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Tissue-Level Inflammation and Ventricular Remodeling in Hypertrophic Cardiomyopathy

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Introduction

Hypertrophic cardiomyopathy (HCM) is a cardiac condition caused primarily by sarcomere protein mutations with several distinct phenotypes, ranging from asymmetric septal hypertrophy, either with or without left ventricular outflow tract obstruction, to moderate left ventricular dilation with or without apical aneurysm formation and marked, end-stage dilation with refractory heart failure. Sudden cardiac death can occur at any stage, including in the absence of a clinically evident phenotype.

The phenotypic variability and heterogeneous remodeling observed in HCM is the end-result of many factors to include loading conditions and wall stress; however, inflammation, apoptosis, proliferation of matrix proteins, myocyte hypertrophy, micro-vascular dysfunction and thrombosis, and impaired or dysfunctional regulatory pathways intrinsic to the myocardium contribute as well. The pathological transition from hypertrophy to dilation has not been studied extensively and we believe that points of divergence for cardiac-specific hypertrophic, inflammatory and fibrotic pathways may be the key to understanding distinct phenotypes of the disease. Similarly, a better understanding of these events and their association could be the basis of new diagnostic tools and treatment paradigms.

The following brief review summarizes our current understanding of the developmental stages, pathobiology and natural history of HCM with emphasis on the expanding role of tissue-level inflammation that may include extracellular traps (ET) in myocardial fibrosis and ventricular remodeling (Figure 1). We also present several working hypotheses for ongoing and future investigation.

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Scope of the Problem and Scientific Premise for Investigation

Hypertrophic cardiomyopathy (HCM) is a cardiac condition affecting one of every 500 persons in the general population. The condition most often transmits as an autosomal dominant trait caused by mutations in genes encoding sarcomere proteins; however, spontaneous mutations and other heritable causes of HCM, including mutations in genes involved in calcium signaling or development of the myocardial cytoskeleton must also be considered¹. The specific gene mutation carries phenotypic and prognostic implications; however, the underlying mechanism(s) have not been determined fully.

The classic phenotypic description of a hypertrophied myocardium with a small left ventricular (LV) cavity and normal-to-supernormal systolic function has undergone a revision over the past two decades to include several well-characterized phenotypic variations such as HCM localized to the septum or apex with or without apical aneurysms and progressive left ventricular remodeling, dilation and systolic dysfunction often referred to as “end-stage HCM”. In fact, many clinicians and scientists active in the field have called for a recognition of the “endophenotype spectrum” of disease in HCM, ranging from regional myocardial hypertrophy to papillary muscle anomalies, mitral valve elongation and either localized or diffuse fibrosis in a model of convergence that concomitantly serves as a foundation for focused investigation².

The available evidence suggests that serious clinical events, including sudden death are more likely to occur during pathologic points of transition and phases of remodeling^{3, 4}. While progressive myocyte hypertrophy represents a stage of remodeling and in many patients represents a dominant phenotype, our area of research interest and focus in the current review will be on early stages of disease characterized by tissue-level inflammation and intra-mural vasculopathy that we believe collectively and ultimately trigger ETs, platelet-derived transforming growth factor (TGF)- β expression, fibroblast activation and fibrosis and ventricular remodeling.

Phenotypic Distinction in Men and Women

A consistent observation in studies of HCM is a male predominance of ~ 60%. Among individuals with mild left ventricular hypertrophy, women may be under-represented due to diagnostic bias since they tend to have less hypertrophy and fewer electrocardiographic abnormalities. They are more likely to have outflow track obstruction, smaller LV cavity size, stroke volume and LV mass, higher indexed maximum wall thickness (IMWT) and more hyperdynamic function as gauged by LV ejection fraction⁵. The male predominance persists across a broad degree of left ventricular hypertrophy and clusters at the severe end of the spectrum, suggesting that additional factors linked to the sex of affected individuals play a role in clinical penetrance and expression of disease. The hearts of men with HCM tend to have relatively more inflammation and fibrosis on histological examination than their women counterparts, but women more frequently develop heart failure symptoms and, in addition, experience lower survival rates than men⁶ {Lorenzini, 2019 #8560}.

Inflammation in HCM

Kuusisto and colleagues⁷ described the presence of low-grade inflammation in the myocardium of patients who had a single HCM-causing mutation in the alpha-tropomyosin gene (TPM1-D175N) and who developed cardiac hypertrophy, myocyte disarray and myocardial fibrosis. They further described invading inflammatory monocytes undergoing cardiac trans-endothelial migration in tissue samples and concomitantly increased plasma levels of proinflammatory cytokines, including interleukin (IL) IL-6 and C-reactive protein (CRP) in patients with HCM compared to age-matched controls. There was a significant association between the degree of myocardial inflammatory cell infiltration and fibrosis in histopathological samples and myocardial late gadolinium enhancement (LGE) by cardiac magnetic resonance imaging (cMRI). Peripheral blood levels of high-sensitivity (hs) CRP, tumor necrosis factor (TNF)- α and interleukin (IL)-1 were associated with LGE and histopathological myocardial fibrosis, respectively.

The basis of or trigger for early inflammation in HCM has not been determined to date and is a focus of our laboratory research. The initial trigger(s) may be linked to cardiomyocyte disarray with mechanical stress and sarcomere injury, mitochondrial oxidative stress and micro-vascular disease with tissue injury.

Do Leukocyte-derived Extracellular Traps Participate in HCM Phenotypes?

Evidence has emerged supporting a key role of leukocyte extracellular release of its nuclear contents that physically “trap” cells, including erythrocytes and platelets, as well as fibrinogen and a variety of proteins actively engaged in proinflammatory, prothrombotic and profibrotic processes, all operational in human cardiomyopathies including HCM. In response to strong stimulation, neutrophils (and occasionally monocytes and eosinophils) release (ETs), consisting of DNA and histones in a process that involves histone (H) citrullinated (Cit) by peptidylarginine deiminase (PAD)-4, chromatin unwinding, breakdown of nuclear membranes and cytolysis⁸⁹. PAD-4 is normally found in the nuclear, cytoplasm and cytoplasmic granules of neutrophils and eosinophils where it contributes to inflammatory and immune responses. This is achieved through post-translational modification with citrulline¹⁰. The chromatin fibers serve as a strong scaffold for platelet adherence and activation and thrombus formation through several complementary mechanisms (Table 1).

Tissue-level inflammation and neutrophil ETs or NETs have been detected in humans with acute myocarditis and in animal models of autoimmune myocarditis. Weckbach and colleagues¹¹ identified NETs in paraffin-embedded sections of endomyocardial biopsies obtained from 14 patients diagnosed with myocarditis. NETs were detected by the co-localization of the H2A–H2B–DNA complex, myeloperoxidase and H3Cit. They also reported that inhibition of NET formation in a murine model of autoimmune myocarditis reduced inflammation in the acute phase of the disease. By inhibiting the midkine (MDK), a cytokine involved in many different processes, including growth and differentiation, repair, migration, inflammation and NET formation both locally and following its secretion through extracellular interactions with a wide range of receptors (reviewed in¹² with an anti-

N-MK antibody, there was reduced tissue-level NET formation, infiltration of PMNs and fibrosis. A detailed protocol for the detection of tissue-level NETs is available¹³.

Mitochondrial DNA may also represent a trigger for inflammation¹⁴ after its internalization by cardiomyocytes and resulting upregulated expression of proinflammatory signaling pathways.

Platelets can trigger NET formation and bind to histones to form platelet-NET attachments¹⁵. Histones activate platelets through toll-like receptor (TLR)-dependent mechanisms to generate the release of polyphosphates¹⁶, which are prothrombotic¹⁷, increase inflammation and causes tissue injury. Employing Western Blot and immunoprecipitation, a dose-dependent increase of fibronectin levels was identified in samples from stimulated neutrophil-like cells. Coimmunolocalization studies employing confocal microscopy revealed that fibronectin and H3Cit co-localized inside the lattice structure of NETs. DNA-histone complexes orchestrated the adsorption of different cell types and, in turn, provided specific binding sites on a common substrate, fibronectin, for integrin-mediated cell adhesion of neutrophils, platelets and endothelial cells¹⁶.

Von Willebrand factor is believed to be a “linker molecule” for the binding of NETs to areas of vascular and tissue injury (Figure 2). Inflammation provokes the formation of ultralarge VWF fibers, particularly under shear stress conditions, that are immobilized on the endothelial cell surface where they are transformed to highly adhesive strings. The interaction between DNA and VWF takes place at the A1-domain of VWF¹⁸. In animal models of ischemia-reperfusion, myocyte injury causes an increase in plasma nucleosomes (chromatin and histone bundles), abundant neutrophil infiltration and H3Cit at tissue-specific locations of injury. This observation supports an active transport of leukocytes from the intravascular to the extravascular space through a process known as diapedesis, with subsequent NET release. It also provides a foundation for investigating tissue-level inflammation, NETs and VWF in human disease to possibly include HCM. Extracellular chromatin released through NETosis worsens the effect of ischemia-reperfusion injury on the ventricular myocardium and inhibiting both VWF-mediated leukocyte recruitment and chromatin removal reduces ischemia-related cardiac damage¹⁹. This suggests that targeting VWF and DNase might be an effective treatment approach under these conditions.

NETs and Microvascular Thrombosis

NETs represent part of a continuum of sterile inflammation and thrombosis^{20–31} (Table 1). In the context of HCM, NET-mediated microvascular dysfunction and thrombosis may contribute to tissue injury, at least in part associated with recurring cycles of ischemia and reperfusion, inflammation, fibrosis and ventricular remodeling^{32, 33}.

In a Wistar rat model of ischemia-reperfusion injury, DNase and tissue plasminogen activator (t-PA) reduced NET density, “no-flow” area in the ischemic region, infarct size and left ventricular remodeling. The beneficial effect did *not* occur in rats treated with DNase or t-PA alone³⁴.

Microvascular thrombosis³⁵ has been observed in several animal models of cardiomyopathy and typically occurs in presence of active myocardial inflammation. Witsch and colleagues³⁵ investigated the impact of a recombinant human (rh) disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13 (ADAMTS13) on cardiac remodeling, scarring, and contractile function under conditions of chronic LV pressure overload. This particular model of heart failure, caused by ascending thoracic aortic constriction (TAC), produces a profound inflammatory response and microvascular dysfunction, resulting in fibrotic remodeling and cardiac failure in a murine model of heterozygous myosin-binding protein C gene mutation³⁶. Mice treated with either rhADAMTS13 or vehicle were assessed for coronary vascular inflammation and ventricular function at several postsurgical time points, as well as for cardiac fibrosis after 4 weeks. Early after induction of pressure overload under rhADAMTS13 treatment, there was less endothelial-lumen-associated VWF, fewer platelet aggregates, and decreased activated transforming growth factor (TGF)- β 1 levels than in vehicle-treated mice. There was also significant preservation of cardiac function and decrease in fibrotic remodeling with rhADAMTS13 administration. The findings suggest that decreased VWF-mediated recruitment of platelets, the predominant source of activated TGF- β 1, and preservation of microvascular perfusion substantially attenuate ventricular fibrosis and remodeling.

Kormaz and colleagues studied intravascular thrombosis, inflammation, NET formation and the coagulant state within the microvasculature following skin burns in two animal models and in patients³⁷. Intravascular thrombi, the presence of intravascular NETs and the number of tissue factor (TF)-positive blood vessels within the burn wound were determined. In rats, a significant increase in intravascular thrombi and TF expression was observed 14 days post-burn, that in a majority of animals coincided with intravascular NETs. In swine, a significant increase in intravascular thrombi and TF expression was found over time (up to 60 days post-burn) that coincided with NETs. In the eschar of burn wound patients, a significant increase in intravascular thrombi was found, and a majority coincided with the presence of NETs as early as 12 hours from the acute event.

Patients with an acute ischemic stroke and a concomitant elevation of cardiac specific troponin often have widespread myocardial, cerebral and pulmonary microthrombosis with H3Cit expressed within the thrombi. Thalín and colleagues³⁸ reported that plasma obtained from patients with cancer contained high H3Cit levels and an increase in granulocyte colony-stimulating factor, known to “prime” neutrophils toward NETosis. Acute thrombotic microangiopathies (TMA) are also characterized by excessive microvascular thrombosis and are associated with markers of NETs in plasma. Jiménez-Alcázar showed that NETs generated *in vitro* were efficiently degraded by plasma from healthy donors. However, NETs remained stable after exposure to plasma from TMA patients. The inability to degrade NETs was linked to a reduced DNase activity in TMA plasma. Plasma DNase I was required for efficient NET degradation and TMA plasma showed decreased levels of this enzyme³⁹. The DNA and histone components of NETs may be the primary drivers of microvascular thrombosis¹⁵. An association between tissue-level inflammation, NET formation, microvascular thrombosis and heightened disease activity has been reported in patients with Crohn’s disease⁴⁰.

Neutrophil Susceptibility to Form NETs Increases with Age

The natural history of HCM while heterogeneous is influenced by the age of those afflicted, with the likelihood of myocardial fibrosis and ventricular remodeling increasing over time. Martinod and colleagues⁴¹ examined blood and neutrophils from young (8–16 week) and old (20–27 months) mice obtained from the National Institutes of Health's C57BL/6NIA Aged Rodent Colony. Platelet-neutrophil complexes were found more often in older mice than in younger mice, suggesting a greater extent of platelet activation and platelet–neutrophil interactions in the older animals. Using H3Cit as a biomarker of PAD4 activity and neutrophil priming for NETosis, the investigators quantitated basal levels of circulating H3Cit⁺ cells and found that a greater percentage of neutrophils were primed toward NETosis in older mice. They also found that after incubation, either with or without stimulation, neutrophils from older mice had a greater propensity to form NETs as quantified by microscopy.

NETs and Myocardial Fibrosis

The release of NETs, orchestrated by PAD4, damages organs in acute inflammatory models. NETosis is more prevalent in aged mice; however, reduced fibrosis occurs in the hearts and lungs of aged PAD4^{−/−} mice compared with PAD4^{+/+} mice. An increase in left ventricular interstitial collagen deposition, a decline in systolic and diastolic function and remodeling occur only in PAD4 proficient mice, and not in PAD4^{−/−} mice. In an experimental model of cardiac fibrosis, LV pressure overload-induced NETosis and significant platelet recruitment occurred in PAD4^{+/+}, but not within PAD4^{−/−} myocardium. DNase 1 and PAD4 deficiency similarly protected hearts from fibrosis, supporting an important role for NETs and chromatin in cardiac fibrosis and ventricular remodeling⁴² as occurs in HCM.

Antigen–antibody complexes are capable of inducing NET formation. FcγRIIIb-induced NET formation presents distinct kinetics from phorbol 12-myristate-acetate (PMA)-induced NET formation, suggesting differences in signaling. Because FcγRIIIb also induces a strong activation of extracellular signal-regulated kinase (ERK) and nuclear factor Elk-1, TGF-β-activated kinase 1 (TAK1) has been implicated in ERK signaling. Aleman and colleagues determined the role of TAK1 in the signaling pathway activated by FcγRIIIb leading to NET formation. FcγRIIIb was stimulated by specific monoclonal antibodies, and NET formation was evaluated in the presence or absence of pharmacological inhibitors. The antibiotic LL Z1640–2, a selective inhibitor of TAK1 prevented FcγRIIIb-induced NET formation⁴².

In the early phase of inflammation, proinflammatory cytokines, including tumor necrosis factor (TNF)-α, IL-6^{43,44}, interferon (IFN)-γ and TGF-β1 are secreted by macrophages, lymphocytes and platelets, contribute to cardiac injury. In addition, these locally produced cytokines have been found to possess both autocrine and paracrine properties that can influence neighboring tissues to enhance vascular permeability recruitment of invasive leukocytes and reactive oxygen species (ROS) production.

NETs and Remodeling of the Myocardium

Inflammatory proteins are involved in the molecular pathogenesis of acute lung injury (ALI). The complement cleavage product, C5a, is a peptide acting as a potent anaphylatoxin.

C5a triggers the formation of NETs and the release of histone proteins to the extracellular compartment during ALI. NETs activate platelets and augment the release of TGF- β 1, which then contributes to tissue remodeling during the later phases of ALI. Interception of C5a signaling or blockade of extracellular histones has shown promising protective effects in small animal models of ALI, rescuing the phenotype⁴⁵. A similar process may be operative within the myocardium among patients with HCM, however, ongoing research will determine potential similarities or differences.

Myofibroblasts treated with NETs demonstrate increased connective tissue growth factor expression, collagen production, and proliferation/migration. The fibrotic effects decrease after degradation of NETs with DNase, heparin or myeloperoxidase inhibitors. IL-17 expressed in NETs promotes the fibrotic activity of differentiated LF but not their differentiation, suggesting that priming by DNA and histones is essential for IL-17-driven fibrosis.⁴⁶

Concluding Thoughts and Next Steps

HCM, a cardiac condition affecting one of every 500 persons in the general population, is caused primarily by sarcomeric protein mutations with several distinct phenotypes, ranging from asymmetric septal hypertrophy, either with or without left ventricular outflow tract obstruction, to moderate left ventricular dilation with or without apical aneurysm formation and marked, end-stage dilation with refractory heart failure. Sudden cardiac death can occur at any stage, including in the absence of a clinically evident phenotype.

While the focus of HCM has traditionally been on cardiac hypertrophy, myocyte disarray and myocardial fibrosis, inflammatory and micro-vascular components to the condition and its phenotypic variability have received less attention. Animal models of HCM provide an opportunity to determine if and when tissue-level inflammation occurs, the underlying mechanisms that may involve ETs and whether it participates in the phenotypic variation of HCM-specifically, pathological remodeling and transition from a hypertrophic state, characterized by myocyte hypertrophy to a dilated state with diffuse myocardial fibrosis and impaired systolic performance (Figure 3). A better understanding of these events and their association through dedicated research programs could be the basis of a new treatment paradigm that targets one or more intermediate phenotypes and favorably impacts the outcomes of patients with HCM.

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Figure 1:

Proposed staged and general mechanistic events in the metamorphosis of hypertrophic cardiomyopathy to heterogeneous phenotypes, to include in some cases dilated, end-stage heart failure with heart failure and clinical events.

Figure 2:
The attachment of neutrophil extracellular traps (NETs) to areas of cardiomyocyte injury, vascular injury and tissue inflammation is mediated by Von Willebrand Factor (see text).

Figure 3:

Stages of hypertrophic cardiomyopathy that include an early phase characterized by tissue-level inflammation and neutrophil extracellular traps or NETs; an intermediate phase with platelet activation, microvascular thrombosis and transforming growth factor (TGF) β 1 signaling and a late phase of myocardial fibrosis, ventricular remodeling and ultimately heart failure.

Table 1

Neutrophil Extracellular Trap (NET)-derived molecules that link inflammatory and thrombosis phenotypes.

NET component	Potential Mechanism
DNA	FXII - dependent thrombosis ¹⁷
	Activation of intrinsic pathway of coagulation ¹¹
Chromatin	Platelet Activation ¹²
	Factor VII activation ¹³
Histones	Platelet aggregation and platelet-dependent thrombin generation ²²
	Platelet aggregation ²¹
	Inhibit protein C activation (and inhibition of FVa, FVIIIa) ²⁰
Elastase	Vascular injury and VWF binding ¹⁴
	TFPI Cleavage ¹⁶
	Platelet Adhesion ¹⁴
Cathepsin G	TFPI Cleavage ^{16,17}
	Fibrin deposition ¹⁸
	Endothelial Cell Injury ¹⁹
TF	Thrombin generation through extrinsic system of coagulation ¹⁷

TF (Tissue Factor); TFPI (Tissue Factor Pathway Inhibitor); F (Factor)