CELLULAR IRON METABOLISM AND FOAMY MACROPHAGE DEATH: MECHANISMS UNDERLYING ACELLULAR LIPID CORE FORMATION IN ATHEROGENESIS

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Macrophage Death and Abnormal Iron Metabolism in Atherogenesis

Acellular lipid cores and dead macrophages are important features of vulnerable atherosclerotic plaques. Macrophage apoptosis is often identified at sites of plaque rupture and contributes to the enlargement of necrotic core and plaque vulnerability \[1-4]\. The suggested causative agents responsible for plaque macrophage death are toxic moieties of oxidized lipids, including oxidized low-density lipoproteins and oxysterols. We have proposed that toxic materials in the death zones of the atheroma may impair the clearance of cholesterol and promote apoptotic death of phagocytic cells, thereby resulting in persistent development of atheroma lesions \[5]\.

With gender differences taken into account in heart disease incidence, especially the fact that body iron storage is associated with atherosclerosis and myocardial infarction, cellular iron has been suggested to be involved in atherogenesis \[6]\. We earlier reported that human atherosclerotic lesions often contain large amounts of ferritin associated with apoptotic macrophages and foam cells \[7]\. Storage iron is strictly required for normal cellular metabolism but also serves as a reservoir from which ample amounts of toxic redox-active iron can be liberated under atherogenic stimuli and result in lipid peroxidation and oxidative cell damage. In this process cellular iron metabolism and proinflammatory cytokines such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-1 (IL-1) are closely interconnected. The cytokines regulate cellular iron homeostasis by affecting the expression of proteins involved in the uptake, storage, and export of cellular iron. TNF caused a significant increase of iron uptake but decrease of iron release in murine macrophages \[8]\. In human monocytes the proinflammatory cytokines also increase the uptake of non-transferrin-bound iron via stimulation of divalent metal transport-1 expression and cause iron retention by down-regulating ferroportin synthesis \[9]\. Of many unknown questions regarding the role of cellular iron in atherogenesis, causes of iron accumulation in atheroma and its pathological implications have been recently uncovered.

Erythrophagocytosis and Ferritin Accumulation in Atherosclerotic Plaque Destabilization

In several earlier studies prominent iron deposition has been described in atherosclerotic lesions \[6]\, however the source of iron and ferritin accumulated in the lesions and their implications are investigated until recently. We once hypothesized that since atherosclerosis is an inflammatory disease, trivial hemolysis and micro-hemorrhage in the atherosclerotic process may lead to the catabolism and release of hemoglobin (Hb) following erythrophagocytosis by macrophages. After oxidation and subsequent dissociation from methemoglobin, the heme-iron can catalyze the formation of reactive oxygen species (ROS). Free Hb may also avidly bind to the acute-phase serum protein haptoglobin (Hp) and is subsequently eliminated and metabolized by monocytes/macrophages. This is a crucial process for the rapid elimination of free Hb and heme iron and, more importantly, for the controlling of redox-active iron. Epidemiological studies
have lately revealed an association among Hp polymorphism, body iron, and atherosclerosis-related vascular diseases [10-11]. Based on above hypothesis we earlier demonstrated that erythrophagocytosis and iron release by macrophages are of importance in LDL oxidation [12-13]. Several recent studies suggested that in the process of erythrocyte-iron recycling by macrophages ferroportin 1 and natural resistance-associated macrophage protein 1 (Nramp1) were upregulated and responsible for resultant iron release [14-16].

Subsequent histological studies further established that erythrophagocytosis is the origin of pathological iron-deposition and ferritin accumulation in atheroma and revealed its possible pathogenic implications in atherogenesis. In experimental animals, the erythrophagocytosis was evident in lesions from cholesterol-fed rabbits and apoE-deficient mice where the iron deposition was also detectable in the same regions. Heme oxygenase-1 (HO-1), ferritin, and iron accumulated in same areas of atheroma with positive immunoreactivity for hemoglobin [17]. In the early human atherosclerotic tissues, evident immunoreactivity with Hb and ferritin antibodies mainly in CD68-positive cells (macrophages) suggests an ongoing degradation of erythrocytes is an early event in atherogenesis [18]. On serial sections from human carotid and coronary atherosclerotic arteries the immunoreactivity of Hb significantly corresponded to the same regions of ferritin immunoreactivity [17,18], the staining intensities of which were significantly correlated with iron [19]. Such Hb deposition often emerges in micro-vessel-rich areas, near the lipid core in advanced lesions. The perivascular foam cells not only contained erythrocytes (hemoglobin, iron) but also platelets (glycoprotein Ibalpha) [19]. In the Hb accumulated regions of both early and advanced lesions, Hb scavenger receptors (HbSR/CD163) were frequently noted [18]. It has been suggested that intraplaque microhemorrhages initiate platelet and erythrocyte phagocytosis, leading to iron deposition, macrophage activation, ceroid production, and foam cell formation [19].

In coronary lesions from patients who had died suddenly of coronary causes, larger amounts of both glycophorin A (a protein specific to erythrocytes that facilitates anion exchange) and iron (Mallory’s stain) were associated with larger necrotic cores and greater macrophage infiltration that critically affect plaque stability. Furthermore, accumulation of erythrocyte membranes significantly contributes not only to the iron deposition but also to free cholesterol accumulation, macrophage infiltration, and enlargement of the necrotic core, thereby increasing atherosclerotic plaque instability [20].

**Ferritin Induction and Labile Iron-driven Oxidative Damage in Apoptotic Foam Cells**

Our recent study [21] and data from our ongoing projects uncovered why foam cell death often associates with ferritin accumulation in atheroma lesions [7], which may be relevant to the formation of atheroma acellular lipid cores. In Linköping Carotid Study we found that intense ferritin deposition frequently occurred at regions adjacent to the acellular lipid cores in human carotid atherosclerotic lesions and was significantly correlated with macrophage infiltration (p < 0.0001, n = 59). Many CD68 positive foam cells usually at the periphery of acellular lipid cores were rich in ferritin and apoptotic (p < 0.0037, n = 60). To explore the relationship between oxidized lipid-induced apoptosis and ferritin accumulation in foam cells *in vitro*, human monocytic cells (U937), murine J774 macrophages, and human monocyte-derive macrophages were exposed to 7β-hydroxycholesterol (7β-OH) to see whether it causes lipid accumulation, apoptosis, and ferritin accumulation. The 7β-OH can induce typical foam cell formation in human macrophages. Such foam cells were apoptotic being cell shrinkage, nuclear condensation
and fragmentation and also showed increased ferritin expression as detected by immunocytochemistry and western blot. More importantly, ferritin induction was significantly correlated to the occurrence of apoptosis.

The intracellular free iron pool is a redox-active and regulatory form of intracellular iron and is often named as the labile iron pool (LIP). Trace amounts of “free” iron can catalyze the production of highly toxic hydroxyl radicals via Fenton/Haber-Weiss reactions. In above cell model, 7β-OH induced expansion of intracellular LIP and induction of ROS in apoptotic foamy cells. Iron chelators, 10-phenanthroline, apoferritin, and desferrioxamine, afford significant reduction of the 7β-OH-induced LIP and ROS and cell death, while, addition of iron compounds enhanced 7β-OH-induced effects.

The involvement of redox-active iron in 7β-OH induced apoptosis was further confirmed by the observations regarding Nramp1 expressions in vitro and in vivo. Nramp1 has antiport activity and transports Fe^{2+} to acidic late endosomes and lysosomes where toxic radicals are generated via Fenton and/or Haber-Weiss reactions. Functional studies in Nramp1 transfected macrophages have demonstrated that the Nramp1 protein plays a vital role in macrophage iron metabolism, early macrophage activation and also determines macrophage functions in inflammation [22]. Nramp1 regulates macrophage iron handling and facilitates iron release from macrophages undergoing erythrophagocytosis in vivo [16]. The increases in Nramp1 mRNA were seen in the apoptotic foamy macrophages in atheroma lesions from hyperlipidemic rabbits [23]. In vitro experiments showed that both iron compounds and 7β-OH considerably enhanced the levels of Nramp1 mRNA that mainly appeared in apoptotic nuclei. The iron chelator DFO clearly decreased the 7β-OH-induced Nramp1 mRNA.

It is known that oxysterol promotes the synthesis and secretion of TNF-α [24] that mediates cell death [25]. In a TNF-induced cell death model, ferritin heavy chain is induced and mediates inhibition of c-Jun N-terminal kinase (JNK) signaling, depending on suppressing ROS accumulation and iron sequestration [26]. In the process, LIP may serve as a common denominator of ferritin both in the enhancement of cell death by basal steady-state H-ferritin and in protection against cell death by induced H-ferritin [27]. These findings provide a plausible explanation for upregulated ferritin and associated apoptosis in human atherosclerotic lesions. Under inflammation conditions cytokies TNF-α or IL-1 alter cellular iron metabolism, including ferritin induction. However, such induced ferritin may be less protective [28] than we expected and sometime even associated with enhanced cell death as we see in human atherosclerotic lesions.

**Summary and Perspectives**

Macrophage erythrophagocytosis and hemoglobin catabolism by macrophages occurs in micro-vessel-rich regions and contributes to iron deposition and ferritin induction in human atheroma. Oxidized lipid 7β-OH induces not only foam cell formation but also oxidative damage-related apoptosis with abnormal metabolism of cellular iron. The increased redox-active iron through regulation of Nramp1, perhaps other iron transporters as well, in atheroma lesions may enhance oxysterol cytotoxicity and lead to the acellular lipid core formation in atheroma progression. These findings establish several mechanistic links between the abnormal iron metabolism and macrophage death described in human atherosclerotic lesions, and suggest modulation of abnormal iron metabolism as a potential approach for anti-inflammatory therapy of atherosclerosis.
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References