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Haptoglobin genotype and the iron hypothesis of atherosclerosis

Hilla Lee Viener and Andrew P. Levy

Rappaport Faculty of Medicine, Technion Israel Institute of Technology, Haifa, Israel

Jerome Sullivan first proposed 30 years ago that iron deficiency may be protective against the development of ischemic heart disease [1]. Enthusiasm for this hypothesis was dampened by a series of studies which failed to show that conventional measurements of total body iron status were associated with increased atherosclerosis in man. However, one potential reason for the failure to demonstrate an association between iron and atherosclerosis in these studies may have been due to a failure to focus on the molecular form of iron which is most likely responsible for inducing atherosclerosis. Iron is a reasonable candidate for carrying out the oxidative modifications which are believed to be paramount in the atherosclerotic process [2]. Iron is capable of generating highly reactive label oxygen free radicals such as superoxide radical and hydroxyl radical under physiological conditions. However, most of the iron in our bodies is stored bound to professional iron binding proteins-transferrin or ferritin-in which the iron is redox inactive and not capable of inducing oxidation of substrates. Over the past 10 years it has become appreciated that there exists a small but tightly regulated pool of iron which is not bound to transferrin or ferritin and which is redox active (referred to as labile plasma non-transferrin bound iron or NTBI) [3,4]. NTBI represents a number of molecular species very few of which have been identified. One type of NTBI which has been identified is the iron contained within the Haptoglobin-hemoglobin complex [5].

Haptoglobin (Hp) is an abundant plasma protein which binds with high affinity to hemoglobin [6]. While the heme iron in hemoglobin is a very potent oxidant, the binding of Hp to hemoglobin serves to decrease the ability of iron derived from hemoglobin from carrying out oxidative reactions [7]. In man there exists two possible alleles at the Hp genetic locus, denoted 1 and 2. The protein product of the Hp 2 allele is defective in its ability to block oxidative reactions mediated by iron derived hemoglobin. Therefore, while the Hp 1-Hb complex is relatively redox inert, the Hp 2-Hb complex contains by definition non-transferrin bound redox-active iron [5].

The Hp genotype has been shown in multiple independent longitudinal studies to be an independent determinant of the risk of incident cardiovascular disease in individuals with Diabetes Mellitus (DM) [8-13]. These studies have shown that DM individuals with the Hp 2-2 genotype have a 2-5 fold increased risk of myocardial infarction, stroke, and cardiovascular death as compared to Hp 1-1 or Hp 2-1 individuals. This association between

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*Corresponding author: Tel: 97248295202; fax: 97248514103; alevy@tx.technion.ac.il.

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the Hp genotype and cardiovascular disease has been recapitulated in mice made transgenic for the Hp polymorphism [14].

Currently we believe that the reason why Hp 2-2 DM individuals have more cardiovascular disease is due to the increased damage caused by hemoglobin bound to the Hp 2-2 protein. One target for the Hp 2-2-hemoglobin complex with which a direct connection can be made with atherosclerotic disease is HDL [15]. Epidemiological studies have demonstrated that HDL is protective against CVD. However, Hp can bind to HDL (most likely via an interaction with helix 6 of ApoA1) and thereby serve to tether hemoglobin to HDL. In Hp 2-2 DM individuals there is an impairment in the clearance of Hp-hemoglobin due to a decreased uptake of the complex by the CD163 Hp-hemoglobin scavenger receptor present on monocytes and kupfer cells [16,17]. This results in an increase in the steady state plasma concentration of Hp-hemoglobin in Hp 2-2 DM individuals thereby increasing its association with HDL. Hemoglobin bound to Hp 2-2 which is bound to HDL is redox-active and has been shown to result in the oxidative modification of HDL lipids and protein in Hp 2-2 DM individuals. HDL in these Hp 2-2 DM individuals is dysfunctional in terms of its ability to promote reverse cholesterol transport which is believed to be the primary function of HDL. Proof of concept experiments that the oxidative modification of HDL by iron derived hemoglobin is responsible for dysfunction HDL in Hp 2-2 individuals has been provided by the demonstration in mice and in man that antioxidant therapy with vitamin E can reduce oxidative modification of HDL in Hp 2-2 and correct HDL dysfunction in this cohort [17]. Interestingly there appears to be a pharmacogenomic interaction between the Hp genotype and vitamin E on HDL function so that vitamin E results in an improvement in HDL function in Hp 2-2 individuals or mice but not in individuals or mice with the other Hp genotypes. This may provide some insight into the apparent pharmacogenomic interaction between Hp genotype and vitamin E on cardiovascular disease in DM [8].

Another major target for the Hp-hemoglobin complex is related to the action of the complex outside of the vascular compartment after hemorrhage, in particular after intraplaque hemorrhage [18]. Intraplaque hemorrhage has been identified as a major component of the unstable plaque. It has been proposed that this may be due to the hemoglobin released from lysed red cells after the hemorrhage. As the Hp 2-2 protein clears hemoglobin at a slower rate this would appear to suggest that intraplaque hemorrhage in Hp 2-2 individuals might be associated with increased plaque iron which is capable of carrying out oxidative reactions. Recently such an interaction between hemoglobin derived plaque iron and the Hp genotype has been demonstrated in brachiocephalic arteries of Hp transgenic mice in an ApoE knockout background [14,19]. In man in a cadaveric study increased amounts of plaque iron were identified in the atherosclerotic plaques of Hp 2-2 DM individuals [20]. In the current issue of *Atherosclerosis*, Lioupis and colleagues [21] have demonstrated in a consecutive series of individuals undergoing carotid endarterectomy that increased iron exists in atheroectomy specimens from Hp 2-2 DM individuals. The precise location of this iron in the atherosclerotic plaque is not clear. Lioupis and colleagues suggest that there is an increase in both intracellular and extracellular iron in Hp 2-2 DM plaques. While a decrease in the efficiency of uptake of Hp 2-2-hemoglobin might explain why there is more extracellular plaque iron [22] it is unclear why macrophages within the plaque might harbor increased iron in Hp 2-2. This increased retention of iron in Hp 2-2 macrophages may be due to an inability to catabolize the Hp 2-2-hemoglobin complex in lysosomes or perhaps due to an inability to export the iron from these cells due to elevated hepcidin levels [23]. Present studies examining intracellular iron stores by transmission electron microscopy in Hp 2-2 DM individuals and mice coupled with molecular analysis of macrophages isolated from Hp 2-2 DM plaques may allow us to understand why intracellular trafficking of the Hp 2-2-hemoglobin complex is impaired.

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