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Cytoglobin at the crossroads of vascular remodeling

Matthew B. Amdahl[1,2], **Anthony W. DeMartino**[1,3], **Jesús Tejero**[1,3], and **Mark T. Gladwin**[1,3,*]

[1]Heart, Lung, Blood, and Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA 15213, USA

[2]Department of Bioengineering, University of Pittsburgh, PA 15213, USA

[3]Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA

Since its discovery approximately fifteen years ago, cytoglobin (Cygb) has been studied extensively. Because it is found outside the red cell, Cygb is categorized as a non-erythroid globin, along with (in humans) proteins such as myoglobin (Mb), neuroglobin, androglobin, and hemoglobin α (Hb α). The putative functions of these non-erythroid globins are linked to tissue protection from conditions such as hypoxia, ischemia, and oxidative stress.[1] Cygb not only fulfills these functions, but also has been related to other roles including tumor suppression and the regulation of fibrosis in cell and animal models.[2–7] Like other heme globins, Cygb can reversibly bind oxygen and other small molecules. The ability of Cygb to store and sense oxygen, as well as its involvement in nitrite and nitric oxide (NO) metabolism, being able to both scavenge NO and produce NO from nitrite, are probably key to its function(s).[8, 9] However, in spite of significant progress in understanding the structure, localization, and functional characteristics of Cygb, the central physiological roles of this protein have yet to be fully elucidated.[10–12]

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Jourd'heuil et al. examine the role of Cygb in controlling apoptosis and vascular remodeling after injury. Cygb appears to be the predominant globin in vessel walls of humans, rats, and mice with expression levels substantially higher than those of Mb. The protein is found in medial smooth muscle cells (SMCs), and dedifferentiation of SMCs by culture or vascular injury leads to a loss of Cygb expression, although this loss is only temporary after injury, with Cygb expression recovering after several days.

The authors use two different injury models: a rat model of unilateral carotid artery balloon angioplasty and a mouse model of unilateral carotid artery ligation. Following either vascular injury, animals that do not express Cygb show substantially impaired remodeling, specifically decreased neointima formation. Analyses of apoptotic and proliferative markers suggest higher levels of apoptosis and cell death with Cygb loss but unchanged levels of

*Address correspondence to: Mark T. Gladwin, Pittsburgh Heart, Lung, Blood, and Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA 15261; Division of Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA 15213; gladwinmt@upmc.edu.

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None.

proliferation, indicating increased apoptosis as the primary cause of disrupted neointima formation.

In experiments with rat aortic SMCs, the authors observe increased Cygb expression under hypoxic conditions or after treatment with inflammatory cytokines. While hypoxia is known to increase Cygb expression, the induction of Cygb by cytokines has not been documented before and suggests Cygb may modulate inflammatory responses to non-ischemic tissue damage. Cygb silencing significantly increases rates of cell death, indicating a cytoprotective role. 1400W, a selective NOS2 inhibitor, largely reversed this increase in cell death, suggesting NO-dependent cytotoxicity that can be prevented by Cygb expression. Worth mentioning, Cygb-KO mice show exacerbated expression of NOS2 and inflammation markers, suggesting a link between Cygb function and immune response.[10] The increase in cell death was also reversed with the use of the reducing agent N-acetyl cysteine or a pan-caspase inhibitor, implicating oxidative stress in promoting apoptosis. Specifically, Cygb loss appears to activate caspase-3, a finding that had previously been observed in animal models of brain ischemia-reperfusion injuries but is novel in the context of vascular injury.[13]

The implication that Cygb protects cells from NO-dependent toxicity is particularly compelling as numerous researchers have explored Cygb's nitric oxide dioxygenase (NOD) activity, which was first proposed by Jourd'heuil et al.[14] NO dioxygenation occurs when oxygen-bound Cygb reacts with NO, resulting in the production of nitrate and the oxidation of the heme iron from the ferrous the ferric state.[15–17] This reaction is extremely rapid for globins (nearly diffusion-limited).[18] NO dioxygenation is considered to significantly contribute to NO metabolism *in vivo*, with physiologic effects including cytoprotection and regulation of vascular tone.[16, 19–23] For example, endothelial Hb α, localized to myoendothelial junctions, has been shown to consume NO generated in the endothelium, regulating vascular tone.[21] Inhibition of this NO consumption results in significant decreases in blood pressure.[22] Loss of Cygb in the SMCs appears to elicit similar effects, showing increased vasodilation and decreased blood pressure in Cygb knockout mice.[23]

Catalytic NO dioxygenase activity is limited by the reduction of the heme iron. A reducing system has been characterized for Hb α; inhibition of CYB5R3, a reducing enzyme present in endothelium, slows NO consumption by Hb α.[21] Recent data from our group and others show a highly efficient reduction of Cygb by the NADH/CYB5/CYB5R3 reducing system; in fact, the reduction of Cygb is at least an order of magnitude faster than that of other heme globins.[23, 24] Taken together, these results suggest the existence of a Cygb/CYB5/CYB5R3 metabolon in vascular SMCs, enabling rapid consumption of NO and thus modulating NO bioactivity and signaling. Interestingly, Mb in vascular smooth muscle has previously been shown to contribute to hypoxic vasodilation via nitrite reduction to NO.[25] The responses of Mb and Cygb to oxygen and the relative efficiency of their reducing systems could underlie different roles for both proteins on vascular wall NO signaling.[16, 24]

This work by Jourd'heuil et al. showcases a new role for cytoglobin as an important regulator of apoptosis and vascular remodeling in SMCs after injury, acting independent of other globins. This role is supported by the novel observation that inflammatory cytokines trigger re-expression of Cygb in de-differentiated SMCs *in vitro*, preventing NOS2-

dependent cytotoxicity and promoting proper remodeling of vascular tissue. Rapid NO consumption by Cygb in vascular SMCs, which has previously only been shown to influence vascular tone and blood pressure, may mediate this effect. This work provides compelling evidence that Cygb may be a key regulator of vascular function under both normal and pathologic conditions, indicating that Cygb and other non-erythroid globins may have value as therapeutic targets or agents for myriad disease states. The particular properties of the non-erythroid globins keep revealing new possibilities, from the potential of modulating NO signaling to their use as potential carbon monoxide scavengers and/or oxygen carriers.[26, 27]

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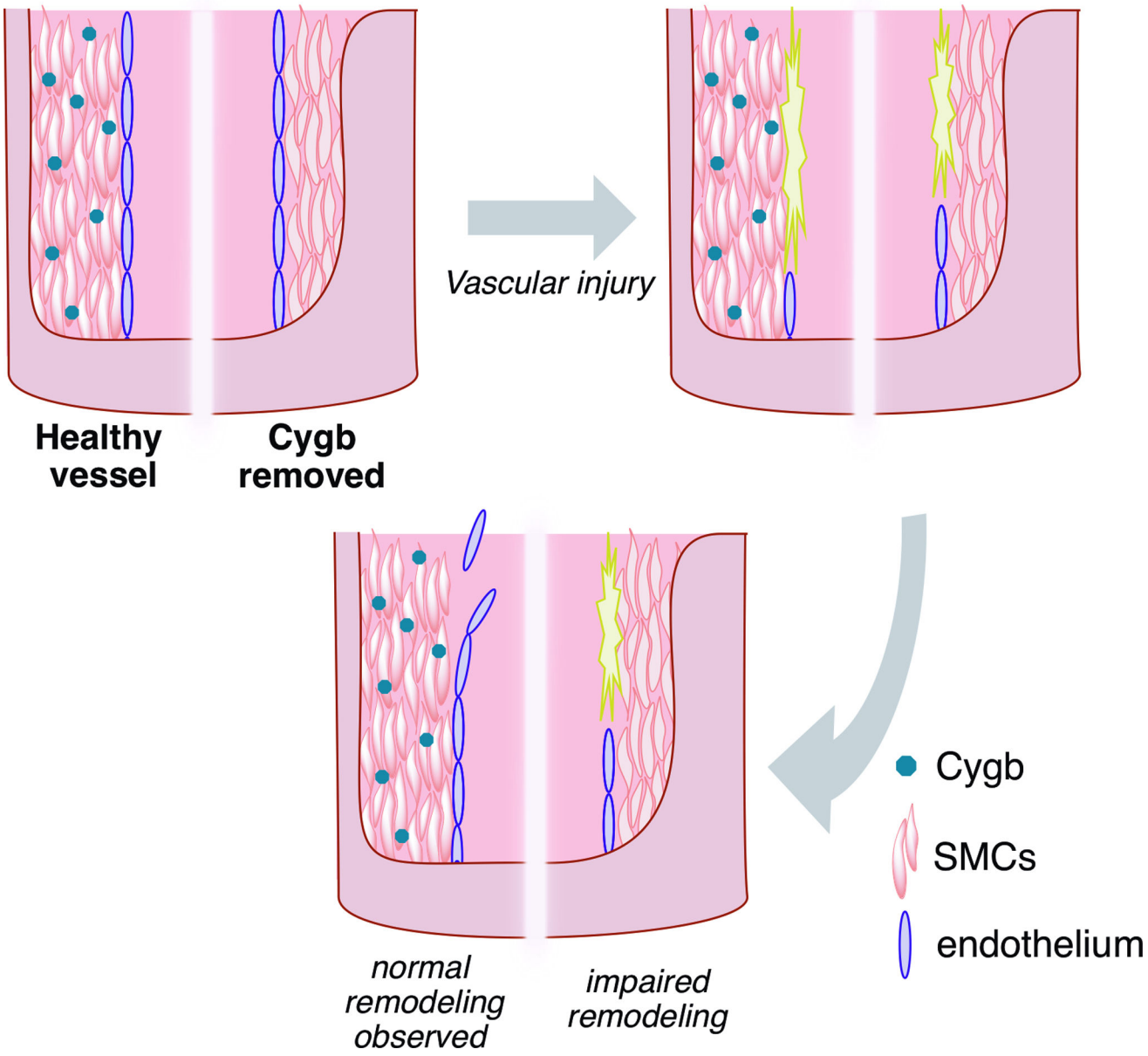

Figure 1.

Murine vascular injury is induced via arterial balloon angioplasty, leading to denuding of the endothelium. In a healthy vasculature (left half of vessel), such an injury results in remodeling (neointima formation) after several days. However, the loss of cytoglobin (Cygb) via either RNA silencing or genetic knockouts (right half of vessel) results in impaired remodeling over the same period.