amount of iron sufficient to meet its metabolic needs and to be capable of protecting itself from the toxicity of excess intracellular iron. The former is achieved by transferrin receptor-1 (TfR1)–dependent or –independent iron uptake at the cell surface, while the latter is accomplished by sequestering intracellular iron in ferritin, the iron storage protein shell composed of 24 subunits of H- and L-ferritin chains. TfR1 and cytoplasmic ferritin syntheses are controlled by iron regulatory proteins (IRP1 and IRP2) that function as cytoplasmic sensors of cellular iron status. These IRPs regulate the translation of TfR1 and ferritin mRNAs through their interaction with iron regulatory elements (IREs) present on the untranslated regions of the respective mRNAs.

The importance of iron in mitochondrial function is well known. However, the role of mitochondrial iron trafficking in the regulation of cellular iron homeostasis is not understood. In mitochondria, iron is used for the synthesis of heme and the generation of iron-sulfur [Fe-S] clusters. The latter are essential components of flavoenzymes and of proteins involved in the citric acid cycle and the electron transport chain. Recently, Levi et al reported the existence of ferritin in mitochondria (MtFt). MtFt is composed of 22-kDa ferritin subunits that display similarity to H-ferritin. However, although MtFt resembles H-ferritin, its function is unknown.

In this issue of Blood, Nie and colleagues examine the role of MtFt in cellular iron uptake and distribution using a cell line stably transfected to express the murine MtFt gene under control of a tetracycline-responsive promoter. They show that overexpression of MtFt results in an increase in IRP-IRE mRNA interaction, an increase in TfR1 levels, and a decrease in cytoplasmic ferritin synthesis. This constellation of findings provides direct evidence that the expression of MtFt produces a state of relative cytoplasmic iron deprivation. MtFt expression is also shown to decrease the enzymatic activities of [4Fe-4S] cluster-containing cytosolic aconitase and mitochondrial aconitase.

An intriguing finding in their study is that when compared with noninduced cells, MtFt-overexpressing cells take up a greater amount of iron that is preferentially incorporated into MtFt rather than into cytoplasmic ferritin. Moreover, iron in MtFt appears to be less accessible to chelation than iron sequestered in cytoplasmic ferritin. These elegant studies help bring into focus a role for MtFt as a protein that not only sequesters iron but also modulates the trafficking of iron through the cytoplasm.

Also in this issue of Blood, Napier and colleagues provide an up-to-date review of our current understanding of iron trafficking and processing in mitochondria, illustrating how studies of genetic diseases associated with mitochondrial iron overload have led to advances in this field. These diseases include X-linked sideroblastic anemia (caused by a defective erythroid-specific 5-aminolevulinic acid synthase), sideroblastic anemia with ataxia (caused by mutations in the ABCB7 gene that encodes a transporter for Fe-S clusters), and Friedreich ataxia (caused by a mutation in the gene for frataxin). The review includes a discussion of heme synthesis, Fe-S cluster biogenesis, MtFt, and frataxin. Building on observations made in mitochondrial iron overload disorders, the authors propose an interesting and testable model in which frataxin plays a regulatory role in the trafficking of mitochondrial iron for heme synthesis, Fe-S cluster formation, and storage in MtFt.

Clearly, mitochondrial iron metabolism is an exciting area for investigation. We look forward to future studies that will further illuminate the role this powerhouse plays in cellular iron metabolism in health and disease.

REFERENCE

Comment on Zhang and McCrae, page 1964

Annexin A2: better left alone

Alisa S Wolberg and Robert A S Roubey UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Zhang and McCrae demonstrate that APLA/b2GPI-mediated endothelial cell activation occurs via dimerization of annexin A2 molecules on the cell surface.

The association of antiphospholipid antibodies (APLAs) with thrombosis is well established, and there is growing evidence that APLAs themselves contribute to hypercoagulability. The mechanisms of APLA-associated hypercoagulability are less clear, however. A confusing variety of antibody effects has been proposed. One important line of research involves the activation of endothelial cells by certain APLAs, specifically those directed against β2-glycoprotein I (β2GPI). In their current study, Zhang and McCrae make an important next step in this endeavor.

Previously, APLAs, in a β2GPI-dependent manner, were shown to stimulate endothelial cells to a procoagulant phenotype by increasing monocyte adhesion and the expression of E-selectin, vascular cell adhesion molecule-1, and intracellular adhesion molecule-1. These events presumably require the transduction of a signal into the cell and, therefore, the involvement of a cell surface receptor. Since the effects of APLA/β2GPI do not appear to be mediated by Fc receptors, investigators posited the existence of a cell surface receptor for β2GPI. In previous work, McCrae and colleagues identified
 annexin A2 as a high-affinity receptor for β2GPI on endothelial cells (Ma et al). The current paper ties this finding to the earlier functional studies, demonstrating that cross-linking of annexin A2 by APLA/β2GPI activates endothelial cells (see figure).

The key observation by Zhang and McCrae is that cross-linking of annexin A2 on the endothelial cell surface induces the expression of cell adhesion molecules. Cross-linking was achieved using bivalent anti-annexin A2 antibodies or APLA/β2GPI. The critical role of bivalent cross-linking was demonstrated using monovalent antibody fragments that (a) did not activate cells, and (b) blocked the effects of bivalent antibodies.

These findings establish at least 2 important points. First, a cell surface receptor (ie, annexin A2) is critical in mediating the in vitro effects of APLA on endothelial cells. Second, the data highlight the role of immunoglobulin G (IgG) bivalency. Antibody bivalency has been shown to explain how antibodies to β2GPI, a relatively weak phospholipid-binding plasma protein, lead to high avidity binding of IgG–β2GPI complexes to phospholipid membranes. Now, bivalency has been shown to play a critical role in cross-linking annexin A2 and transducing an activation signal to cells.

As noted by the authors, important questions remain. How does annexin A2, which does not have a transmembrane domain, transduce a signal? Recently, Raschi et al have demonstrated that APLA activation of endothelial cells occurs via the MyD88 pathway that is commonly associated with toll-like receptor (TLR) signaling. Does annexin A2 signal through the MyD88 pathway? Is annexin A2 physically associated with a TLR? In addition to transducing a signal, what is the effect of APLA/β2GPI on annexin A2’s role as a coreceptor for plasminogen and tissue plasminogen activator? Does binding of APLA/β2GPI to annexin A2 inhibit plasmin generation? Once annexin A2 is cross-linked by APLA/β2GPI, what is the fate of this complex? If it is internalized or shed, the amount of annexin A2 available to support plasmin generation may be decreased. Finally, and perhaps most importantly, might the APLA/β2GPI/annexin A2 complex provide a molecular target for antithrombotic therapies in the antiphospholipid syndrome?

**REFERENCES**


---

**HEMATOPOIESIS**

Comment on Guthrie et al, page 1916

**NO role in EPC function**

**Mervin C. Yoder  INDIANA UNIVERSITY SCHOOL OF MEDICINE**

The nitric oxide pathway plays an important role in modulating endothelial progenitor cell function, playing a critical role in blood vessel repair in response to injury.

A variety of studies in the murine system provide substantial evidence that blood and endothelial progenitor cells (EPCs) arise from a common progenitor, the hemangioblast, during embryogenesis. Recent evidence that adult murine bone marrow hematopoietic stem cells (HSCs) possess hemangioblastic activity has also been reviewed. From this work, one would predict that the hemangioblasts give rise to circulating EPCs that participate in vessel homeostasis throughout murine life. Understanding the role for hemangioblasts and EPCs in the repair of damaged vessels or in pathogenic tumor neangiogenesis has been complicated by apparent differences in the involvement of these cells depending upon the type of host injury or tumor model used.

Ischemic or traumatic injury alone is not sufficient to induce retinal neovascularization in the adult mouse. However, ischemic retinal injury in mice exposed to high concentrations of intraocular vascular endothelial growth factor (VEGF) develop a robust proliferative retinopathy similar to that seen in human diabetic retinopathy. Using such a model, Grant...
Annexin A2: better left alone

Alisa S. Wolberg and Robert A. S. Roubey