Defensin-chemokine heteromeric complexes derived from heterocellular activation—a possible target to inhibit CCL5 in cardiovascular settings

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Ischemic heart disease is one of the leading causes of death all over the world even after several medical technologies such as revascularization therapy including catheter intervention and surgery, and drug treatment have been developed. Among them, acute myocardial infarction (AMI) is caused by sudden occlusion of coronary arteries induced by atherosclerotic plaque rapture or vasospasm. Consequently, cardiomyocytes downstream of the culprit site are exposed to hypoxia and eventually die, which induces acute pump failure, fatal arrhythmia, acute valvular dysfunction, cardiac rupture and so on. Even after surviving this acute period, pathological cardiac remodeling changes left ventricular size, mass and function, and leads to several chronic complications such as heart failure and ventricular arrhythmia, which finally increases the mortality. Therefore, development of new treatment strategy for this life-threatening disease is required.

Hypoxia due to marked reduction of blood supply impairs barrier function of endothelial cells. As a result, vessel permeability is enhanced and leukocyte infiltration through vessel walls is subsequently augmented (1,2). Injured or dead cells and damaged extracellular matrix due to prolonged hypoxia release danger associated molecular patterns (DAMPs) which activate innate immune system to release inflammatory mediators such as cytokines, chemokines and adhesive molecules via pattern recognition receptors (PRRs) including toll-like receptors (TLRs) and subsequently recruit immune cells into the infarcted site (2,3). DAMPs also activate the complement cascade which induces further chemotaxis (2,4). Recovery of blood flow during this period induces reperfusion injury because the sudden supply of oxygen in this scenario with excess succinate released by damaged cells allows the reverse flow of electrons through complex II of the electron transport chain, succinate dehydrogenase, such that reactive oxygen species (ROS) are generated in excess (5). This ROS production amplifies tissue damage and additional activation of the complement pathway (1,2).

When blood supply to the tissue is markedly reduced, neutrophils are initially recruited into the ischemic zone. They release proteolytic enzymes and more ROS to perpetuate local cytotoxicity. They also secrete further inflammatory mediators and induce subsequent monocyte migration into the ischemic site (2,3,6,7). Humans and mice have at least two monocyte subsets. One is the classical inflammatory monocyte which is typically identified as $CD14^{+}CD16^{-}$ monocyte in humans and as $Ly6C^{high}$ monocytes in mice (which like human monocytes are $CD14^{+}CD16^{-}$ (8). The other subset is the non-classical inflammatory monocyte, which is typically identified as $CD14^{low}CD16^{+}$ in humans and $Ly6C^{low}$ monocyte in mice (9). These human and mouse monocyte subsets have gene expression similarities (8,10). After coronary ligation of mice, classical monocytes are recruited to the

infarcted site and perform proteolytic and inflammatory activity initially. When healing goes well, these monocytes differentiate into cells that at least transiently resemble non-classical monocytes that in turn become repair-promoting macrophages (11,12). In skeletal muscle injury, blockade of monocyte recruitment prevents effective healing (11). Often, however, recruited monocytes develop detrimental phenotypes, particularly when their recruitment leads to the replacement of cardiac resident macrophages which have inherent regenerative potential (13). In this case, inhibition of monocyte recruitment preserves the population of these resident macrophages and attenuates cardiomyocyte injury in mice (13) and chronic heart failure (14).

Chemokines are a group of chemotactic heparin-binding cytokines. Many chemokines like CCL2, which attracts CCR2-expressing classical monocytes, are released from the infarcted site and play a critical role in disease processes. Among the many chemokines characterized is CCL5 (also known as RANTES: regulated on activation, normal T cell expressed and secreted). CCL5 recruits monocytes, neutrophils and lymphocytes through binding to multiple chemokine receptors, CCR1, CCR3 and CCR5. Blocking CCL5 with a neutralizing monoclonal antibody attenuates neutrophil and macrophage infiltration in the ischemic site and improves survival and cardiac function after coronary ligation in mice (15). However, *Ccl5*$^{-/-}$ mice show a decrease in antigen-specific T cell proliferation and production of interferon gamma (IFN-γ) and IL-2 by T cells (16), and are immune compromised due to delayed viral clearance (17). Therefore, direct inhibition of CCL5 in a therapeutic setting may have an adverse effect on immune response against pathogen infection. Moreover, CCL5 is reported to be required for ischemia-induced angiogenesis (18), which can limit the healing effect of CCL5 inhibiting therapy. Thus, if it were possible to selectively affect certain activities of CCL5, therapeutic potential of targeting the role of CCL5 in cardiovascular disease would be greater.

A recent paper by Alard *et al*. raises such a possible, more selective approach (19). The authors noted that CCL5 secreted from platelets and human neutrophil peptide 1 (HNP1, α-defensin) derived from neutrophils form heteromers to enhance classical inflammatory monocyte adhesion and recruitment to cultured endothelial cells via CCR5. Neither CCL5 nor HNP1 alone is sufficient to augment monocyte recruitment and adhesion. Thus, the activation of different cellular players, platelets and neutrophils, during inflammation might lead to the formation of a defensin-chemokine complex, pointing to a high level regulatory process that might keep monocyte recruitment in check unless multiple activation checkpoints are crossed. Activated platelets aggregate during ischemia and reperfusion, augment tissue injury by releasing platelet-derived mediators and are transported to the inflammatory site by polymorphonuclear leukocytes (1,20). It has been known that platelets can release CCL5 (21). HNP1 belongs to the α-defensin family and is contained within azurophilic granules of neutrophils with other HNPs (HNP 2–4). When these granules fuse with phagosomes, HNPs are thought to be released to pathogen or phagocytic surfaces and cause disruption of microbial membrane to kill bacteria (22).

The authors realized that a novel means to affect CCL5-mediated recruitment of monocytes might be to block the interaction of CCL5 with HNP1. Thus, the group developed a short peptide (referred to as the SKY peptide) which specifically disturbed the interaction between human CCL5 and HNP1. When both human CCL5 and HNP1 were overexpressed in the murine heart by adeno-associated virus (AAV), classical monocytes showed exacerbated recruitment into the heart after ischemia-reperfusion of the left coronary. When cardiac function was assessed, left ventricular end-diastolic pressure (LVEDP), contraction velocity (dP/dt_{max}) and relaxation velocity (dp/dt_{min}) deteriorated. Administration of SKY peptides completely abolished the worsening of cardiac function in this context. By contrast, overexpression of either CCL5 or HNP1 alone had no augmenting effect on monocyte accumulation in the heart after the procedure.

These data are consistent with the possibility that use of the SKY peptide may allow for a more selective therapeutic to block monocyte recruitment in the context of myocardial infarction and ensuing heart failure. There are, however, several critical questions that the field must address beforehand. First, is it always desirable to inhibit classical monocyte recruitment, given that these cells can promote healing in some settings? Second, is the heteromeric complex formed by HNP1-CCL5 only acting to recruit monocytes or is it also required to act with CCL5 in mediating T cell functions in adaptive immunity or angiogenesis? Third, might HNP1-CCL5 inhibition also find utility to treat atherosclerosis by blocking monocyte recruitment to plaque? Finally, the authors point out that the *in vivo* data implicating HNP1-CCL5 heteromeric complexes arises from *in vivo* overexpression systems,

making it unclear if the heteromeric complexes truly assemble and function in natural settings. The latter is a critical issue that we suggest can be addressed by using non-invasive molecular imaging technique such as the development of SKY peptide-based positron emission tomography (PET) tracer to detect the formation of HNP1-CCL5 complexes in different organs and settings. More importantly, this PET reagent can be used not only as a diagnostic probe to read out the existence of a novel bioactive heteromer, but also provide key information about its dynamic concentration to better understand the therapeutic efficiency.

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Footnote

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