Review Article

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Insights into Cardiovascular Diseases: The Vicious Platelet-Immune System Loop

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#: Equal Contribution

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Abstract

Cardiovascular disease (CVD) is the leading cause of death today and a burden on a country's economic growth. Management of this disease continues to depend on dietary control and statins. However, the disease burden refuses to decline. It is probably because treatment regime addresses the effect and not the cause. Augmented LDL level has been traditionally perceived as the major risk factor contributing to the development of atherosclerosis, the main cause of several coronary ailments. Recent research also suggests that pre-existing chronic inflammation leads to oxidation of LDL and thus makes it pathogenic. This inflammatory repertoire, apart from leukocytes, also includes the anucleated cell fragments called platelets. Platelets conventionally associated with clotting phenomenon, also express array of inflammatory mediators creating a crucial link between immune response and thrombotic complications. In this review we will examine the role of inflammation as a primary causative agent of atherosclerotic as well as non-atherosclerotic cardiovascular diseases.

Keywords: Oxygenated blood, Deoxygenated blood, Immune response, Treatment, Atherosclerosis, Cardiovascular disease.

Introduction

Cardiovascular disease is a collective term that refers to maladies associated with the heart and the vasculature. The principal function of the heart is to simultaneously pump oxygenated blood to the tissues and deoxygenated blood to the lungs following a cardiac rhythm. This rhythm is initiated by electronic impulses emanating from the sino-artrial (SA) node and is crucially maintained by the myocardium [1].

Myocardial function is adversely affected by ischaemia, inflammation and enhancement in size of myocardial tissue fibers causing myocardial infarction. Ischaemia is usually a result of either coronary artery spasm or atherosclerosis. Coronary artery spasm refers to abrupt vasoconstriction in a major coronary artery as a result of dysregulated smooth muscle contraction. Psychological stress and few drugs can spike the release of hormones, epinephrine and norepinephrine, the main causative agents of these spasms [2]. Atherosclerosis however is a very complex process. It is an admixture of increased level of low-density lipoprotein, loss of endothelial integrity, oxidative stress, inflammation, thrombosis and ultimately plaque formation that leads to blood vessel occlusion. Inflammation is also triggered by infection in the heart muscle and autoimmune response against some cardiac proteins, thus contributing to non-atherosclerotic cardiac diseases such as Myocarditis, Endocarditis, Pericarditis and other inflammatory heart diseases [3].

It is thus evident that inflammation is an event central to cardiovascular diseases. It features as the cause, the consequence and even both simultaneously. Inflammation under its canopy has the leukocytes, the cytokines, the chemokines, granulocytes and a quasi-immune cell type, the platelets. The role of platelets has always been highlighted in clotting phenomenon and thrombosis. However the huge body of literature suggests that its role in regulating immune response and vice-versa remains underrated in cardiovascular diseases. In fact, neurological stress also affects platelet functions and is often the reason why stress is a risk factor for development of cardiac complications.

In this review, we will explore the role of inflammation in the development of atherosclerotic as well as non-atherosclerotic cardiovascular diseases. We will also try to understand how other immunological pathways exclusive to some common inflammatory diseases dispose the patients towards cardiovascular diseases. Apart from molecular mechanisms, since coronary diseases often require surgical intervention, brief inclusion of commentaries authored by experienced practitioners will also be a part of this review and how new avenues of treatment are being explored.

Atherosclerosis

Atherosclerosis is the term given to a plaque developed in the coronary arteries as a result of lipid deposition, endothelial activation, of inflammatory cells' accumulation, smooth muscle cell proliferation, calcification and connective tissue deposition [4]. However, these plaques may remain asymptomatic for a long period of time as the muscles in the vessels continue to provide

the expansion. These are stable plaques- content of extracellular matrix and smooth muscles cells is typically high. However some plaques are highly infiltrated by inflammatory cells and are rich in foam cells, called vulnerable plaques [5]. They tend to make the vessel wall in the affected area stiff and also more prone to rupture. Increased stiffness and reduced lumen size of the vessel, both partially obstruct the flow of blood due to stenosis. It can thus result in ischaemia. The plaques can even rupture and thus initiate the clotting cascade. These clots can completely obstruct blood flow and even travel to smaller arteries to create blockage resulting in Myocardial infarction.

Site of atherosclerotic plaque

The aorta, coronary and carotid arteries have been found to be highly susceptible in comparison to other arteries [6]. It mainly affects branching points of coronary arteries where the normal hemodynamic shear stress faces a deviation. Usually, the shear stress reduces thus increasing time of interaction between mediators of plaque formation and the glycocalyx of the endothelia. In fact in healthy individuals, basal LDL deposition is observed mainly at these branch points. Studies, both in vitro and in vivo have asserted that not increased but decreased hemodynamic shear stress is associated with development of atherosclerosis [7]. In the vessel, the exact location of plaque is observed to be the tunica intima, located between the endothelia and the tunica media. The inflammatory cells infiltrate these regions with the help of chemokine receptor-ligand coupling due to damaged endothelia [8].

Physiological risk factors of atherosclerosis

The most well explored risk factors associated with atherosclerosis are – blood lipid profile, chronic inflammation and hemodynamic shear stress. These are factors that are modulated by an individual's life style including food intake, sedentary habits, smoking and alcohol abuse, exposure to infectious agents and persistence of any autoimmune disease.

Onset of Atherosclerosis

Cholesterol is an indispensable component of the cell membrane as it helps in maintaining its fluidity. It is also a precursor to steroid hormones and bile acids, which help in fat emulsification. However cholesterol is a hydrophobic molecule and thus has minimal dissolution capacity in blood. It is synthesized mainly by the liver and thus dissemination throughout the body is very important. This is mediated by discoidal amphiphilic protein-lipid bodies called the Lipoproteins.

They are classified on the basis of density of lipids into very-low density (VLDL), low-density (LDL) and high-density (HDL). A high LDL to HDL ratio is considered to be a risk factor for atherosclerosis as oxidised LDL can lead to activation of endothelial cells beyond baseline which can lead to damaging consequences [9]. The endothelial cells lining the innermost wall of the arteries express array of molecules specific to stimulus. They express LDL receptors (LDLr) as well as scavenger receptors like SRA which bind to oxidised LDL (oxLDL). LDL bound LDLr is endocytosed and the components are released into the lysosome due to the acidic environment. This is a mode of cholesterol obtainment by the endothelial cells. But cholesterol accumulation beyond the need of the cell gives rise to oxidative stress i.e ROS generation [10]. One of the most stable ROS is Hydrogen peroxide and it can easily diffuse across cell membrane and oxidise the LDL. Source of LDL oxidation can however be multivarious including cigarette smoke, wood smoke, stress, infection induced inflammation etc. Oxidised LDL activates the endothelial cells to produce integrins like P-Selectin, ICAM-1 and VCAM-1 and chemokines like CCL-2 and CCL-5 [11]. This activation can also be brought about by cytokines in the milieu and abnormal hemodyanamic shear stress.

Monocytes are always present in blood circulation and they express surface molecules that aid in binding to the endothelia. However, under normal hemodyanamic shear stress these interactions are too feeble to initiate any inflammatory response. Monocytes express PSGL-1, VLA-4 and LFA-1, which under low shear stress readily interact with their cognate receptors on the endothelial cell surface [12]. These interactions thus help in monocyte tethering, rolling and firm adhesion. In fact, once intergrins bind to their cognate partners, their structure changes to enable tighter binding and increased response. CCR2 and CCR5, cognate receptors for CCL-2 and CCL-5 on monocytes respectively ensure firmer binding. It is postulated that these chemokines also help in extravastion of monocytes to the vessel wall but with no or feeble experimental evidence.

Monocytes can be classified into the following- classical monocytes ($CD14^{++}CD16$-), inflammatory monocytes ($CD14^{++}CD16^{+}$), and patrolling monocytes ($CD14^{+}CD16^{++}$). In CVD monocytosis is observed especially in the inflammatory subset ($CD14^{++}CD16^{+}$) [13]. The main function of the classical subset is to perform phagocytosis and differentiate into macrophages. While inflammatory monocytes produce inflammatory cytokines like TNF-α and play a major role in T cell activation and patrolling monocytes as the name suggests patrol the vessel wall. Recently a RNA Binding Protein Quaking has been identified to play a major role in monocyte differentiation and migration. It was observed that the level of this protein was increased in response to modified LDLs [14]. Endothelial cells upon activation upregulate MHC-class II molecules which transduce differentiation signaling in monocytes by ligating the CD4 molecules [15]. The classical monocytes, inflammatory monocytes and macrophages invade the sub-endothelial region through CX3CR1/CCL3 and LFA-1/ICAM-1 interaction. The macrophages uptake the oxLDL through scavenger receptors like CD68 etc and become cholesterol loaded and the vessel wall gives a streaked appearance due to fat streaks. These macrophages are called foam cells and are rich in atherogenic molecules like tissue factor and thus can make the plaque more vulnerable.

Platelets are cells crucial in the commencement of clotting phenomenon and atherosclerotic plaques are found to be rich in CD41 and P-selectin, platelet surface markers [16]. Activation of platelets is marked by upregulation of surface Pselectin, ROS and

Superoxide generation and change in shape. CD36, a scavenger molecule, present on platelet surface binds to oxidized LDL and activates Src proteins [17]. This in turn activates NOX2 and results in ROS and superoxide generation. It also activates Myosin Light Chain kinase which in turn phosphorylates Myosin Light chain thus enabling platelet contractility [18]. Under normal condition, the endothelial glycocalyx prevents platelet adherence. But upon injury, endothelia release the protein von Willebrand factor and also upregulates Pselectin [19]. These help in platelet binding through molecules, GPIb-IX-V complex present on platelet surface. Platelets then deposit CCL5 on endothelia and thus bind to CCR5 on monocytes and enable their tethering and rolling on endothelial surface. Platelet factor 4 (PF4) released by platelets give rise to a M4 kind of macrophage phenotype, it lacks CD163 but otherwise resembles M2, the anti-inflammatory phenotype of macrophages. Platelets bind to circulating monocytes through P-selectin/PSGL-1, GP1bα/CD11b-CD18 and ICAM2/LFA-1 [20]. Under inflammatory and thrombotic conditions monocytes in the blood flow are often embellished by platelets. Thus, platelets form a bridge between endothelia and circulating monocytes.

The endothelia, monocytes, macrophages and platelets together initiate the process of plaque formation. However, the maturity of the plaque to a disease prone lesion involves other immune cells like dendritic cells, granulocytes and adaptive cells. The specific role of platelets in activating these cells and cell surface molecules involved will be further described in the review in detail.

oxLDL or Inflammation, Who Came First?

As we try to understand the development of atherosclerosis, a major question arises, can abnormal LDL level alone commence plaque formation? The answer is subtle. It can alone orchestrate an inflammatory response only if native LDL binding can activate endothelia beyond a threshold. Evidence in this regard is poor. So the next question is what is the source of oxidation of LDL? Cigarette smoking and other xenobiotics certainly contribute. But, chronic smoking and exposure to xenobiotics also contribute to the development of an inflammatory milieu. Another crucial question is raised by the site of plaque formation in the vessel, which is the sub-endothelial region. Such infiltration is possible only if the integrity of the endothelia has been perturbed. Such perturbations, evidentially, arise from infection and inflammation. In fact cardiac surgeons often report that when they remove plaques surgically, they observe that the arteries look like they have been insulted regularly for a long period of time. Such insult can ensue only from chronic inflammation.

Causes of Chronic inflammation – CVD As A Co-Morbidity

Chronic inflammation can be caused by cigarette smoking, pollutants, processed food intake and other inflammatory diseases like Rheumatoid Arthritis (RA), Systemic Lupus Erythromatosus (SLE), Chronic Obstructive Pulmonary Disease (COPD), Depression and Type II Diabetes. Population studies have been performed on osteoarthritis(OA) patients to check parameters like chest pain(CP) and shortness of breath (SOB) (cardinal of CVD) to understand the role of noncardiovascular diseases as risk factor. It was observed that OA indeed augmented both CP and SOB [21].

Rheumatoid arthritis

It is often characterized by aberrant expression of MHC class II, an event often associated with CVD. CD4 T cells in SLE and RA often show down-regulation in the co-stimulatory molecule CD28. These cells release IFN-γ and also contribute to increase in arterial intimal thickness and thus predisposing to atherosclerosis. Increased ESR in RA has been associated with coronary complications but causality is yet to be established [22].

Depression

It is characterized by decrease in Serotonin level, which is known to decrease platelet aggregation. Depression also reduces Nitric oxide availability. NO helps in unwanted adherence of platelets to the endothelia. Arginine metabolism produces NO but in plasma of patients suffering from depression; Arginine metabolism gets skewed towards production of agmatine and thus reduced NO level [23].

Chronic obstructive pulmonary disease

COPD is an inflammatory disease of the lower respiratory tract interrupting the smooth flow of air. It is diagnosed by the drop in Forced Expiratory Volume 1 (FEV1). It can be caused by long-term cigarette smoking and prolonged exposure to pollutants. The inflammatory molecules are upregulated in COPD [24]. Gradually, it gives rise to systemic inflammation. Of which, IL-1, IL-6 and TNF-α significantly contribute to endothelial dysfunction and enhances endothelial permeability. Patients of COPD exhibit arterial stiffness which is directly proportional to frequency of exacerbations. This phenomenon has been correlated to increased level of troponin and N-terminal pro-brain natriuretic peptide (BNP) observed. Also, COPD contributes to coagulopathy due to elevated levels of factor VIIa, tissue factor and thrombin-antithrombin complex. These factors can destabilize the otherwise stable atherosclerotic plaques. Neutrophil secreted matrix metalloproteases (MMPs) not only disturb endothelial integrity but can also rupture pre-formed atherosclerotic plaques [25].

Diabetes

Type II diabetes is characterized by hyperglycemia and also hyperinsilunemia, which can ensue into superoxide generation, inflammation and neuropathy [26]. Superoxides are generated from mitochondrial respiration as a result of elevated intracellular glucose. Hypergylcemia leads to glycated proteins through polyol intermediates called Advanced Glycation End (AGE) products. The AGEs can ligate scavenger receptors present on endothelial cells and macrophages, leading to NF-κB translocation to the nucleus. Also, intracellular increased glucose level leads to Diacylglycerol upregulation thus activating Protein Kinase C (PKC) [27]. These events lead to the upregulation of adhesion molecules like ICAM-1, Eselectin on the endothelia and other pro-inflammatory mediators. Also the hexoaminase pathway that converts fructose-6 -phosphate to glucosamine-6-phosphate, leads to elevated levels of PAI-1 and TGFβ1. Hyperinsulinemia also upergulates coagulative molecules

Plasminogen Activator Inhibitor – 1 (PAI-1) and fibrinogen and reduces fibrinolysis [28].

Role of Surface Molecules on Activated Platelets in Immunomodulation

The major adhesion molecules expressed by platelets and their functions will be briefly described in the following paragraph.

P-selectin: A membrane glycoprotein required for the adhesion of platelets to the endothelium. It interacts with PSGL1, present on immune cells to form plateletleukocyte complex which enhances leukocyte adhesion at the site of inflammation [32].

Integrins: Transmembrane receptors, important for cell to cell and cell to extracellular matrix [ECM] interactions. Platelets express many integrins- αvβ3, α2β1, α5β1, αIIbβ3 or GPIIb/IIIa. GPIIb/IIIa not only plays a crucial in platelet adhesion, aggregation and thrombus formation but it has also an important role in the establishment of interaction with immune cells via CD40L [29,32].

CD40L (CD154): A transmembrane protein of the tumor necrosis factor [TNF] family. Other than immune cells it is also expressed by activated platelets and act as a central player in communication with dendritic cells, B cells and T cells and modulating their functions. It induces dendritic cell maturation, B cell isotypeswitching and also provides help to CD8+ T cell mediated responses [29,32,33]. Dendritic cells have significant effect on adaptive immune cell response. CD40L is thus important for establishment of platelet and innate immune interactiom and later with adaptive immune cells via dendritic cells during atherogenic inflammatory response [33].

CD40: A trimeric transmembrane protein and other than platelets is expressed by various immune cells, mainly antigen presenting cells i.e monocytes, dendritic cells and B cells [33,34]. Platelet expressed CD40 interacts with B cells, monocytes, macrophages, dendritic cells and endothelial cells via CD40L and stimulate multiple inflammatory immune responses. For example,CD40 interacts with activated T cell via CD40L, leads to platelet activation and secretion of RANTES, (Regulated on activation, normal T cell expressed and secreted) which enhances T cell recruitment and also triggers the synthesis of cytokines needed for various inflammatory immune response [34].

Intracellular Adhesion molecule 2 (ICAM2 or CD102): It is continuously expressed on platelets and on a wide range of immune cells. It plays a major role in adhesion and migration of leukocytes by interacting with its cognate ligands β2 integrins like LFA 1 [CD11a/CD18] and ICAM-grabbing non integrin [DC SIGN] which mediate neutrophil adhesion and migration [32,35].

TLRs (Toll like receptors): Type 1 integral membrane protein contains extracellular leucine rich repeats, a single transmembrane domain and intracellular IL-1 receptor domain. It is well known that it is expressed by most of the immune cells and recognize conserved molecular motifs expressed by pathogens. TLRs (1-9) expressed by platelets, act as connecting link between platelets and inflammatory response [33,36,37]. Interaction of platelets with LPS via TLR4 enhances its cross talk with neutrophils which leads to increased neutrophil inflammatory response i.e. neutrophil degranulation and releases extracellular trap [33].

Chemokine receptors: These are G-protein linked receptors that induce signal transduction by interacting with several cytokines and chemokines and involved in multi-functional cellular response. Platelets express range of chemokine receptors (CCR1, CCR3, CCR4, CXCR2, CXCR4, CXCR6 and CXCR7) to differential extents.

Role of activated platelet secretome in immunomodulation

Platelets house variety of secretory molecule in its granules. Platelets have mainly three types of intracellular granules: α-granules, dense granules and lysosomes.α- granules are the most abundant and contain multifunctional biologically active molecules like coagulation factors, chemokines, adhesive proteins, mitogenic factors and regulators of angiogenesis [38]. Secretory molecules contributing to the development of atherosclerosis will be discussed briefly in the following paragraph.

CXCL7: It binds to CXCR1 and CXCR2, helps in neutrophil migration.

CXCL4: It is the maximally expressed chemokine in atherosclerotic lesion of the carotid artery and has multiple atherogenic functions. It induces interactions of platelets and T cells and increases the secretion of IFN-γ. It prolongs monocyte and neutrophil survival by arresting apoptosis [39,40] and also increases the infiltration by macrophages and alters monocyte differentiation to M4 type in the absence of M-CSF which is associated with atherosclerosis [41].

CCL5: It activates CCR5 and CCR1 receptors and increase the vessel adherence of monocytes eg: Platelets release CCR5, which upon being deposited on the endothelium stimulates monocyte activation and adhesion [32,39].

CXCL12 (SDF-1): It is well known that CXCL12 is associated in the development of coronary artery disease (CAD) and is found to be highly expressed in the atherosclerotic lesion. It is expressed by various cell types including platelets. In CAD patients, platelets augment the secretion of CXCL12, CXCR4 and CXCR7. Therefore in these patients platelets get activated in an autocrine manner and further indulge the disease [42].

MIF (macrophage migration inhibitory factors): It has chemokine like structure and function and is involved in monocyte recruitment and arrest. It is known that recruitment, adhesion and transmigration of monocyte at vessel wall has important role in atherogenesis. MIF is secreted by T cells, B cells, endothelial cells (ECs) and smooth muscle cells (SMCs) during atherogenesis [43].

Inflammation and Non-atherosclerotic CVD Development

Myocarditis, inflammation of the myocardium is usually caused by infectious causing agents like viruses. But the inflammation often

continues to plague even after remission of the infection. Dilated cardiomegaly [DCM] is another cardiac disease characterized by enlargement in the size of heart due to thinning and stretching of ventricular walls. Several recent studies suggest that apart from infection [in case of Myocarditis] and hypertension [in case of DCM], disease development is ensued by autoimmunity too [3]. Usually autoreactive T cells are eliminated during thymic selection of T cells. However, few cells may escape the scrutiny and also body may be due to injury being exposed to neo-antigens. Autoimmune reaction is suppressed by the peripheral tolerance mechanism under such conditions. However, breakdown in tolerance does occur and contribute to disease pathogenesis. The exact mechanism(s) of breach of tolerance remains largely elusive.

Myocarditis and DCM patients often harbor autoantibodies against beta1 adrenergic receptors and mitochondrial antigens like M7. Anti-adenine nucleotide transporter (ANT) antibodies are also observed. Interestingly, ANT shares homology with CVB3 virus probably suggesting molecular mimicry in the development of autoimmunity. Proper functioning of the heart relies on cell action potential, maintained by Sodium and Potassium ions. Autoantibody against Na+- K+ ATPase detected in these patients contributes to malfunctioning of the heart and is probably due to breakdown in thymic selection [44-48].

Present prevention and disease management strategy and recent advancements

The present treatment regime focuses on normalizing cholesterol level using Statins. Statins inhibit the enzyme HMGCoA reductase, which catalyses the irreversible step in the synthesis of cholesterol. This treatment indeed lowers risk of cardiac complications by stabilization of the plaque. The other drug used is Aspirin which prevents platelet activation thus lowering the risk reducing vascular complications. These two drugs are now used in combination to help patients with dysregulated cholesterol level. However if cardiac complications arise, the plaque[s] are removed surgically [47].

The inflammatory heart diseases are usually treated with corticosteroids to suppress inflammation and drugs like cyclosporine A are used to tackle autoimmune response. But these drugs have systemic immunosuppressing effect and thus leaving the patients susceptible to further infections and several other complications. The present treatment strategy is aimed at specifically targeting pathogenic autoantibodies. Immunoadsorption is a technique being employed to absorb autoantibodies from the circulation. Drug molecules are being developed to directly interact with the active site of the autoantibodies. Intravenous immunoglobulins is a mixture of polyreactive antibodies that can be anti-idiotype as well as bear the capacity to neutralize autoantibodies. IA and IVIG are being currently researched upon and have shown optimistic results by improving the hemodynamic condition of the patients [48].

Shortcomings in present disease management

It is only common practice to prescribe statins and aspirin. But the surgeons like Dr. Lundell and Dr. Browntein have openly opposed usage of statin under every circumstance. Several observations made from patients on statins for a prolonged period show that they may develop muscle pain, fatigue and even disturbed sleep. Statins can cause myopathy and in extreme cases rhabdomyolysis. However, none of these side effects are taken into consideration by the cardiologists. Apart from side-effects, in several cases like high LDL if accompanied by poor HDL, statins are ineffective [49].

There is now debated that aberrant level of cholesterol cannot singularly develop vulnerable plaque and result in plaque rupture. Inflammation is indispensible. Inflammation can stem from several sources [as mentioned in the review] including reduction in level of ionized magnesium in the blood [50]. Scarcity in magnesium can also contribute to increased stenosis. Animal studies have shown magnesium treatment reduces lipid deposition in the aorta [51].

Also, LDL by itself cannot lead to activation of endothelia and platelets. It is clear from molecular evidence that oxidation of LDL is the real culprit in giving rise to inflammation. Also, the diseases in which CVD is considered to be a co-morbidity it is clear that ROS and superoxide ion generation is what plays a crucial role in the cross-talk. However, the treatment and prevention regime still does not include antioxidants and inflammation subsiding agents. Some studies have observed that consumption of Vitamin E rich food indeed reduced the risk of CVD but not when consumed as a supplement, may be pertaining to poor absorption from the alimentary canal [52]. Though, enough clinical trials need to be done to substantiate inclusion of antioxidants as a part of the medication regime [53]. To quote Dr. Lundell, “I have peered inside thousands upon thousands of arteries. A diseased artery looks as if someone took a brush and scrubbed repeatedly against its wall. Several times a day, every day, the foods we eat create small injuries compounding into more injuries, causing the body to respond continuously and appropriately with inflammation [49].

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