stranded and double stranded RNA of viruses (Eisenacher et al., 2008). Dendritic cells are subsequently activated to mature by mast cells and move into the lymph nodes to present antigens to naïve T cells, which differentiate to induce T\(_h\)2 or T\(_h\)1 cytokine responses (Wen et al., 2008). The instruction signals from mast cells tend towards T\(_h\)2 cytokine responses. Hp acting as a ligand for mast cells (El-Ghmati et al., 2002) may have a significant role in the dendritic cell activation process. It appears that the default cytokine profile stimulated by Hp is T\(_h\)2. However, the evolution of the HP\(^2\) allele seems to have modulated this function by shifting the cytokine profile towards a T\(_h\)1 dominant phenotype. Such an important evolutionary function must have been acquired following severe environmental challenges that have continued to sustain the selection of the HP\(^2\) allele.

4. Inflammation and Hp

Inflammation describes the processes involved in the disturbance of tissue homeostasis as a result of acute or chronic stimuli from an infection, stress, autoimmune reaction or mechanical injury (Gabay, 2006). The homeostatic immune surveillance is largely mediated by polymorphonuclear leucocytes (PMN), with the disturbance eliciting PMN migration through the T\(_h\)1/T\(_h\)2 cytokine profile (Kato and Kitagawa, 2006). Hp actively participates in all the processes from PMN recruitment and free radical quenching, to tissue repair and regeneration. Not surprisingly, the reduction or absence of Hp protein, as occurs in hypohaptoglobinemia or ahaphtoglobinemia, is associated with allergic (skin and lungs) and anaphylactic transfusion reactions, respectively (Gilstad, 2003; Larsen et al., 2006; Shimada et al., 2002).

A model for the role of Hp in inflammation is depicted in Figure 2. In this model, danger signals stimulate Hp expression through the activity of IL-6. In Hp1-1 subjects, Hp significantly reduces ROS generation through its potent antioxidant, Hb-binding and anti-inflammatory role as previously elaborated. This activity then triggers a T\(_h\)2dominant response initiating healing and repair. In Hp2-2 subjects, however, weak ROS quenching would allow the persistence of the inflammatory stimulus, leading to a T\(_h\)1 response and increased oxidative stress. Hb in this model is a modulator, as its binding to Hp reduces the generation of ROS by preventing haem and iron release and also depletes the available Hp in circulation. This model could explain the effect of Hp phenotype on the progression and outcome of parasitic diseases, infectious diseases and non-infectious diseases as outlined below.

5. Hp and disease associations

The gene frequencies of the HP\(^1\) and HP\(^2\) alleles differ geographically (Langlois and Delanghe, 1996). In West Africa, East Africa and South America, the HP\(^1\) allele is predominant, while North America, Europe, Asia and Australia have a predominant HP\(^2\) allele. The HP\(^2\) allele appears to be gaining a selective advantage, having derived from the HP\(^1\) allele in India (Langlois and Delanghe, 1996). Hp gene polymorphism has been investigated in case-control and cross-sectional studies of several diseases in relation to their incidence and clinical expression. In particular, diseases of parasitic origin (malaria) (Atkinson et al., 2007; Elagib et al., 1998; Minang et al., 2004; Quaye et al., 2000), infectious diseases (pulmonary tuberculosis, HIV) (Delanghe et al., 1998; Kasvosve et al., 2000) and non-infectious diseases (diabetes, coronary artery disease and obesity, among others) (Chieffini et al., 2004; Levy, 2004; Quaye et al., 2006) have been studied. These studies provide some insights into the role of HP\(^2\) and HP\(^2\) alleles in susceptibility/protection against infectious and non-infectious diseases and the selective advantage of the HP\(^2\) allele in various populations.
In principle, host-pathogen interaction is defined by resistance to (the ability to limit pathogen burden) or tolerance of (the ability to resist disease severity) a given pathogen (Raberg et al., 2007). This interaction leads to the evolution of both the host and pathogen for survival. In the recent history of the evolution of the human genome, malaria has been identified as the strongest known selective pressure (Kwiatkowski, 2005). The role of Hp gene polymorphism in malaria disease has been controversial. Some studies (cross-sectional and case-control) suggest that the Hp2-2 phenotype is protective against severe Plasmodium falciparum infection and placental parasite burden (Cox et al., 2007; Elagib et al., 1998; Minang et al., 2004; Quaye et al., 2000). Other case-control studies did not find significant associations (Aucan et al., 2002; Bienzle et al., 2005). Two recent prospective cohort studies have also supported the view that the Hp2-2 phenotype affords protection against malaria that increases significantly in children 3 years or older (Atkinson et al., 2007; Cox et al., 2007).

It has been suggested that the presence of the A-61C mutation in the Hp promoter region, which is associated with the HP2 allele, may confound genotyping for Hp0 or Hp2-2 phenotypes (Cox et al., 2007). This might explain, in part, the controversy over the role of the Hp2 and Hp1 alleles in malaria protection and susceptibility. These issues notwithstanding, two questions of interest are: how might homozygosity for the HP2 allele confer protection in P. falciparum infection, while posing a risk in the predominantly inflammation-related disease states described previously and how might these influence HP2 allele selective advantage? Functionally, the HP2 allele is a weaker antioxidant, promotes iron retention, has a weaker HBC, is lower in concentration in circulation, and in immunomodulatory function promotes a Th1-dependent cytokine profile. The cumulative effect of all of these factors could be a more pro-oxidant environment, arising from decreased quenching of ROS following infection or injury. In malaria disease, persistent haemolysis, which is also associated with the Hp2-2 phenotype (Atkinson et al., 2006) could increase ROS burden and decrease antioxidants. Eventually, sustained ROS generation, to which the Plasmodium species is vulnerable, could lead to a reduction in parasite burden. This initial respiratory burst and parasite destruction might promote an accelerated processing of parasite antigens for presentation to naive T cells. In a malaria-endemic environment, this activity could boost host immunity over an extended period and prevent severe disease or afford immunological tolerance. In a study relating malarial anaemia and Hp genotype, it was also observed that the Hp2-2 phenotype was associated with asymptomatic malaria at the end of the malaria season (Atkinson et al., 2006). Thus, the increasing prevalence of the HP2 allele in both Africa and Asia (Teye et al., 2006) may be largely attributed to selective pressure from malaria, as has been the case for HbS, HbC and /H9251 and /H9252 thalassaemia. Additionally, the added shift towards Th1 cytokine profiles contributed by the HP2 allele provides a balance in the immune response against pathogens, which might add to its selective advantage.

In the case of non-infectious diseases (diabetes, coronary artery disease, obesity), endogenous danger signals arising from disturbed metabolic pathways and tissue damage may favour a less pro-oxidant cytokine profile as engendered by the HP1 allele, thus making the HP2 allele a risk factor. This scenario could similarly apply in HIV and pulmonary tuberculosis infections, where increased iron retention and reduced antioxidant factors lead to poor prognosis in subjects with the Hp2-2 phenotype (Delanghe et al., 1998; Kasvosve et al., 2000). The Hp0 phenotype is associated with anaphylactic transfusion reactions from IgG and IgE Hp antibodies (Gilstad, 2003; Shimada et al., 2002). Hp0 individuals are also at risk for iron loading in kidney proximal tubules due to glomerular filtration of Hb (Fagoonee et al., 2005).
Based on the collective phenotype-dependent activities of Hp described here, one might propose that eventually, routine screening for Hp phenotype could impact the treatment of diseases with inflammatory etiologies.

6. Conclusions

Hp gene polymorphism has featured prominently in diseases of parasitic, viral and bacterial origin. Additionally, it has been associated with non-infectious diseases involving inflammation. The unique and peculiar functional activities defined by the phenotypes make consideration of Hp phenotypes in the clinical setting during treatment important. It also calls for intensified research to determine the precise activity of the phenotypes in regulating the pro-oxidant and antioxidant environments at injury sites that lead to: inhibition or promotion of pathogen growth through antigen processing; mitigation or promotion of disease risk and/or outcome; and tissue damage or wound healing and repair. Clearly, more studies of Hp and disease associations are required, particularly with respect to its immunomodulatory function. Studies involving Hp and parasitic diseases, as well as prospective studies with Hp knockout mice in non-infectious disease models will be valuable.

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