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The Potential Roles of Von Willebrand Factor and Neutrophil Extracellular Traps in the Natural History of Hypertrophic and Hypertensive Cardiomyopathy

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Abstract

Inflammation is often applied broadly to human disease. Despite its general familiarity, inflammation is highly complex. There are numerous injurious, immune and infectious determinants, functional elements and signaling pathways, ranging from genetic to epigenetic, environmental, racial, molecular and cellular that participate in disease onset and progression, phenotypic heterogeneity, and treatment selection and response. In addition, inflammation can be tissue and organ specific, adding a layer of complexity to achieving a detailed and translatable understanding of its role in health and disease. The following review takes a close look at inflammation in the context of two common heart diseases, hypertrophic cardiomyopathy and hypertensive cardiomyopathy.

Keywords

Tissue-specific inflammation; Von Willebrand Factor; neutrophil-derived extracellular traps; hypertrophic cardiomyopathy; hypertensive cardiomyopathy

Introduction

Inflammation is an area of significant interest and importance in human cardiovascular health and disease. There are several reasons that include, but are not limited to the following: first, inflammation is a dynamic and complex genetic, epigenetic and cellular process involved with many cardiovascular diseases, disorders and conditions; and second, targeted therapies can be selected and tested to determine their impact on measurable

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processes and tissue phenotypes. Our interest in tissue-level inflammation in hypertrophic cardiomyopathy (HCM) in adults and early hypertensive cardiomyopathy (HTCM) in youth is the foundation for the following review focused on the potential role of inflammation, Von Willebrand Factor (VWF) and leukocyte-derived extracellular traps (ETs) in these common cardiomyopathies associated with significant morbidity, mortality and health care expenditures worldwide[1].

Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a common condition affecting one of every 500 adults. It is transmitted as an autosomal dominant trait and caused primarily by mutations in genes encoding sarcomere proteins; however, mutations [2] in genes involved in calcium signaling or development of the myocardial cytoskeleton can also cause HCM. Mutations in the cMyBP-C gene *(cMyBP-C3)* are associated with ~34% of all HCM cases [3] and affect ~60 million people worldwide [4]. While many cases are heritable, spontaneous mutations also occur. There is considerable phenotypic variability, ranging from asymmetric septal hypertrophy and isolated apical hypertrophy to moderate left ventricular dilation and end-stage dilation [5]. Sudden cardiac death is a feared and devastating complication that occurs most often in patients with ventricular fibrosis. Indeed, even small areas of fibrosis increase the risk substantially [678].

Hypertensive Cardiomyopathy

Systemic hypertension, most often referred to as essential hypertension, is the most common acquired cardiovascular risk factor in the world-affecting from one-half to two-thirds of adults greater than 40 years of age[9]. Essential hypertension can also begin during youth [10–12]. Regardless of age of onset, elevated blood over time can cause target organ damage that includes concentric left ventricular hypertrophy and impaired diastolic relaxation collectively also known as hypertensive cardiomyopathy (HTCM) [13]. Similar to HCM, HTCM is characterized by ventricular remodeling and myocardial fibrosis on cardiac MRI [14]. Coronary microvascular disease also occurs in both conditions [15]. The extent of fibrosis and its diffuse pattern, as well as heightened global longitudinal strain tends to be greater in HCM than HTCM, as does the heterogeneity of phenotypic expression [16].

Von Willebrand Factor: Fundamental Constructs and Biology

Von Willebrand Factor (VWF) is a large and complex glycoprotein ranging in size from 600,000 to 20 million Da that participates actively in platelet *adhesion* to either injured or disrupted vascular surfaces, platelet *activation* and platelet aggregation, particularly under high shear-stress conditions [17]. VWF also possesses a wide variety of other biological properties that will be summarized in subsequent sections.

The VWF gene is located on the short arm p of chromosome 12 (12p13.2) with 52 exons that span 178kbp. Genetic regulation of plasma and vesicular VWF is complex and influenced by several non-VWF quantitative trait loci (reviewed in [18]. Analysis of the VWF

sequence can also be complicated by a non-coding partial VWF pseudogene located on chromosome 22q11.2, which shares 98% sequence homology with exons 23-34 of VWF [19].

Following its synthesis within bone marrow megakarocytes and endothelial cells (EC), VWF is packaged in EC Weibel-Palade Bodies (WPB) and platelet α-granules and released employing several distinct pathways. The *first* represents a constitutive pathway linked directly to synthesis. This applies primarily to vascular EC. The *second* is a regulated pathway that responds to EC or platelet stimulation by histamine, leukotriene D4, platelet-activating factor, vascular permeability factor, the terminal component of complement, epinephrine, fluid mechanical forces, blood vessel dynamic forces, factor VIIa, thrombin and fibrin [20]. In addition, WPB rapidly translocate to the cell surface of vascular EC following activation [21]. The size, complexity, function and redundancy of VWF, coupled with its secretion and activation underscore its biological and teleological relevance in human health and disease.

VWF associates with the luminal surface of EC by vitronectin receptors and constituents of the WPB itself[22] (summarized in a subsequent section). The nature (or stimulus) of release also carries important functional ramifications. For example, thrombin stimulates the appearance of high-molecular weight multimers (HMWM) of VWF with increased functionality e.g. platelet binding and aggregation capacity among others [23].

Neutrophil Extracellular Traps: Pathogenesis, Structure and Function in Human Disease

In response to strong stimulation, neutrophils, and to a lesser degree monocytes and eosinophils, release extracellular traps (ETs), consisting of DNA and histones in a process known as NETosis. The process involves histone (H) citrullination (Cit) by peptidylarginine deiminase (PAD)-4, chromatin unwinding, breakdown of nuclear membranes and cytolysis [2425]. There is also a vital or non-lytic NETosis, wherein nuclear materials (DNA and histones) are released without cellular suicide [26, 27]. Tissue-level inflammation and NETs have been detected in humans with acute myocarditis and in animal models of autoimmune myocarditis [28]. The bioactive proteins released from NETs that cause tissue injury include: MPO (myeloperoxidase), NE (neutrophil elastase), matrix metalloproteinase (MMP)-9, high-mobility group box (HMGB)-1, proteinase (PTN)-3 and properdin [29–31]. To the best of our knowledge, the presence and functionality of NETs in HCM have not been investigated previously. The available information suggests that a heightened sterile inflammatory response is *necessary*, but in the absence of NETs may not be *sufficient* to cause tissue injury, fibrosis and ventricular remodeling.

NET Architecture

Pires et.al [32] investigated the nanoscale properties of NETs following vascular injury. They combined fluorescence and atomic force microscopy and identified branching filament networks arranged in an organized porous structure with openings of 0.03+/− 0.04 μm^2. Topological profiles were up to 3.0 ± 1.0 nm in height, supporting a “beads on a string” model of nucleosome and chromatin strands. Typical branch lengths of 153+/− 103 nm

appeared as rigid rods and height profiles of naked DNA and NETs of 1.2 +/− 0.5 nm were indicative of extensive DNA super-coiling throughout the NETs. The existence of DNA duplexes was supported by force spectroscopy with the occurrence of force plateaus that ranged from ~65 pN to 300 pN. The findings suggested that NETs function as microscopic mechanical sieves, with elastic properties that stem from their DNA-protein composition and size. We hypothesize that these central features may be particularly important and determine their ability to interact with other cells, including myocytes, fibroblasts and platelets in diseases and conditions like HCM and HTCM (Fig. 1).

VWF in Hypertrophic Cardiomyopathy and Hypertensive Cardiomyopathy

Von Willebrand factor levels are elevated in patients with HCM [33] and correlate with progressive disease and major comorbidities. The same is true among patients with HTCM and end-organ damage [34]. The responsible mechanisms and potential impact on cardiovascular disease progression are summarized below:

Vascular and Endocardial Injury

The early stages of HCM and HTCM are characterized by heightened endocardial and vascular wall stress that causes altered pulse wave velocity, EC injury and increased permeability [353336–38]. Vascular EC under stress exhibit changes in adhesion receptors for leukocytes and platelets. Endothelial cell WPB maturation, secretion of adhesive proteins, local environmental responses and exocytosis are altered substantially [39]. In turn, VWF kinetics are impacted, leading to increased surface concentrations and availability for platelet, leukocyte and leukocyte-derived extracellular trap (ET) (primarily of neutrophil origin or NETs) binding to injured or altered EC and endocardial cells.

Weibel-Palade Body Mobilization

Weibel-Palade bodies are endothelial cell-derived elongated organelles that contain VWF and numerous functionally active proteins [40]. Their unique structure and architecture is due to the arrangement of VWF as tubules and, based on local environmental factors, strings of varied length [22, 41, 42]. In the presence of vascular and tissue injury, WPBs themselves can be organized and exocytosed in an elongated state, highlighting the dynamic nature and overall structural-functional plasticity of these organelles (Fig. 2).

In a two-step process, histones and DNA have been shown to promote WPB release from vascular endothelial cells and the former induces the release of VWF from WPB as well [4344]. The ability of histones to stimulate WPB release is caspase-, Ca^{2+} - and charge-dependent. Peripheral blood levels of WPB-released proteins, including VWF correlate with both the degree and duration of inflammation [43].

Endothelial cell activation causes a rapid exocytosis of WPB [45]. In addition to the release of VWF, WPB can release a host of adhesion molecules, micro-vesicles and proteins involved directly with maintaining vascular tone, vascular permeability, membrane/protein transport and Ca^{2+}- ATPase signaling [46]. Mechanical stretch is associated with inflammation and the exocytosis of WPB through vascular endothelial growth factor (VEGF) receptor-2 signaling

pathways [47]. Vascular stretch in a hypertensive mouse model was shown to cause a rapid release of VWF and interleukins (IL) [4849].

Tissue-Specific Inflammation and VWF

Acute States

Platelets play a pivotal role in the recruitment of neutrophils to sites of inflammation as well as their subsequent trans-endothelial migration. VWF may be a key determinant of this event. Anti-VWF antibodies have been shown to stabilize the endothelial barrier [50]. The interaction of platelets and neutrophils, to include NET formation, occurs through several signaling pathways independent of platelet aggregation and thrombosis (reviewed in Pitchford) [51]. Accumulation of platelets and VWF within microvessels is a unifying step for EC cell activation, impaired vascular integrity, leukocyte recruitment, trans-endothelial migration, tissue inflammation and target organ injury [52] (see subsequent section).

While platelets dissociate from leukocytes during trans-endothelial migration in high shear stress conditions, platelet-leukocyte complexes can remain intact under low mechanical stress [53]. Inflammation and its widely varied triggers provoke the formation of ultra-large VWF fibers, which are immobilized on the EC surface and transform to highly adhesive strings under shear conditions [54].

Not all neutrophils release NETs. The available evidence suggest that 20-25% of neutrophils have the capacity to release NETs and those that do are characterized by low density (low density granulocytes) (LDGs) found to co-localize with mononuclear cells [55]. LDGs and NETs found within the circulation and tissues of patients with inflammatory myopathies correlate with disease activity and the degree of muscle damage. In addition, LDGs isolated from the peripheral blood display a condition-specific gene signature and have heightened NET formation capabilities [56]. An ability of sera from inflammatory diseases to stimulate NET formation in neutrophils obtained from healthy controls supports the premise of tissue and condition-specific inflammatory responses [57].

Tissue-specific inflammation may be directly influenced by tissue-specific EC and vascular beds that typically possess a genetic signature identical to the surrounding tissues [58]. In heart failure with reduced ejection fraction, inflammation tends not to be a dominant histological feature and more often reflects concomitant metabolic inflammatory stimuli than a disease-related sterile inflammation as is often the case with HCM and HTCM {reviewed in Paulus)[59]. Tissue-specific NET release is an area of interest and ongoing investigation for our group.

The hemodynamic-mechanical stress that characterizes HCM and HTCM leads to impaired autophagy [60]. Consequently, there is an accumulation of damaged organelles, proteins and DNA that trigger danger-associated molecular patterns (DAMPs), NETs, alarmins and mitochondrial DNA that activate inflammatory pathways [61]. Self-renewal of resident cardiac macrophages regulate the accumulation of these injurious molecular and protein materials and limits remodeling following myocardial injury[62].

Biomarkers of myocardial remodeling, fibrosis and endothelial cell dysfunction are increased among proteins with HCM and HTCM [63], correlate with cMRI-determined left ventricular mass and inversely correlate with global myocardial perfusion [64].

In a murine heterozygous myosin-binding protein C (*cMyBP-C3*) mutation model subjected to thoracic aorta constriction (TAC), members of our group showed that cardiomyocytes undergo marked hypertrophy and have impaired force generation, higher Ca^{2+} sensitivity, and blunted length-dependent increase in force generation. RNA sequencing revealed several differentially regulated genes between *cMyBP-C3* heterozygotes and wild-type mice, including regulators of inflammation (e.g. heat shock protein β−1 and tumor necrosis factor), hypertrophic response, myocardial fibrosis and ventricular remodeling. [65]. Employing a well-established homozygous *cMyBP-C3* mutation model, the group also observed that upregulation of inflammatory pathways and evidence of proinflammatory M1 macrophages on immunofluorescence staining of tissues persisted over time as the phenotype was transitioning from a hypertrophic state to a dilated cardiomyopathy [66]. There are several initial triggers for inflammation in HCM, however, the available data suggest that oxidative stress stemming from disorganized sarcomere structure and contractile dyssynchrony are pivotal [67] early contributors [68].

Our group performed RNA-sequencing on peripheral blood samples obtained from adolescents with either hypertension or normal blood pressure [67, 69]. In adolescents with elevated blood pressure and increased left ventricular mass by transthoracic echocardiography compared to those with normal blood pressure and normal left ventricular mass, there were 270 differentially expressed genes (unpublished data).

Employing a gene ontology database, differentially expressed gene clusters in several biological pathways, including inflammation, EC activation, systemic blood pressure regulation, vascular remodeling and regulation of apoptosis were identified in participants with elevated blood pressure and increased left ventricular mass. A KEGG pathway analysis revealed upregulation in TGF-β, relaxin and tumor necrosis factor (TNF)-a signaling and mitophagy. There was concomitant upregulation in miRNA involved with cardiovascular remodeling (JAK-STAT, TNF-α, TGF-β and Wnt), fibrosis (fibroblast growth factor receptor) and blood pressure (adrenergic signaling in cardiomyocytes and nitric-oxide synthase) (unpublished data).

In support of the scientific premise that sarcomere abnormalities as observed in HCM with accompanying myocyte dyssynchrony lead to oxidative stress, tissue inflammation and fibrosis are several observations: first, there is a reciprocal augmentation of myosin contractility in response to a step-wise loss of cMyBP-C3 [70] and, second, mavacamten, a myosin modulator that reduces steady-state ATPase activity by inhibiting the rate of phosphate release of β-cardiac myosin-S1 and stabilizing an autoinhibited state of two-headed cardiac myosin [71], suppresses the development of ventricular hypertrophy, cardiomyocyte disarray, and myocardial fibrosis and attenuates hypertrophic, inflammatory and fibrosis gene expression in mice harboring heterozygous human mutations in the myosin heavy chain [72].

Chronic States

Levels of VWF antigen predict clinical events among patients with chronic heart failure [73] independently of other well-known risk factors. A relationship between reduced ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity, responsible for cleavage of VWF HMWM, and heart failure has also been reported [74].

A traditional view of inflammation places neutrophils at the forefront of acute inflammation, but down-plays their participation in chronic inflammatory states. The study of NETs and NETosis has changed this long-held paradigm significantly as remnants of leukocytes continue to play important roles in chronic inflammation, tissue injury and fibrosis after the parent cells are no longer present histologically [75].

Neutrophils are released into the blood in response to a wide variety of stimuli and circulate for 6-8 hours. By contrast, once they undergo endothelial cell transmigration and enter surrounding tissues, neutrophils can survive for days in duration [76]. In tissues, NETs occupy a space several-fold larger than the cells from which they were derived and cause tissue injury. Additional injury can occur if the concentration of NETs is high and clearance mechanisms are not fully operational. The bioactive proteins released from NETs that are responsible for tissue injury include: MPO (myeloperoxidase), NE (neutrophil elastase), matrix metalloproteinase (MMP)-9, high-mobility group box (HMGB)-1, proteinase (PTN)-3 and properdin [29].

While NET formation is most robust in acute inflammatory states, chronic inflammation is also characterized by the presence of functional NETs. Several well-known examples include chronic obstructive pulmonary disease, cystic fibrosis and systemic lupus erythematosus [77]. The same may be true in HCM and HTCM where ongoing hemodynamic-tensile wall stress, oxidative stress, and tissue ischemia from impaired perfusion-energetics, small-vessel vasculopathy and reduced capillary density for the existing ventricular mass are operative (see subsequent section). This is an area of ongoing research in our laboratory.

Platelet Intercellular Transfer

An important question when contemplating tissue-specific inflammation is the portal of entry from the intravascular to the extravascular space. As summarized previously, trans-endothelial migration of leukocytes plays an important role. In addition, platelets contain a major source of growth factors and inflammatory mediators that can also participate.

Platelets contain functional RNA that can be transferred to other cells in a process referred to as horizontal transfer (reviewed in Clancy) [78]. The transfer of platelet cytosolic RNA to nucleated cells increases protein translation and biological effects at the vascular level and, if the recipient cell undergoes trans-endothelial migration, at the tissue level [79]. An example of the functional signature of platelet horizontal transfer and its biological and pathological effects is hepatocyte proliferation that follows platelet internalization by hepatocytes and functional transfer of RNA in non-alcoholic fatty liver disease [80]. Platelet micro-vesicles are also an important source of RNA that can be transferred to a variety of cells, including

neutrophils, T lymphocytes, monocytes, macrophages and smooth muscle cells (reviewed in Edelstein)[81].

VWF-NET Interactions

As summarized previously, VWF associates with the luminal surface of EC and is activated by shear stress, inflammatory cells and secreted proteins [40].

VWF Anchors NETs to the Vessel Wall and to Inflamed Tissues

NETs "trap" and kill bacteria [82], but, as discussed previously they can also injure host tissues. In animal models of methicillin-resistant Staphylococcus Aureus bacteremia, neutrophils release NETs into the liver vasculature, where they remain anchored to the vascular wall by VWF and display significant neutrophil elastase proteolytic activity [83]. Similar processes have been described in the heart. For example, ischemia-reperfusion injury of the myocardium causes an increase in plasma nucleosomes, but in addition, there is abundant neutrophil infiltration at the tissue level and citrullinated histone H3 at the site of injury. [84, 85]. We posit that targeting VWF-mediated leukocyte recruitment or NET adhesion may represent novel therapeutic strategies to reduce cardiac injury in HCM and HTCM.

Potential VWF Binding Site(s)

In studies performed by Grassle et.al [54] both functionalized surfaces and intact cell layers of human umbilical vein EC were perfused with isolated, protein-free DNA or leukocytes from whole blood at distinct shear rates. Isolated DNA and DNA released by stimulated leukocytes was able to bind to *shear-activated,* but not *inactivated* VWF. As previously described, VWF is known to mediate leukocyte adhesion to the vessel wall and facilitate extravasation into the perivascular space, a process that is augmented by DNA released from leukocytes and likely involved in tissue-level injury [50]. The VWF *A1 domain* is believed to be the binding site for NETs-specifically for DNA (Fig. 3) [86].

NET Clearance and Regulation

While rapidly evolving, the available data suggest that NETs have an early protective effect following acute injury, particularly from infectious causes. This might not be the case in all tissues and in the setting of fungal infections [87]. Emerging data also suggest that the regulation of NET formation and clearance is equally *protective* and *necessary* to prevent compounding injurious effects from an initial injury and an excessive inflammatory response. For example, NETs that are *not* removed from inflamed tissues or from the circulation can be cytotoxic or trigger an autoimmune response [88]. DNase is one mechanism for NET degradation, but in physiologic amount's is *not* sufficient for complete regulation. Human monocyte-derived macrophages and dendritic cells [89] are able to engulf NETs in a cytochalasin D-dependent manner. Moreover, recombinant C1q, found in human plasma, opsonizes NETS and facilitates their clearance. Once within macrophages, NETs are degraded in lysosomes. The regulation of NETs within tissues requires both normal macrophage activity and DNase, but ADAMTS-13 activity is important for the degradation of VWF and thus the ligand that allows binding of any residual NETs to site(s) of injury. We

are currently investigating whether failure at any step in the process could lead to persistent tissue injury, inflammation and fibrosis in HCM and HTCM.

The modulation of cardiac macrophages is increasingly recognized for its importance in chronic disease, including heart failure. Tissue-resident and recruited monocyte-derived macrophages subsets play a particularly important role in HCM, HTCM and ischemia-related cardiac remodeling [90]. Phenotypic expressions of disease are influenced by differing macrophage populations. For example, M1 macrophages, in general, are programmed to regulate inflammation while M2 macrophages play a pivotal role in reparative processes [91]. Members of our group have previously shown in a murine model of homozygous *cMyBP-C3* mutation that upregulation of inflammatory pathways and evidence of proinflammatory M1 macrophages on immunofluorescence staining of tissues persisted over time as the phenotype was transitioning from a hypertrophic state to a dilated cardiomyopathy [66].

VWF and Histone Interactions

The composition of NETs includes a high concentration of histones [92]. Analysis of VWF modulators and the marked charge asymmetry of amino acid sequences within the *A1 domain* has led to an electrostatic model for VWF modulation. Both VWF and the 39/34-kDa VWF fragment bind strongly to histones [92]. Histone binding to VWF neither activates nor inhibits VWF binding to platelets; however, in complex with DNA or platelets, attachment to the vessel wall facilitates its injurious effects. In addition, VWF derived from EC and platelets can trigger NET formation and subsequently bind to histones forming platelet-NET attachments. Histones activate platelets through toll-like receptor (TLR)-dependent mechanisms to generate the release of polyphosphates [93], which, in turn, increase both tissue-level inflammation and tissue injury.

VWF and Small Vessel Disease

Patients with HCM and HTCM commonly have microvascular disease and dysfunction referred to as *small vessel vasculopathy* [9495]. In many instances, the overall perfusion network of microvessels is insufficient for the burden of myocardial mass, while in others there is attendant dysfunction with impaired endothelium-dependent and-independent regulation of vessel tone [96, 97]

Pathological studies have shown that intra-mural arteries undergo typical changes observed during vascular injury (fibromuscular dysplasia), which include myofibroblast transdifferentiation and activation, medial layer hypertrophy, smooth muscle cell intimal infiltration and elaboration of extracellular matrix, and breakdown of the internal elastic membrane [98]. Studies using human subendocardial biopsies have shown that HTCM is associated with precapillary arteriole smooth muscle hyperplasia, whereas HCM hearts are characterized by a decreased density of such vessels without significant changes in the medial layer content [99]. This difference may be one of many factors that explain the phenotypic variations in the site(s) and extent of myocardial hypertrophy between the conditions.

The investigation of myocardial remodeling in HCM and HTCM requires an in-depth understanding of early microvascular remodeling, dysfunction and thrombosis [100]. The current evidence in humans indicates an early remodeling process with increases in circulating cathepsin S and endostatin that, in turn, correlate with diastolic dysfunction. Evidence of transcriptional changes in the pre-hypertrophic stage have been described in a thick filament mouse model of HCM with upregulation of TGFB1, CTGF, and periostin and also downregulation of SERCA2a, phospholamban, and sarcolipin—markers of fibrosis and cellular remodeling, respectively [101].

Early changes in myocardial energetics in HCM and HTCM may play an important role in the developing phenotype. Because diastole is crucial for myocardial perfusion, the subendocardium is prone to ischemia with increased flow velocities required to supply this layer. As discussed in a prior section, increased flow velocities with high shear stress mediates VWF release from WPB and flow-induced arterial remodeling. The extensive collateral network of the coronary microvasculature tends to mask the effects of vascular remodeling found dispersed throughout the myocardium in HCM and HTCM [102].

Betoni et. al investigated 20 patients with congenital thrombotic thrombocytopenic purpura (cTTP) who are unable to cleave VWF multimers because of a genetic *ADAMTS13* deficiency [103]. Using assays of ex *vivo* serum-induced C3 and C5b-9 deposits on EC, the group documented that in cTTP, complement is activated via the alternative pathway on the cell surface. The abnormality was corrected by restoring ADAMTS13 activity in cTTP serum, which prevented VWF multimer accumulation on EC. The same effect was observed with administration of an anti-VWF Ab. In mechanistic studies the group found that VWF interacted with C3b through its three *type A* domains and initiated activation of the alternative pathway, although assembly of active C5 convertase and formation of the terminal complement products C5a and C5b-9 occurred solely on the VWF-A2 *domain.* They showed that in ADAMTS13 deficiency, VWF-mediated formation of terminal complement products, particularly C5a, altered normal EC properties and induced microvascular thrombosis in a perfusion system.

Zheng et.al employed engineered microvessels to determine the pathological responses to EC activation [104]. They found that endothelial-secreted VWF was able to assemble into thick bundles or complex meshes, depending on the vessel geometry and flow characteristics. Assembly was most robust in vessels of diameter ≤300 μm subjected to high shear stress, strong flow acceleration and with sharp turns. VWF bundles and webs bound platelets, leukocytes and erythrocytes, obstructed blood flow and also caused erythrocyte fragmentation.

Microvascular Thrombosis, Interstitial Fibrosis and Ventricular Remodeling

In the hearts of patients with HCM and HTCM, NET-mediated microvascular thrombosis may contribute to inflammation, further NET formation, tissue fibrosis and ventricular remodeling [84]. Microvascular thrombosis [105] has been reported in several animal models of cardiomyopathy and typically occurs in the presence of active myocardial inflammation. This can be either infectious or non-infectious (sterile) in nature. The role of VWF in sterile

inflammation is of particular interest to our group. Witch et.al described a profound inflammatory response and microvascular dysfunction in a mouse model of HCM employing TAC that led to fibrotic remodeling and cardiac failure. The down-stream effects were prevented by pre-TAC administration of rhADAMTS13. Early after the induction of pressure overload under rhADAMTS13 pre-treatment, there was less EC-associated VWF, fewer platelet aggregates, and decreased TGF-β1 levels than in vehicle-treated mice. There was also significant preservation of cardiac function and decreased fibrotic remodeling with rhADAMTS13 administration [106]. The findings suggest that in the setting of pressure-overload-induced cardiomyopathy and associated sterile inflammation, decreased VWF-mediated recruitment of platelets, a major source of activated TGFβ1, and preservation of microvascular perfusion substantially attenuate ventricular fibrosis and remodeling.

Human in vitro Heart Tissue Models for Investigation

The ability to model human organs and specific diseases, disorders and conditions is vital to our understanding and advancements in both diagnosis and treatment. Human pluripotent stem cells (hiPSC), derived from patients with HCM and HTCM can be differentiated into cardiomyocytes to fabricate 3-dimensional cardiac organoids. They can be decorated with vascular channels or vascular organoids can concomitantly be differentiated via mesoderm induction of hiPSC aggregates composed of endothelial cells and pericytes to form a human vascular system for experimentation [107, 108]. The complementary application of *in silica* modeling [109–111], systems-based pharmacology [112] and platelet-like particles[113] or nanovesicles [114] could also offer a means to more fully and accurately capture the complexity and dynamic nature of HCM and HTCM from molecular, biological and physiological perspectives (reviewed in Becker [1]). Gene manipulation of the cells, to include in the precardiac development stage of the first and second heart fields may provide insights [115]. Similarly, iPSCs from PAD deficient mice[116]developed to spheroids/oranoids under proinflammatory and profibrotic conditions may provide a model in which to test therapeutic agents. We have developed human cardiac organoids in our laboratory for the investigation of HCM and HTCM.

Concluding Thoughts and Future Directions

Acute and chronic inflammation are common in cardiovascular disease, including cardiomyopathies such as HCM and HTCM. Changes in cardiomyocyte structure, intra- and extra-cardiac myocardial hemodynamic forces, microvascular dysfunction and capillary density-myocardial mass mismatches with impaired perfusion and cyclic ischemia-reperfusion injury predominate the pathobiology landscape. Sterile inflammation, through several well-characterized mechanisms triggers NET formation with inherent cytotoxic, pro-inflammatory and pro-fibrotic effects. VWF is a pivotal participant in each step to include NET binding and both its localized (vascular and tissue-level) and systemic detrimental effects.

The collective response to acute and chronic inflammation, coupled with impaired macrophage-and dendritic cell-mediated NET and DAMP protein regulation may represent key determinants of the phenotypic expression of HCM and HTCM. Heart and vascular

organoids are available and, coupled with computer-based modeling and systems-based pharmacology could provide important mechanistic information. We believe that a more in-depth understanding of the roles of VWF, tissue-level inflammation and NETs would advance the field and, most importantly, benefit patients affected by these common heritable and acquired diseases.

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Highlights for Review

The pathogenesis and natural history of hypertrophic cardiomyopathy and hypertensive cardiomyopathy are considered to be distinct and highly complex. While they share some phenotypic similarities, the potential role of inflammation in these common and life-threatening heart diseases has not been defined fully. We provide an overview and offer a theoretical construct for the potential contributions of Von Willebrand factor and neutrophil extracellular traps.

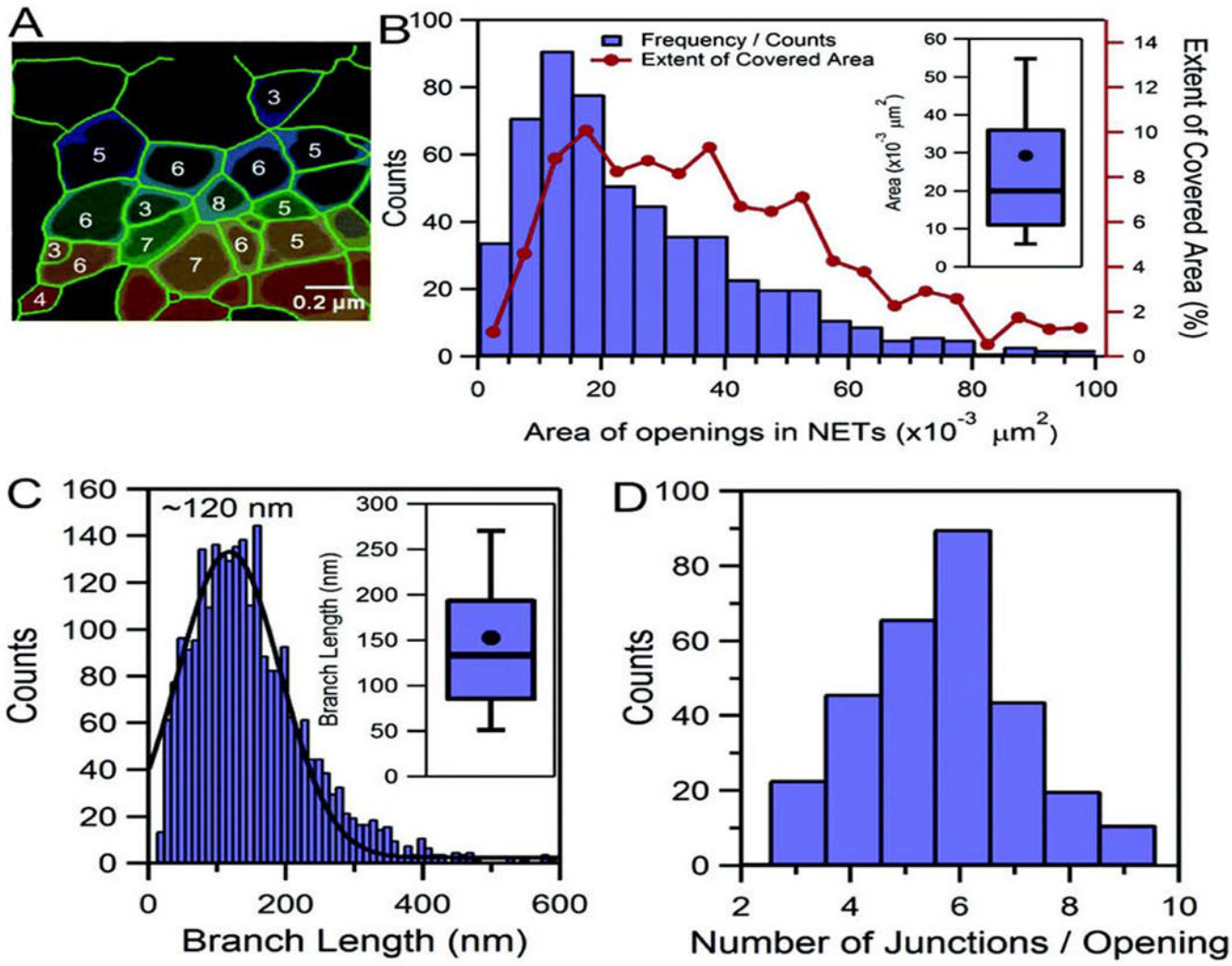

Figure 1:

Filaments that only partially resemble NETs, exhibiting several orders of branching, but otherwise not forming a porous matrix (A). More advanced states of NET decomposition, in which DNA appears to exhibit a much more relaxed conformation (B). NETs treated with thermally inactivated trypsin (C). In partially digested NETs, the average branch length was 278 ± 189 (s.d.) nm, indicating that proteins within NETs participate in regulating branch-length and architecture. From Pires RH. Nanoscale 2016; 8: 14193-14202. With permission.

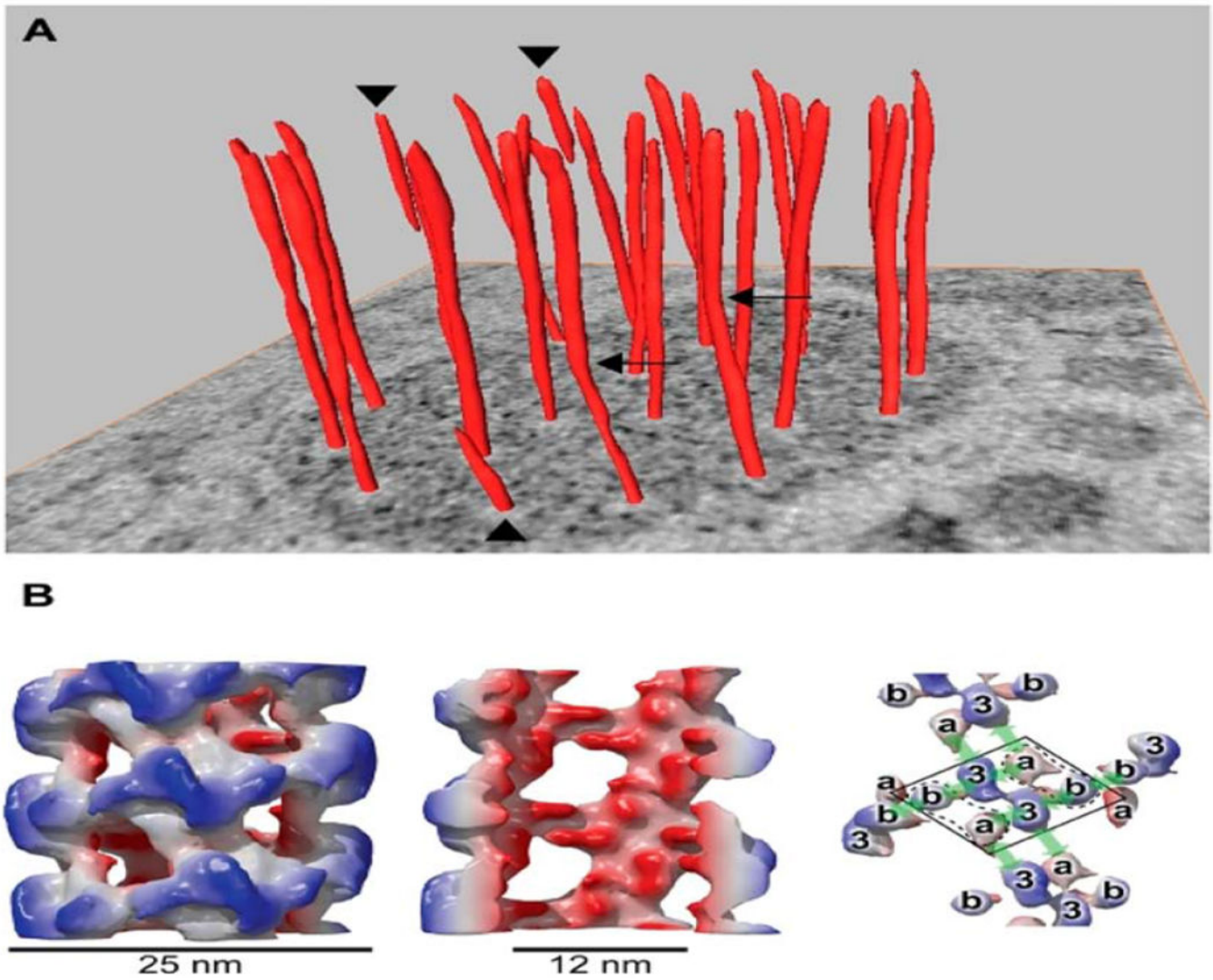

Figure 2:

Helical arrangement of VWF in tubular striations of WPB. (A) orderly twisting of the tubules within WPBs is well-illustrated on a tomographic slice. Several tubules (see arrowheads) stop halfway into the WPB and others (arrows) display kinks. In panel B, a reconstruction of VWF tubules is shown with an outside diameter of 25nm (left), cutaway view of the internal diameter of 12nm (middle) and different domains within the helix (right). From Valentijn KM et al. Blood 2011; 119: 5033-5043. With permission. VWF (Von Willebrand Factor), WPB (Weibel-Palade Bodies).

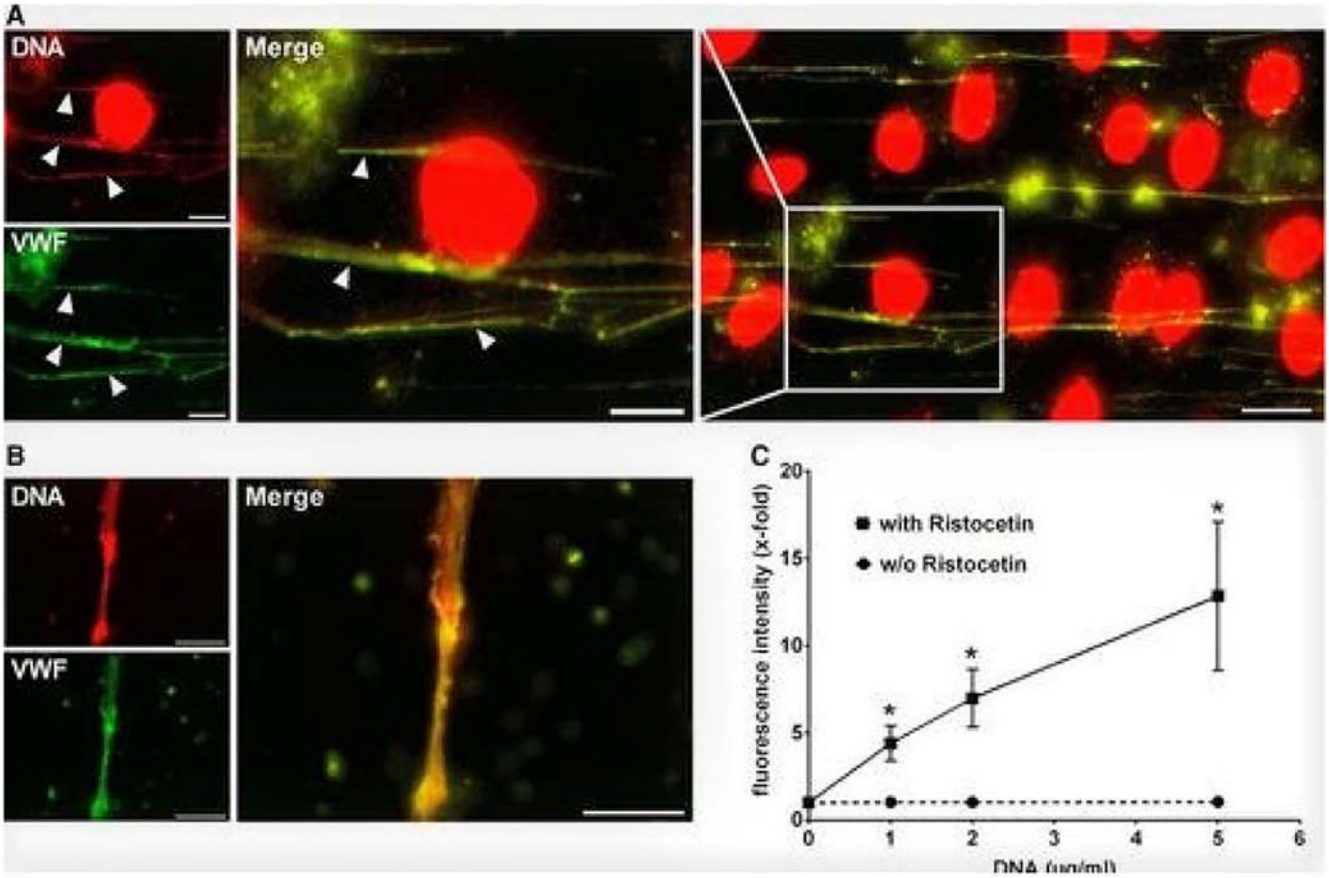

Figure 3:

DNA binds to Von Willebrand Factor (VWF) under shear conditions. Panel A. DNA is localized with VWF strings (arrowheads) (Green represents VWF and red DNA); Panel B. Isolated DNA is perfused over VWF at 2 dyne/cm^2. Binding only occurs with activated VWF. Panel C. Biding of DNA to VWF is Ristocetin-dependent. From Grassle S et al. Arter Thromb Vasc Biol 2014:34:1382-1389. With permission. Von Willebrand Factor (VWF).