Role of Microvascular Tone and Extracellular Matrix Contraction in the Regulation of Interstitial Fluid Implications for Aortic Dissection
Ziad Mallat, Alain Tedgui, Daniel Henrion

Abstract—The pathophysiology of aortic dissection is poorly understood, and its risk is resistant to medical treatment. Most studies have focused on a proposed pathogenic role of transforming growth factor-β in Marfan disease and related thoracic aortic aneurysms and aortic dissections. However, clinical testing of this concept using angiotensin II type 1 receptor antagonists to block transforming growth factor-β signaling fell short of promise. Genetic mutations that predispose to thoracic aortic aneurysms and aortic dissections affect components of the extracellular matrix and proteins involved in cellular force generation. Thus, a role for dysfunctional mechanosensing in abnormal aortic wall remodeling is emerging. However, how abnormal mechanosensing leads to aortic dissection remains a mystery. Here, we review current knowledge about the regulation of interstitial fluid dynamics and myogenic tone and propose that alteration in contractile force reduces vascular tone in the microcirculation (here, aortic vasa vasorum) and leads to elevations of blood flow, transmural pressure, and fluid flux into the surrounding aortic media. Furthermore, reduced contractile force in medial smooth muscle cells coupled with alteration of structural components of the extracellular matrix limits extracellular matrix contraction, further promoting the formation of intramural edema, a critical step in the initiation of aortic dissection. The concept is supported by several pathophysiological and clinical observations. A direct implication of this concept is that drugs that lower blood pressure and limit interstitial fluid accumulation while preserving or increasing microvascular tone would limit the risk of dissection. In contrast, drugs that substantially lower microvascular tone would be ineffective or may accelerate the disease and precipitate aortic dissection. (Arterioscler Thromb Vasc Biol. 2016;36:1742-1747. DOI: 10.1161/ATVBHA.116.307909.)

Key Words: actin ■ aneurysm ■ extracellular fluid ■ pericyte ■ vasa vasorum

Genetic mutations that predispose to thoracic aortic aneurysms and aortic dissections (TAADs) affect structural components of the extracellular matrix (ECM), transmembrane structures involved in mechanical integrity, mechanosensing, and signal transduction, or intracellular components of the cell contractile apparatus.1–3 Concepts and studies are being developed to explain how such alterations may impact the response of the aorta to hemodynamic stress and drive abnormal aortic remodeling.4 Here, we propose a focus shift toward the small vessels (arterioles and vasa vasorum) to explain, based on current knowledge about the (patho)physiology of blood flow regulation and fluid dynamics, how alterations in ECM and the cell contractile apparatus may instigate the frightening process of aortic dissection.

Microcirculatory Vascular Tone and the Autoregulation of Blood Flow: The Example of Myogenic Tone
Microcirculatory vascular tone is the main determinant of vascular resistance and ensures tissue perfusion in response to changing metabolic demands. An increase in pressure induces a rapid and reversible vasoconstriction of small resistance vessels, an intrinsic property because of their ability to develop myogenic tone (MT).5 Besides protecting downstream capillaries from abrupt increases in blood pressure occurring in larger vessels, MT has a key role in the inward eutrophic vascular remodeling in response to a chronic rise in pressure.

Pressure induces deformation of cell membrane proteins such as stretch-activated ion channels; conformational changes in matrix and cytoskeletal proteins; activation of ECM and integrins; and activation of various cellular junctions (Figure 1). Vascular smooth muscle cell (SMC) depolarization and calcium entry are necessary for the development of pressure-induced contraction and myogenic tone. Although the nature of the channel(s) involved in MT development remains debated, canonical transient receptor potential (TRP) channels are strong candidates. Interactions of TRP polycystin 1 and 2 (TRPP1 and TRPP2) with filamin A, an actin crosslinking protein, are critical for stretch-activated ion channel regulation and MT development.6 Indirect evidence for a role of chloride...
channels in MT has also been suggested. G-protein–coupled receptors like the angiotensin type 1 receptor possess mechanosensitive properties, independent of ligand binding, and may activate TRPs. Recent studies also showed that interactions between SMCs and ECM are involved in MT. Pressure-induced deformation of ECM elements may activate integrins (eg, β1, β3) and induces cytoskeletal remodeling and contraction through modulation of stretch-activated ion channel/TRP and membrane depolarization. Calcium sensitization of the contractile apparatus through RhoA/Rho-kinase and actomyosin crossbridge cycling allows force maintenance without high intracellular calcium concentration. In addition to vascular SMCs, pericyte constriction in precapillaries involves similar transduction pathways and may play a significant role in the regulation of tissue perfusion.

**Interstitial Fluid Pressure**

Transcapillary fluid flux is also highly controlled by the level of interstitial fluid pressure (Pif) exerted on the outside of the capillaries. A reduction of Pif promotes and maintains edema formation (Figure 2). Composition and functional properties of the ECM are major determinants of the level of Pif. Briefly, polyanionic glycosaminoglycans (GAGs) of the ECM are osmotically active, whereas collagens, elastic fibers, and microfibrils play important roles in ECM contraction and oppose the decrease of Pif. In steady state, GAGs are underhydrated. When fluid gains access to the interstitial space, the osmotically active GAGs absorb water molecules and swell, which tends to limit the increase of Pif because of an increase of interstitial volume. This phenomenon sustains edema formation. In that case, opposing contraction of the interstitium, driven by the physical properties of the microfibril/collagen network and force generation by its cellular constituents, plays a critical balancing effect and limits edema formation.

**Genes Associated With TAADs Are Involved in the Regulation of Microvascular Tone and Interstitial Fluid Pressure**

**Microvascular Tone**

Genetic mutations that predispose to TAADs affect components of the ECM and proteins involved in cellular force generation. It is remarkable that many of those pathways are involved in the regulation of microvascular tone. This is the case of mutations altering pressure sensing (filamin A [FLNA] and polycystins [PDK1 and PKD2]) or mutations altering SMC contraction (myosin light chain kinase [MYLK]). Other pathways/genes involved in TAADs are expected to alter microvascular tone. This is the case of ACTA2 (actin alpha 2, smooth muscle) and MYH11 (myosin heavy chain 11, smooth muscle), which are involved in SMC contraction, gain-of-function mutation in PRKG1 (protein kinase, cGMP-dependent, type I, involved in SMC relaxation), or mutations in PLOD1 (procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1, associated with severe muscle hypotonia). Mutations in TGFBR2, TGFBR1, TGFBR2, and

**Figure 1.** Summary of the mechanism of myogenic tone with a focus on pathways associated with thoracic aortic aneurysms and aortic dissections. In response to an increase in blood pressure (P), a resistance artery contracts in response to the activation of cationic influx through stretch-activated channels such as transient potential (TRP) channels, possibly TRPC. Pressure may activate several pathways, which may act simultaneously and synergistically. Beside the direct activation of TRP channels by pressure, the interaction of polycystin 1 and 2 (TRPP 1 and 2) with filamin A and the actin cytoskeleton may induce TRP opening. TRP activation leads to Na⁺ and Ca²⁺ entry, membrane depolarization, and contraction. Deformation of the extracellular matrix (ECM) by pressure may be transmitted to the integrins and the cytoskeleton and transmitted to the surrounding smooth muscle cells (SMC). Alternatively, pressure may also activate G-protein–coupled receptor such as AT1R and subsequently stimulate TRP either directly or through phospholipase C (PLC) activation and diacylglycerol (DAG) production. DAG activates protein kinase C and SMC contraction. Phospholipase A₂ (PLA₂) has also been shown to play a role through the synthesis of 20-HETE (hydroxyeicosapentaenoic acid) from arachidonic acid (AA). Finally, activation of RhoA in association with caveolin-1 (Cav-1) and of Rho-kinase allows sensitization of the contractile apparatus to calcium with modulation of myosin light chain phosphorylation (pMLC) as a critical downstream event.
**SMAD3**, which are associated with Loeys–Dietz syndrome, do not increase but rather limit transforming growth factor (TGF)-β signaling in vitro.\(^14\) In that regard, it is interesting to note that TGF-β activity regulates the arteriolar myogenic response,\(^15\) suggesting that reduced TGF-β signaling in Loeys–Dietz syndrome may impair microcirculatory tone. Other TAAD-related mutations affecting ECM components (eg, COL3A1) have not been related directly to alteration of vascular tone. However, it is intriguing to note that COL3A1 may couple GPR56, an atypical G-protein–coupled receptor, to the G\(_{αq12/13}\) family of G proteins and may activate RhoA,\(^16\) a prominent pathway with essential role in the generation of vascular tone.

**Interstitial Fluid Pressure**

As summarized above, given the major roles of microfibril/collagen network in the regulation of Pif,\(^12,13\) both direct (fibrillin-1 \(FBNI\) in Marfan syndrome, collagen type III A1 \(COL3A1\) in Ehlers–Danlos syndrome, collagen type III A1 \(COL1A\), etc) and indirect (genes involved in TGF-β signaling) alterations of ECM components are expected to alter Pif. Depending on the context, both \(β1\) and \(β3\) integrins may modulate the generation and maintenance of Pif.\(^17,18\) Contraction of connective tissue is also modulated through force generation by its cellular components. Therefore, alteration in force generation (eg, \(MYLK\)) may significantly impair Pif. Intriguingly, recent data indicate that TGF-β activity also regulates aldosterone levels, and sodium and water retention,\(^19\) suggesting that reduced TGF-β signaling in Loeys–Dietz syndrome or inhibition of TGF-β signaling in Marfan, may both impair microvascular tone and promote interstitial fluid accumulation.

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**Alteration of Microvascular Tone and Interstitial Pressure as Major Determinants of Aortic Dissection**

On the basis of this knowledge, we propose that a decrease of vascular tone (intrinsic or iatrogenic) upstream of or within the aortic vasorum will lead to alteration of blood flow regulation and increased transmural pressure, which will promote the development of intraparietal (adventitial and medial) edema (and potentially hemorrhage, dependent on the degree of alteration of microvessel integrity), which will instigate the process of aortic dissection. Any associated alteration of ECM that prevents an increase of Pif will further promote and sustain intramural edema. Additional alterations in draining lymphatics\(^20\) (including reduced myogenic tone\(^21\) or abnormal pulse transfer\(^22\)) or in force components controlling transcapillary fluid flux\(^23,24\) will act as aggravating factors. Finally, alterations in endothelial cells and glycocalyx (as it may be the case at branching arterial sites or in areas of low shear stress) would alter vessel permeability and increase the porosity and hydration of the medial interstitium, particularly in response to increased intravascular pressure,\(^25–28\) and would further promote interstitial edema formation.

**Pathophysiological Predictions of the Model**

Aortic dissection is believed to originate with a tear in the intima.\(^29–31\) However, a true intimal rupture may be absent in a substantial percentage of aortic dissections. Vasa vasorum only penetrate the outer third of the thoracic aortic media.\(^32\) Thus, the model presented here predicts that a large percentage if not most aortic dissections in humans will...
start by the formation of an intramural edema and a small tear in the outer third of the media. Recurrence and coalescence of small medial tears will eventually induce aortic medial delamination, which may culminate in the formation of a false channel.

This hypothesis is supported by the presence of leaky neovessels in the media of human TAADs and by recent extensive histopathologic analyses suggesting that most (20 out of 21) aortic tears originate in the outer third of the media alongside the vasa vasorum.43

The model also provides an intriguing explanation for the detection of foci of GAGs in the outer media of diseased aortas, which are considered to induce significant stress concentrations and intralamellar Donnan swelling pressures.35,36 The mechanism behind increased detection of GAGs is unknown, but the current paradigm refers to increased GAG production.37,38 The model presented here suggests an additional or alternative mechanism whereby access of fluid to the interstitial space (secondary to alteration of microvascular tone and Pif) will inevitably induce swelling of the osmotically active and underhydrated GAGs, leading to expansion of the vessel area occupied by GAGs. This would limit the increase of Pif (particularly in case of abnormal microfilbrill/collagen network) and would alter stress transfer within the aortic wall, potentially initiating a vicious circle. It is also interesting to note that GAGs may modulate local generation of several active modulators of capillary leakage.44

Because vasa vasorum are normally absent from the human abdominal aortic media, a further implication of the model is that medial dissection would be a rare event in the human abdominal aorta, a prediction supported by ample clinical observation. However, adventitial remodeling (with abnormal lymphatic drainage, inflammatory changes, and neoangiogenesis) may become more prominent in the latter setting.41,42 This would also apply to aortas of small animals (eg, rodents) where we propose that additional factors (eg, hypertension, increased sodium/chloride and water reabsorption, and abnormal endothelial function) may combine together to ultimately increase interstitial fluid retention in adventitia and outer media, the major process that would instigate aortic dissection.

**Clinical Predictions and Implications of the Model**

A potential power of the model is its suitability to preclinical and clinical testing.

A recent study showed that pressure-induced intramural delamation was associated with accumulation of interstitial fluid in aortic media of mice with disrupted TGF-β signaling and could be prevented in part by pharmacological improvement of SMC contractility ex vivo.46 The results are consistent with the present hypothesis on the role of ECM contraction in limiting interstitial fluid retention and delamination. However, further studies are needed to examine whether the same results may apply to subvessel-level SMC contraction in human thoracic aorta.

A major direct implication of the model relates to its potential to predict the effectiveness of medical strategies in patients with TAADs. According to the hypothesis presented here, drugs that lower blood pressure while preserving or increasing microvascular tone and Pif would limit the occurrence of aortic dissection. In contrast, drugs that further lower microvascular tone and Pif or promote interstitial fluid accumulation would be ineffective or may even precipitate aortic dissection.

As such, the model may support the effectiveness of β-blockers in reducing aortic root dilatation in patients with Marfan syndrome and suggests that they could be useful in limiting vascular complications in other TAADs. Interestingly, a recent pilot trial reported a protective effect of β-blockers in patients with Ehlers–Danlos syndrome.49

In contrast, the model predicts that calcium channel blockers, which substantially inhibit myogenic tone, would be ineffective or might accelerate disease progression and aortic dissection in TAADs. This hypothesis merits serious consideration. Interestingly, it is in line with the results of a recent observational and retrospective study by Doyle et al who showed that the use of calcium channel blockers in patients with Marfan syndrome and other forms of TAADs may be associated with increased risk of aortic dissection and need for surgery, in comparison with the use of other antihypertensive agents. However, after a series of mechanistic experimental studies in Marfan mice, Doyle et al attributed the increased risk of aortic dissection to enhanced activation of protein kinase Cβ pathway and showed that the clinically available antihypertensive agent hydralazine both limited protein kinase Cβ activation and normalized aortic growth in Marfan mice. This led the authors to consider hydralazine as an appealing alternative therapeutic strategy for Marfan syndrome.50 In contrast, the model presented here does not support the use of hydralazine in the human setting of Marfan disease or other TAADs given the expected inhibitory effect of hydralazine on myogenic tone. Similarly, our model also suggests that the recent failure of losartan in Marfan patients may be due, at least in part, to the inhibitory effect of angiotensin type 1 receptor blockade on myogenic tone. Thus, we think that modulation of blood pressure in TAAD patients should be considered in light of potential side effects that may result from excessive inhibition of myogenic tone. In the future, other pathways involved in maintenance of vascular integrity and regulation of vascular tone, for example, selective sphingosine 1 phosphate–signaling pathways, may be worth of consideration as potential adjunctive therapeutic targets.

Treatment with diuretics may limit the expansion of interstitial fluid and may constitute a therapeutic option. However, treatment with high-ceiling diuretics (eg, bumetanide and furosemide) should be considered with caution because they may reduce myogenic tone through inhibition of Na-K-Cl cotransporter (NKCC)1.56 Mineralocorticoid receptor antagonists (eg, spironolactone, eplerenone) could also reduce NKCC1 activity and myogenic tone, although to a lower extent.57,58 This is supported by the fact that selective deletion of mineralocorticoid receptor SMCs does not alter myogenic tone in adult young mice. An appropriate diuretic should therefore show a favorable profile between its beneficial effects on interstitial fluid accumulation and its potential deleterious impact on myogenic tone.
Other strategies to modulate Pif may be worth of investigation. This could be achieved through modulation of interstitial fluid flux and interstitial fluid accumulation, while maintaining arterial tone and interstitial pressure play a major role in the progression of TAADs and the initiation of aortic dissection. An effective therapeutic strategy should aim to limit hemodynamic stress on the large arterial conduit, reduce transcapillary fluid flux and interstitial fluid accumulation, while maintaining or increasing microvascular tone.

In conclusion, we propose that alterations of microvascular tone and interstitial pressure play a major role in the progression of TAADs and the initiation of aortic dissection. An effective therapeutic strategy should aim to limit hemodynamic stress on the large arterial conduit, reduce transcapillary fluid flux and interstitial fluid accumulation, while maintaining or increasing microvascular tone.

Acknowledgments
We thank Bernard Lévy for helpful discussions. We apologize for not being able to reference all primary evidence because of lack of space.

Sources of Funding
This study was supported by British Heart Foundation, United Kingdom, and Institut National de la Santé et de la Recherche Médicale, France.

Disclosures
None.

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Highlights

• Genetic mutations that predispose to thoracic aortic aneurysms and aortic dissections affect components of the extracellular matrix and proteins involved in cellular force generation.

• We propose that those alterations lead to reduced myogenic tone in vasa vasorum and defective regulation of interstitial fluid pressure in the aortic wall.

• Those events create increased accumulation of interstitial fluid, and induce intramural swelling pressures and significant stress concentrations around foci of glycosaminoglycans, which may promote aortic wall delamination and dissection.

• Strategies that limit hemodynamic stress on the aortic wall, reduce transcapillary fluid flux and interstitial fluid accumulation, while maintaining or increasing myogenic tone, should be effective in the prevention of aortic dissection.
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Arterioscler Thromb Vasc Biol. 2016;36:1742-1747; originally published online July 21, 2016; doi: 10.1161/ATVBAHA.116.307909
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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Low Flow  
Low Pressure  

*High Pif  
Low fluid flux  

Normal aorta

Low force generation  
Low myogenic tone  
(ACTA2, MYH11, PRKG1, MYLK, FLNA, PDK, etc.)  
Alteration of collagen, microfibrils or elastic fibers (COL3A1, FBN1, TGBR1/2, etc.)

Low Pif  

High transcapillary fluid flux  

Aorta in TAADs

*Low myogenic tone  

High Flow  
High Pressure  

Edema  

GAGs

*Normal myogenic tone  

Low Flow  
Low Pressure  

Low fluid flux  

VV