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The Platelet: Sensing Shear and the Endocrine Regulation of Cardiovascular Sclerosis

Dwight A. Towler, MD, PhD

Diabetes and Obesity Research Center Sanford-Burnham Medical Research Institute Florida
Hospital Translational Research Institute Orlando, FL 32832

Calcific aortic valve sclerosis (CAVS) cause profound morbidity in our increasingly dysmetabolic and aging citizenry¹. Two to five percent of our elderly population will require aortic valve replacement (AVR) surgery to mitigate risk of death due to severe CAVS. Once considered only a passive process of dead and dying cells, arteriosclerotic calcification -- including CAVS -- has emerged as an actively regulated form of mineralized tissue metabolism¹. Preclinical and epidemiological studies reveal that aging, tobacco use, renal failure, bicuspid aortic valve, and the features of metabolic syndrome -- hypertension, worsening glycemic control, hypertriglyceridemia, low HDL cholesterol, and obesity² -- are cumulative risk factors for arteriosclerotic valve calcification¹. Although hypercholesterolemia certainly contributes to risk of arteriosclerotic disease, targeting cholesterol via lipid-lowering statin therapy is insufficient to fully mitigate disease progression¹. Seminal histological studies by Otto and colleagues indicate that while early valve lesions do exhibit intra- and extra-cellular lipid accumulation with inflammation, features of active matrix remodeling are present as well including disruption of the elastic lamina with lamina fibrosa protein accumulation and microcalcification³. With advanced disease, remodeling woven bone can be seen in ca. 13% of specimens⁴ although the molecular fingerprints of active osteogenesis are uniformly present even when amorphous calcium phosphate deposits predominate^{1, 4}. Currently no medical therapies exist for preventing or treating CAVS -- and our capacity to identify those at greatest risk for clinical progression is limited¹. In the Japanese Aortic Stenosis Study (JASS), while warfarin use portended worsening disease treatment of hypertension with angiotensin receptor blockade (ARB) was associated with attenuated risk of CAVS progression⁵. Interestingly, emerging data suggest that ARBs exert beneficial actions in cardiac valve biology in part via inhibition of TGF- β signaling cascades^{6, 7}.

In culture, TGF- β 1 clearly promotes aortic valve interstitial cell calcification⁸, with responses dependent upon the stiffness of the extracellular matrix⁹. However, the contributions of TGF- β 1 signaling to CAVS have not been fully examined *in vivo* -- nor has the precise cellular source of TGF- β 1 important to valve pathobiology been established. In this issue of the Journal¹⁰, Collier and colleagues begin to address these important questions. Implementing the “Reversa” LDLR^{-/-} mouse model of vascular disease¹¹ -- first validated

in CAVS by Miller, Weiss, Heistad and colleagues¹² -- the authors demonstrate that diet-induced hypercholesterolemia results in valve thickening, worsening aortic valve stenosis, increased wall shear stress (WSS; determined by echocardiographic measurement of valve leaflet separation and Doppler velocimetry), and elevated circulating TGF- β 1 levels over a 12 month period. Reversing hypercholesterolemia after 6 months of dyslipidemia attenuates the severity of CAVS observed at 12 months, reflected in reductions in the rate of aortic stenosis and concomitant increases in transvalvular velocity, WSS and plasma TGF- β 1¹⁰. In those animals with progressive valvular disease, a modest but significant positive relationship was observed between WSS and total circulating TGF- β 1 levels -- with a strong trend for correlation exhibited at 12 months between circulating TGF- β 1 levels and the extent of aortic valve fibrosis by histological scoring ($r = 0.78$, $p = 0.08$). More importantly, surgical introduction of an ascending aortic constriction (AAC) in wild type mice -- an experimental mimetic of aortic valve stenosis -- also increased WSS and circulating TGF- β 1 levels in the absence of hypercholesterolemia. The source of TGF- β 1 was firmly established to be the platelet in the AAC model; conditional deletion of TGF- β 1 in the megakaryocyte lineage (PF4-Cre;TGF- β 1(fl/fl) mice) markedly reduced time-dependent increases in total plasma TGF- β 1 following AAC¹⁰. Thus, the relationship observed between total plasma TGF- β 1 levels and WSS in wild-type mice was no longer significant in PF4-Cre;TGF- β 1(fl/fl) mice. Importantly, bioactive platelet-derived TGF- β 1 was responsible for driving Smad2/3 and ERK phosphorylation in response to AAC in both circulating leukocytes and mesenchymal cells of the ascending aorta -- and these same signaling responses were phenocopied by diet-induced aortic valve disease, WSS, and plasma TGF- β 1 upregulation in the Reversa mouse model¹⁰. Ex vivo, stirring-induced shear of whole blood was shown to increase leukocyte Smad2/3 and ERK phosphorylation via mechanisms inhibited by neutralizing TGF- β 1 antibody. Thus, platelet-derived TGF- β 1 is released and signals in response to elevated aortic shear stress, activating pro-sclerotic signaling cascades in the arterial vasculature¹⁰.

Why is this manuscript so intriguing? Firstly, the storage and release of platelet derived TGF- β 1 has significant implications vis-à-vis the emerging bone-vascular axis and the endocrine regulation of arteriosclerosis¹³. By mobilizing and recruiting mesenchymal progenitors capable of directing robust collagen deposition and matrix mineralization, TGF- β 1 signaling coordinates normal bone turnover and fracture repair¹⁴. Cao and colleagues recently identified that with tissue injury, increases in circulating TGF- β 1 mobilizes and recruit bone marrow-derived mesenchymal stem cells (MSCs; Sca1+CD29+CD11B-CD45-) to sites vascular remodeling -- including mechanically initiated neointima formation¹⁵. Therefore, by analogy, increases in WSS that promote platelet-derived TGF- β release are posited to increase circulating levels of MSCs capable of contributing to pro-sclerotic vascular responses including CAVS. Moving forward, it will be important to examine whether circulating MSCs are increased in response to ascending aortic constriction and are recruited only to that site of vascular injury -- or potentially populate other vascular venues with disturbed flow and dependent upon platelet-derived TGF- β 1. Secondly, this study once again points to the limitations of targeting only cholesterol in CAVS -- even though lipoprotein metabolism is one critically important component of disease initiation¹. Once valve hemodynamics are perturbed in CAVS, a vicious “feed-forward” cycle arising from

increased WSS may continue to condition the dysmetabolic milieu with circulating TGF- β 1. It should be noted that (Smad) and noncanonical (ERK) pathway activation persisted in Reversa mice; pharmacokinetic-pharmacodynamic relationships between platelet-derived TGF- β 1 release and arteriosclerotic disease remain to be established. Independent of whether TGF- β 1 functions by paracrine vascular actions or via MSC mobilization, the administration of TGF- β 1 neutralizing antibody is predicted to help mitigate CAVS disease progression, although this remains to be directly tested. Finally, this study suggests that other platelet-derived molecules such as PDGF¹⁶ and Dkk1¹⁷ – the latter participating in arteriosclerotic responses via the Smad-dependent endothelial-mesenchymal transition¹⁸ – will be relevant to CAVS biology. Moreover, in addition to affording useful plasma biomarkers predictive of CAVS progression, targeted inhibition of TGF- β 1 and other pro-sclerotic platelet products may represent effective strategy for mitigating disease in those individuals at greatest clinical risk. Indeed, fresolimumab -- a monoclonal antibody neutralizing all forms of TGF- β and under evaluation for treatment of focal segmental glomerulosclerosis¹⁹ and advanced melanoma²⁰ -- might impact CAVS progression in those individuals with high plasma TGF- β 1 or elevated leukocyte Smad activation. Of note, circulating levels of Dkk1 are increased in patients with severe CAVS and decrease following surgical aortic valve replacement²¹. Intriguingly, Dkk1 levels apparently do not change following transcatheter aortic implantation²¹; whether other platelet-derived signals including TGF- β 1 are differentially impacted by surgical intervention remains to be assessed – and the clinical impact if any upon arteriosclerotic disease progression in other vascular venues remains to be determined. All in all, the seminal work of Collier and colleagues¹⁰ provides us with an enlightened view of CAVS biology, offering new hope for diagnostic and therapeutic intervention in patients afflicted with arteriosclerotic valve and vascular disease.

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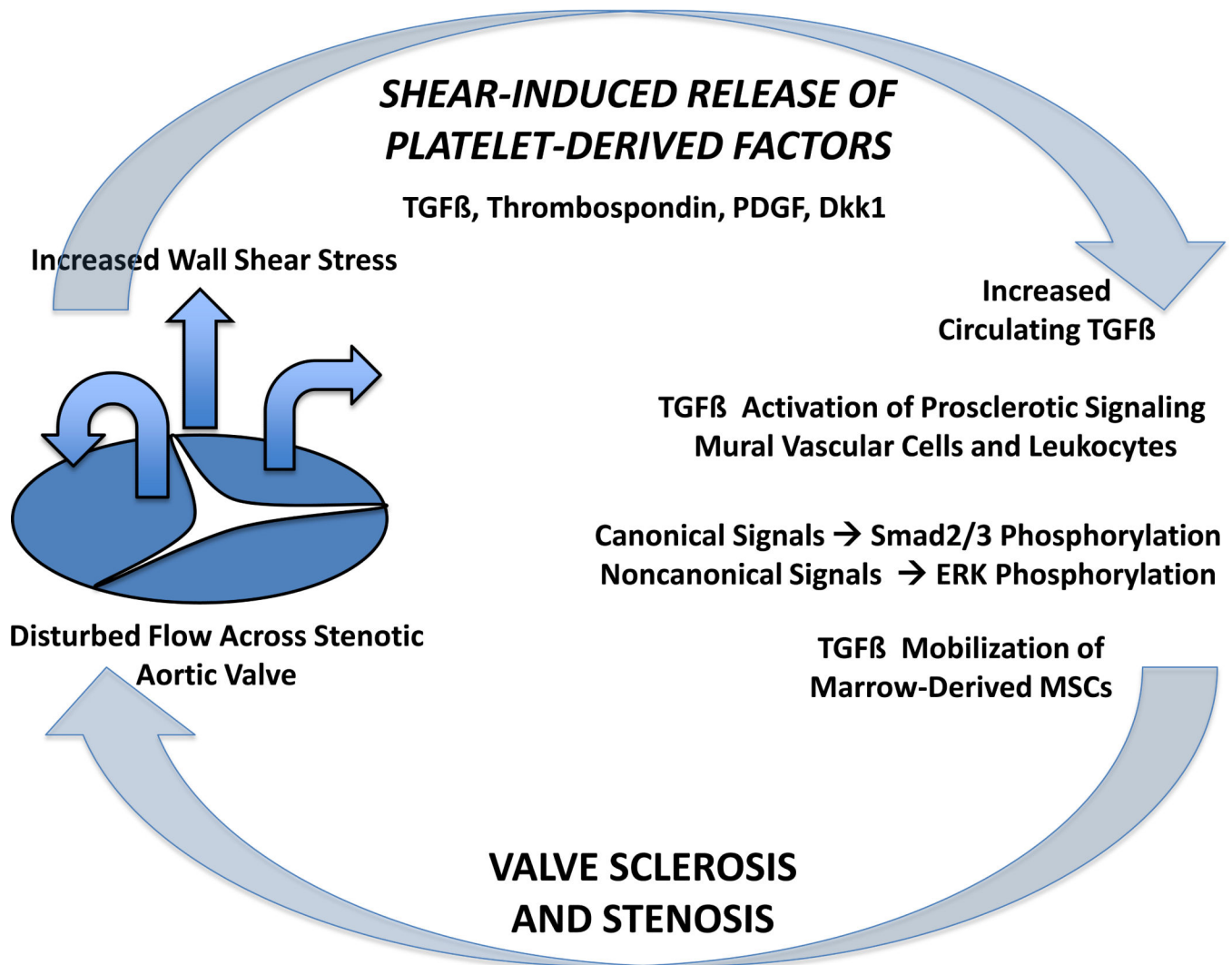


FIGURE. Shear – dependent TGF- β release and the pathobiology of CAVS

In this issue of the Journal¹⁰, Coller and colleagues demonstrate that shear-induced release of TGF- β from platelet alpha granules activates pro-sclerotic canonical and noncanonical signals that can drive systemic arteriosclerotic disease. This portends a feed-forward vicious cycle in CAVS. Thus, in addition to providing potential biomarkers for those individuals at greatest risk for clinical progression, platelet-derived factors such as TGF- β afford novel therapeutic targets for medical treatment of CAVS. See text for details. TGF β , transforming growth factor β ; PDGF, platelet-derived growth factor; Dkk1, Dickkopf-1; MSCs, mesenchymal stem cells.