Activation of Cytokines in CABG Failure

32

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Abstract

Coronary artery disease (CAD) is the most common cause of death in this century. Many interventions have been demonstrated that decrease the rate of mortality and morbidity of CAD, and amongst them is the coronary artery bypass graft (CABG) procedure. Activation of inflammatory cytokines preoperatively and postoperatively is one of the main causes of CABG failure. Many factors during CABG, such as reperfusion injury, will stimulate inflammatory cytokines (e.g., TNF-α[alpha], IL-6), chemokines (e.g., IL-8), the complement system (e.g., C5a), neutrophils, and macrophages. Exposure to high levels of the inflammatory cytokines IL-1beta, IL-2, IL-6, IL-15, or IL-21 stimulates aggressive cytotoxic T cells. These cytokines are responsible for the intensity of the attack against the transplanted graft by the patient on the immune system.

Keywords

Cytokines • Inflammation • Neutrophil • Complement • Coronary artery bypass graft • CABG • Myocardial ischemia • Reperfusion injury

Introduction

Coronary artery bypass grafting (CABG) surgery using cardiopulmonary bypass (CPB) circuit and myocardial cardioplegia strongly stimulates an inflammatory response that may cause temporary organ dysfunction and affect the postoperative course [1].

These systemic inflammatory responses are characterized by excessive secretion and alterations in the serum concentrations of cytokines [2]. The secretion of cytokines perioperatively is the result of a series of events that occur during cardiac surgery with CPB that includes: myocardial ischemia-reperfusion injury (IRI); perioperative administration of drugs and anaesthesia, operation stress, and tissue damage; exposure of blood to artificial non-endothelial surfaces in the extracorporeal circulation; hyperthermia during blood rewarming; and endotoxaemia [3].

These systemic inflammatory responses and oxidative stresses during and after CABG surgery are related to activation of certain natural defense mechanisms. Complement activation and toll-like receptors are strongly involved in these procedures that results in the secretion of a large number of chemokines, cytokines, and other pro-inflammatory mediators including tumor necrosis factor-α(alpha) (TNF-α[alpha]), interleukin (IL)-10, IL-6, and sIL-2R receptor. IL-1b, IL-8, and monocyte chemotactic protein-1 are considered as the main pro-inflammatory mediators related to CPB-induced inflammatory response [4].

Early failure for venous or arterial graft occurs in the first month after surgery and is attributed to errors in the surgical technique and resulting thrombosis. On the other hand, late graft failure occurs due to intimal hyperplasia (IH) or atherosclerosis with its consequent graft stenosis [5]. Endothelial dysfunction or injury will result in an abnormal proliferation of smooth muscle cells (SMCs) that leads finally to IH.

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I.C. Ţintoiu et al. (eds.), *Coronary Graft Failure: State of the Art*, DOI 10.1007/978-3-319-26515-5_32

Mechanical errors can include incompatible anastomosis between the vein or artery to be grafted with the host coronary vasculature, using a diseased vessel, and biological factors that include platelet activation, disturbed local hemodynamics, and interactions between blood and graft material [6].

Biological Factors

Any change in the mechanical forces related to hemodynamics of the blood flow is detected by endothelial cells (ECs). SMC proliferation is caused by the release of growth factors and platelet aggregation due to mitogen release. Foam cells formation results in accumulation of low-density lipoprotein (LDL) in the intima. This causes monocyte attraction, which will develop to macrophages that will phagocyte the LDL with its final rupturing within the intima, resulting in formation of an atherosclerotic plaque. These plaques lead to lesions that increase the rigidity of the arterial vessel wall, causing pushing of the fibrous constitution to further narrow the luminal space. Formation of thrombi will result after rupturing of this fibrous cap with its consequent restenosis [7].

Vascular Injury

Several modes for endothelial injury may occur during the surgical procedure, such as: instruments used to harvest the grafted vessel; checking possible leakage of the graft through using high pressure pumping to the vessel; and anastomoses suturing. Additionally, vein graft implantation in the arterial circulation will expose the vein to high pressures and high blood flow, causing further damage to the venous endothelium because it is not adapted to such an environment. The formation of anti-proliferative products is decreased in the injured endothelium, and the physiological balance in the vessel wall is further disturbed by the release of intracellular growth factors from injured SMCs. SMC proliferation and hyperplasia result from acute injury to the intimal and medial layers. Balloon angioplasty also produces intimal hyperplasia (IH) at the injury sites. IH formation after wound healing has been further documented by Goel et al. and Bechler et al. [8, 9].

Numerous events, potentially generating an inflammatory response, are induced during cardiac surgery. Amongst them, the combination of surgical injury, mechanical manipulation with the heart, the contact of blood components with the artificial surfaces of the cardiopulmonary bypass circuit, transient endotoxemia, and ischemia-reperfusion injury of the heart. One of the main mediators of inflammatory responses are cytokines, which play a crucial role in these responses [10].

Origin of Cytokines

Any inflammatory stimuli will cause stimulation to immune cells as macrophages and T helper cells or vascular cells and adipocytes to produce cytokines. Vascular endothelial cells (ECs) are both a target and also source of cytokines. ECs produce IL-1β(beta) and IL-1α(alpha), while vascular smooth muscle cells (VSMCs) produce IL-1α(alpha), IL-1β(beta), and TNF-α(alpha) [11]. One of the main sources for cytokines are macrophages, which if stimulated will produce many of the important pro-inflammatory cytokines such as IL-1, TNF-α(alpha), IL-6, IL-12, IL-15, IL-18, and IL-32, in addition to anti-inflammatory cytokines such as IL-10 and TGF-β(beta) [12]. IL-12 and IL-18 will stimulate macrophages to produce IFN-γ(gamma) in an autocrine manner. In addition, macrophages have the ability to express many chemokines such as IL-8/CXCL8, MCP-1/CCL2, and MCP-4/CCL13. Platelets are also considered as sources of growth factors, chemokines, and cytokines production. These proteins are stored in α(alpha)-granules, and any activation to platelets will stimulate their release. As in the case of thrombin activation, platelets will produce IL-1β(beta). Platelets also secrete CC and CXC chemokines as MIP-1 (CCL3), mRANTES (CCL5), and PF4/CXCL4. T cells also have the ability to produce cytokines such as IL-4 and IFN-γ(gamma) [13].

Cytokine-Induced Cell Signaling

Cytokines produce their effect through interaction with specific receptors found in affected cells, and activate protein kinases pathways, which in turn activate transcription factors such as NF-κ(kappa)B, signal transducers and activators of transcription (STAT), and SMAD. These pathways are responsible for leukocyte recruitment and adhesion, and for the propagation of the inflammatory process. The mechanisms of activation and negative feedback of these signaling pathways requires a high-quality specific control system that includes suppressors of cytokine signaling (SOCS) proteins, phosphatase regulation of cytokine signaling, and protein inhibitors of STATs (PIAS) [14, 15].

JAK-STAT Pathway

The majority of interferons (IFNs), interleukins (ILs), and colony stimulating factors (CSFs) promote their action through the Janus kinase (JAK)-STAT pathway. When they bind to their receptors, a lot of cytokines cause receptor dimerization and activation for the JAK family members, which cause phosphorylation to each other and the cytoplasmic domains of the receptors on tyrosine residues (JAK activation). After this, STAT transcription factors also are phosphorylated followed by dimerization, then enter the

nucleus and cause transcription for the target genes. Each member of the STAT family have a crucial effect on cytokines' specific functions [16].

NF-κ(kappa)B Pathway

The transcriptional factor NF-κ(kappa)B (nuclear factor kappa B) has a critical role in the inflammation process through up-regulation of genes encoding pro-inflammatory cytokines, chemokines, adhesion molecules as E-selectin, VCAM-1 and ICAM-1, growth factors, cyclooxygenase-2 (COX2), and inducible nitric oxide synthase (iNOS). In turn, TNF and IL-1 family (including IL-1 and IL-18) activate NF-κ(kappa)B in addition to mitogen-activated protein kinase (MAPK) signaling pathways [17].

TGF-β(beta) Pathway

Binding for the ligands of the members of the transforming growth factor (TGF) family as growth and differentiation factors (GDFs) and bone morphogenetic proteins (BMPs) to type II receptors, which are a Ser/Thr kinase, will phosphorelate type 1 receptor, which in turn causes formation of the R-SMAD/coSMAD complex that is a nuclear transcriptional factor cause for the up-regulation of target proteins [18].

Vascular Effects of Cytokines

Differentiation and proliferation of immune cells additionally to the induction of VSMC growth followed by migration is stimulated by many cytokines. Examples are many, such as IFNγ(gamma), which activates macrophages, IL-3, IL-7, and GM-CSF; which stimulate hematopoiesis, IL-4, IL-5, and IL-6; which stimulate proliferation and differentiation of B cells, and IL-2; which stimulates proliferation of antigen-activated T and B cells and IL-1; which activates T cells. Additionally IL-1 induces the growth of VSMC from rat aorta through increased production of endogenous platelet-derived growth factor (PDGF). Also it was found that increased expression of VEGF in a human epithelial carcinoma cultured cell (A431) is mediated by IL-6 and TNF-α(alpha) [19].

Pathophysiologic Mechanisms of CABG Failure

Cellular Effect of Myocardial Ischemia-Reperfusion Injury

A few seconds after coronary arteries occlusion, the dominant source of adenosine triphosphate (ATP) production required for cardiomyocyte activity will go through anaerobic glycolysis; within 1–2 h of ischemia, the phosphates source of energy will be diminished and hypercontracture of the heart will take place due to diastolic failure [20]. Within seconds of reperfusion, the mitochondria will retain its normal oxidative phosphorylation as it was before ischemia, but there will be a mechanical defect in which contractile dysfunction occurs but is short lived. This phenomenon is known as myocardial stunning. If the reductions in the blood supply were intermittent for a longer period of time and associated with contractile impairment, this is known as hibernation [18, 21].

During ischemia and as a result of anaerobic glycolysis, H^+ will accumulate intracellularly leading to a reduction in cell pH (acidic pH). To overcome this situation, the Na^+/H^+ exchanger (NHX) will expel hydrogen ions and exchange it with sodium ions [22]. This increase of intracellular Na^+ will activate the sarcolemmal Na^+/Ca^{2+} exchanger (NCX), which will exchange the intracellular Na^+ with the extracellular Ca^{2+} as Na^+/K^+ ATPase is inactivated due to absence of ATP. Na^+/Ca^{2+} will be exchanged at a high rate and active Ca^{2+} efflux will be reduced paradoxically with the impairment of the endoplasmic reticulum (ER) to reuptake calcium because of the reduction of cellular ATP level, leading to Ca^{2+} overload and cell death [23]. Necrosis in cardiomyocytes may result due to the activation of intracellular lipases and proteases (e.g., calpains). There is a variation in the level of tissue injury depending on severity and duration of ischemia [24].

In the heart, about one-third of the myocardial cell volume consists of mitochondria that are intensively located in the area of myofilament and sarcoplasmic reticulum SR, to accommodate its high requirement of energy representing as ATP. Adenosine triphosphate is produced by the mitochondria via oxidative phosphorylation, normally through the citric acid cycle or what is known also as the Krebs cycle [25]. The electron transport chain of normal mitochondria function through a series of redox reactions resulting in production of ATP and formation of water after transferring four electrons to oxygen in its molecular state. Normally, and during mitochondrial phosphorylation, there is a formation of a small percentage of reactive oxygen species (ROS) due to a small leak of electrons from the electron transport chain [26], but mitochondria possess their antioxidant system represented by reduced glutathione (GSH), glutathione peroxidase, and superoxide dismutase (SOD), which can deal with the ROS in the mitochondria and get rid of it. However, many scavenging molecules are removed after reperfusion. Autophagy can ameliorate the potential negative effect on the cells by removal of dysfunctional mitochondria [27]. On reperfusion of oxygenated blood, there will be a temporary pH imbalance, associated with an interruption for the electron flow in the electron transport chain (ETC), and formation of ROS by dysfunctional mitochondria. At the same time, calcium uptake and swelling continue in normal cells.

This state of imbalance occurs because the myocardial ischemic condition creates an acidic environment, which is immediately neutralized extracellularly after reperfusion, but the intracellular environment remains acidic. This state will cause calcium overload through the stimulation of H^+ excretion via the Na+/H+ exchanger with the uptake of calcium through Na^+/Ca^{2+} exchanger because of the formation of H+ ion gradient between the extracellular and intracellular compartments of the cell membrane [28].

The intracellular elevation of calcium levels will result in the swelling of the mitochondrial matrix and rupture of the outer membrane or generation of permeability transition pores (PTPs). The mitochondrial permeability transition pore (MPTP) opening, which results from the dissipation of the mitochondrial membrane potential, will increase calcium influx to mitochondria along with cessation of ATP synthesis; this will cause the release of pro-apoptotic factor (cytochrome c) through the mitochondrial outer-membrane permeability pore (MOMP) to the cytosol induced by BAX (one of the pro-apoptotic members from the bcl2 family). So mitochondria play a critical role in both the life and death of cardiac cells as it is filled with reactive intermediates, pro-apoptotic and anti-apoptotic signals that have a strong relation with ischemia-reperfusion injury (IRI) [29].

The conversion of reversible ischemia into irreversible cell death occurs through a complex cascade of reactions that involves calcium and sodium overload, ATP depletion, ROS formation, vascular endothelium dysfunction, and inflammatory responses [23].

Endothelial Dysfunction and Role of Neutrophil

Neutrophil

Polymorphonuclear leukocytes are a particular type of phagocytic leukocyte that is responsible for attacking and destroying bacteria, viruses, and other potentially harmful foreign agents in the innate immune system. Neutrophils are the first cells that reach the site of infection and contain cytotoxic granules that kill pathogens. Endothelial dysfunction in myocardial reperfusion injury is mediated by neutrophils through the production of ROS, capillary plugging, and coronary vascular constriction, which alters cardiac performance and causes myocyte injury. During myocardial ischemia-reperfusion, neutrophils are activated by complement (C5a) and inflammatory molecules released by cardiac myocytes, endothelial cells, and mast cells such as IL-6, IL-8, tumor necrosis factor-alpha (TNF-α[alpha]), and histamine [30]. No-reflow is a process through which aggregated neutrophils can occlude micro-vessels and increases capillary resistance to blood flow. This will result in microvascular dysfunction representing as ischemia and even tissue infarction due to decrease of blood supply during reperfusion [31].

Vascular Endothelium

There is a strong involvement of vascular endothelium with neutrophil activities; it inhibits neutrophil adherence via NO and adenosine as well as its involvement with recruiting neutrophils. Endothelial dysfunction is the central factor for neutrophil-induced reperfusion injury [32].

Interaction of Neutrophil with Vascular Endothelial Cell

To reach the inflammatory site in the interstitium, the circulating neutrophil must first pass the endothelial barrier via a process called trans-endothelial migration (TEM) or diapedesis. This process is very important for hematopoietic progenitor immune cells to keep their surveillance, mobilization, and homing [33]. It is a multi-step process including the activation of certain proteins on endothelial cells and their specific receptors on the neutrophil surface [34]. Rolling of neutrophil on the activated endothelium in a short period of adhesive contacts captures neutrophil from the blood stream via L-selectin (CD62L) expressed on the surface of neutrophil with their glycoprotein ligands expressed on endothelial cells. Rolling is also supported by binding of endothelial- E-selectin CD62E and P-selectin (CD62P) to P-selectin glycoprotein ligand-1 (PSGL-1) on the surface of the neutrophil [35].

The rolling process on activated endothelium can be supported by homotypic and heterotypic interactions between activated neutrophils – activated neutrophils, or activated neutrophils –activated platelets, respectively, and this results in secondary capture of polymorphonuclear neutrophils (PMNs) implicated in supporting leukocyte recruitment and thrombus formation [36]. The second step is the change of neutrophil state from rolling to arrest, because of the up-regulation of intercellular adhesion molecule-1 (ICAM-1; CD54) on the surface of activated endothelium and the activation of its counter receptor two integrins, and finally extravasation of activated neutrophils through the vascular endothelium to reach its target [37].

One of the important factors that regulates the cardiovascular homeostasis is the vascular endothelium by keeping the fluidity of blood through the production of anticoagulants and by adjusting vessel diameter in different hemodynamic environments [38]. This process is done by maintaining an appropriate balance between molecules that cause vasodilation such as nitric oxide (NO), prostacyclin,

and adenosine, and vasoconstrictors such as endothelin and angiotensin II [39]. Endothelial dysfunction will take place within a few minutes after the reperfusion, characterized by decreased release of NO, increased ROS production, platelet and neutrophil activation, vasoconstriction, and increased protein and fluid extravasation. NO has important jobs: first is its vasodilating effect, scavenging O_2*– to give ONOO–, inhibiting neutrophil release of O_2*– and histamine released by mast cells, and blocking adhesion and aggregation of platelets [40].

Myeloperoxidase (MPO) enzyme is released from neutrophil azurophilic granules. MPO has the ability to convert H_2O_2 to hypochlorous acid, which is 50 times stronger than H_2O_2. MPO is found in monocytes but is predominantly found in neutrophils, so MPO level is used to detect the degree of neutrophil accumulation [41]. Direct interactions between neutrophil and endothelial cells occur through ROS production that results in conversion of the enzyme xanthine dehydrogenase (XD) into xanthine oxidase (XO). On reperfusion, XO will react with O_2 (acting as an electron acceptor) and produces O_2*$^-$ with more damage to the endothelial cell [42]. The neutrophil role in myocardial ischemia was confirmed by histological studies that clarify the direct relation between the time of ischemia and the size of the infarcted area in the myocardium with the degree of regional neutrophil accumulation. The chemotactic factors, which include complement fragments C5a, C3a, and IL-8 with transforming growth factor, will direct the neutrophil to the site of inflammation. Neutrophils are the main source of the chemotactic factors through their function in an autocrine-like manner [43]. An example of this is of a foreign surface, heparin and heparin-protamin complex to this inflammatory response that leads to activation of complement proteins [44].

Vasoconstriction, elevated vascular permeability, activation of neutrophil leukocytes and mast cells, aggregation of platelets and chemotaxis are facilitated by C3a and C5a proteins arising from the lysis of complement proteins [45]. CPB will activate PMNs, which in turn will release several proteolytic enzymes, produce oxygen free radicals and metabolites of arachidonic acid. The activated cells are the source of the proinflammatory cytokines that enhance inflammatory reactions. So, CPB will stimulate PMN, which is one of the important players in myocardial ischemia reperfusion injury via the expression of adhesion molecules leading to interaction between the endothelial cells, thereby extending the tissue damage. Adhesion molecules have four families that control the endothelial cells and PMNs' interaction with its consequent leukocytes migration toward the site of injury through blood vessels: cadherin, integrin, immunoglobulin, and selectin are these families. The main role during the phase of tight binding and the migration of PMNs is attributed to the integrin family, which is made up of the CD11a and CD18 complex molecule that will form leukocyte function-associated antigen-1 (LFA-1), which is the ligand responsible for inducing endothelial cells to produce intercellular adhesion molecule-1 (ICAM-1), and PMNs migration to the injury site. The processes induced by CPB may affect myocardial function and increase the risk of complications. The LFA-1 expression on leukocytes increases, causing PMN adhesion and migration through the vessel's wall with consequent decreased capillary blood flow [46].

Nitric Oxide, CGMP, and PKG

The simple natural gaseous molecule nitric oxide (NO), which is composed from a single atom of nitrogen and oxygen with a short half-life ranging for a few seconds, is considered to be one of the important signaling molecules in the cardiovascular system [47].

NO synthase (NOS) is the enzyme responsible for the synthesis of NO from its precursor L-arginine. There are three types of NO synthase isoenzymes: endothelial (eNOS) that is found normally in endothelial smooth muscle cells, neuronal (nNOS) expressed in neural tissue, and inducible (iNOS) expressed in response to stress conditions [48].

Vascular endothelial cells are the main source of plasma NO because the high expression level of eNOS and the level of synthesis and release regarding NO is proportional to the increased blood shear stress in the blood vasculature and other stimulators as acetylcholine. NO is also released from neuronal cell terminals as a neurotransmitter [49].

After NO is released, it will pass freely through cell membranes to adjacent cells where it will produce its activity through interacting with the heme moiety present in soluble guanylyl cyclase or other moieties and increasing its activity by 100- to 200-fold toward GTP, with a subsequent increase in the level of cGMP, as sGC is responsible for cGMP formation from GTP [50].

The elevation of cGMP cellular level is responsible for activation of protective enzymatic pathways as protein kinase G I pathway and ERK1/2, which is responsible for protection against myocardial ischemia/reperfusion injury, inhibition of platelet aggregation, vascular and gastrointestinal smooth muscle relaxation, ameliorating symptoms of BPH, inhibiting cardiac hypertrophy, and enhancement of cognitive functions [51].

The cGMP-dependent protein kinase or protein kinase G (PKG) is a specific serine/threonine- kinase stimulated by cGMP binding to its regulatory domain. This results in the inhibition of N-terminal domain from its suppression effect on the kinase domain and causing phosphorylation of substrate proteins with the production of a protective effect as smooth muscle relaxation through the opening of mitochondrial KATPase channels, resulting in hyperpolarization and relaxation, inhibiting the effect of phospholipase C, and

inhibiting the release of Ca^{2+} from SR. There are two nearly identical types of PKG, which are PKGI and PKGII (similarity is up to 90 %), the former located in the cytoplasm and the latter attached to the plasma cell membrane. PKGI has an Iα(alpha) isoform with more selectivity for cGMP than the Iβ(beta) isoform. PKGI is found in vascular smooth muscle cells, platelets, vascular endothelium, and in cardiomyocytes [52].

The NO/cGMP/PKGI protective pathway is started by the effect of NO, and the enzymatic activity of this cascade is amplified at each step resulting in phosphorylation of substrate proteins that will yield the protective physiological response. Alteration of any of the constituents of this pathway will impair the protective response, as in the case of low levels of NO in patients with hypercholesterolemia, hypertension, and diabetes [53].

Inflammation and Role of Immune System in CABG Failure

The crucial role of the innate immune system in myocardial I/R injury pathogenesis have been documented. One of the important branches of the innate immune system are toll-like receptors (TLR), which when stimulated will cause a series of inflammatory responses. Triggering of the TLR4 includes their binding to danger-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), and endogenous substances released from tissues under stress conditions such as heat shock cognate protein (HSC70), which is produced during myocardial I/R when it binds to TLR4; it will induce NF-κ(kappa)B activation, also LPS binding to TLR4, and can induce cardiac myocyte damage [54].

Activated proinflammatory chemokines and cytokines are strong mediators for myocardial I/R injury through TLR4-activation. Myocardial I/R injury due to CPB have many inflammatory contributors and one of the important proinflammatory cytokiens is TNF-α(alpha). TNF- α(alpha) alters Ca+homeostasis-induced production of other cytokines such as IL-1β(beta). TNF- α(alpha) can inhibit alpha- and beta-adrenergic stimulations to cardiomyocytes [55].

IL-1β(beta) is another important cytokine that induces TLR4-mediated myocardial I/R injury. IL-1 induces cardiomyocyte deterioration through p38 and p42/p44 MAPK signaling pathways that cause myocardial arrhythmia. CPB followed by cardiac dysfunction is strongly associated with increased IL-6 levels. IL-6 is a multifunction cytokine produced by different cell types, and is reported to serve as a direct cardio-depressant, inhibiting contractility in the hamster myocardium. IL-6 reduces the transient peak Ca+systolic level through decreased levels of cGMP-mediated L-type Ca+channel. Additionally, IL-6 activates expression of chemokines, intercellular adhesion molecules (ICAM), complements, and C-reactive protein (CRP). These cytokines have a damaging effect on myocardium and lead to cardiodepression [56].

Expression of the adhesion molecule ICAM-1 is done by both endothelial and immune attachment of leukocytes during transendothelial migration. Cardiomyocyte damage after CPB is associated with increased ICAM-1 expression. ICAM-1 actually mediates cardiomyocyte damage via neutrophil-endothelial cell interaction after cardiac I/R [57]. Additionally, the adaptive immune system represented by T cells also has a strong contribution to CABG failure. The inflammatory cytokines such as IL-1β(beta), IL-6, or IL-21 cause progenitor cells to differentiate into aggressive T cells, which attack the grafted tissue, while the anti-inflammatory cytokine, transforming growth factor beta (TGFβ[beta]) induces differentiation into the protective regulatory T cells [58].

Role of Toll-Like Receptors

Inflammatory responses mediated by the immune system during myocardial ischemia and reperfusion leads to a further damage to viable cells around the infarction. Endogenous ligands that are named danger-associated molecular patterns (DAMPs) are released because of injury and initiate inflammatory cascade through binding with toll-like receptors (TLRs) on leukocytes (namely TLR2 and TLR4), which respond to such signals that results in the up-regulation of IL-8, E-selectin, ICAM-1, and GM-CSF [59]. TLR2 is a modulator for leukocyte activity under stress conditions, leading to coronary endothelial dysfunction. Heat shock cognate protein 70 (HSC70) will promote inflammatory response in cardiomyocyte mediated by TLR-4 during MI/R injury [60].

TLR signals through two distinct signaling pathways: the MyD88-independent pathway (TRIF-dependent pathway) and the MyD88-dependent pathway. Both MyD88 and TRIF contribute to the inflammation response occurring after cold I/R that leads to the release of TNF-α(alpha), IL-6, IL-1β(beta), ICAM-1, and MCP-1 under the control of the transcription factor NF-κ(kappa)B [61].

The activated neutrophils release soluble IL-6 receptor α(alpha) (sIL-6Rα[alpha]), which binds IL-6 and then associates with gp130 on the EC surface. This activates additional signaling pathways in ECs that lead to the up-regulation of mononuclear leukocyte, chemo-attractants such as CCL2, and adhesion molecules such as VCAM-1. The engulfment of the apoptotic neutrophils by activated recruited macrophages occur at the end of this cascade. A number of endogenous molecules are released by different immune cells and non-immune cells during myocardial I/R. These endogenous

ligands bind to TLR4 and activate diverse innate immune responses. The immune response processes are mediated by the TLR4 signaling pathway (MyD88-dependent pathway and TRIF-dependent pathway) interacting with other signaling pathways (PI3K/Akt and AMPK, ERK signaling pathway). Finally, transcriptional factor NF-κ(kappa)B is activated and enters the nucleus to promote the inflammatory response. This is the basic mechanism of TLR4 signaling pathway-mediated myocardial I/R injury [62].

The Role of Complement

During myocardial IRI, the complement system can be activated two ways: classical and alternative pathways. Upon activation there will be two main components of the complement system, C5a and C5b-9, responsible for IRI. C5a effects include neutrophils attraction, release of proteases, and initiates the production of TNF-α(alpha), IL-1, IL-6, and MCP-1; while the main role of C5b-9 is related to complement-mediated tissue injury, to activate NFκ(kappa)β(beta), and to induce chemotactic mediators (IL-8, MCP-1) [63].

Activation of Complement in Cardiac Surgery

More than one mechanism is responsible for the activation of the complement system during open heart surgery including the indirect binding of C1q to natural antibodies or C-reactive protein, or direct path by binding of C1q and/or mannose-binding lectin. The direct adsorption of C3 onto the artificial surface of the extracorporeal surface during CPB also causes activation to the alternative pathway. In addition, non-CPB mediated mechanisms produce an activation effect to the complement system during cardiac surgery. For example, tissue injury that leads to plasmin production causes C3 activation. Also, the use of protamine sulphate causes activation of the complement through classical pathways and inhibition for carboxypeptidase N, which exacerbates inflammation via increased kinin and anaphylatoxin levels. C4d-C-reactive protein, which results from complement activation by C-reactive protein, has been related to arrhythmias after coronary artery bypass graft [64].

Complement Activation and Perioperative Myocardial Ischemia/Reperfusion Injury

Cardiac dysfunction after CABG surgery is related to many factors including myocardial IRI, which results from cardioplegic arrest, inadequate protection during aortic cross-clamping, hemodynamic instability, and pre-existing coronary artery disease (CAD) [65]. Complement activation results from exposed basement membranes, subcellular organelles, mitochondrial particles, cardiolipin, certain sensitizing antibodies, or the coagulation/fibrinolytic system. Generation of ROS is directly potentiated through conversion of xanthine dehydrogenase and xanthine oxidase mediated by the effect of C5a in endothelial cells. 76 C5b-9 also causes formation of membrane insertion, which promotes Ca2+ influx and facilitates interactions between leukocyte and endothelial cells, causing endothelial dysfunction [66].

Smooth muscle vasodilatation is mediated by C5a, C4a, and C3a. Neutrophil chemotaxis is mediated by the effect of C5a in addition to the production of thromboxane, prostaglandin E2, leukotriene B4, and formation of ROS. In the coronary arteries, C5a mediates vasoconstriction through the formation of thromboxane, which is produced due to the interaction between neutrophils and platelets. C5a causes attachment of neutrophils to endothelial cells through enhancing platelet activating factor release, which in turn causes the up-regulation of β(beta)2-integrins on neutrophil, which causes shedding of L-selectin and stimulates cytokine production [67].

Additionally, iC3b is responsible for continuous adhesion of leukocytes to vascular endothelium mediated by β(beta)2-integrins and enhances ROS formation and proteolytic enzymes release during neutrophil phagocytosis. C5b-9 is related to the interaction between endothelial cells and neutrophils through activation of the endothelial cells to release the von Willebrand factor and translocation of P-selectin from Weibel-Palade bodies to the endothelial cells. Endothelial NF-κ(kappa)B activation by C5b-9 increases leukocyte adhesion molecule transcription expression [68]. C5b-9 also changes vascular endothelium tone through decreasing cGMP level and inhibiting endothelium-dependent relaxation. C5b-9 facilitates chemotaxis and activation of neutrophils to release ROS, proteolytic enzymes, and arachidonic acid derivatives (leukotriene B4, prostaglandin E2, thromboxane). Any inhibition to these effects induced by the complement system gives protection from inflammation to the vascular endothelium [69].

Oxidative Stress and Role of ROS

On reperfusion, the hypoxic state of cardiomyocytes will change to normal oxygen tension rapidly after reintroduction of molecular oxygen; this will cause O_2 reduction and formation of ROS [38] as superoxide anions (O_2^-), which is the most common radical, hydroxyl radicals (OH^-), and hypochlorous acid (HOCl), which will cause the oxidation of membrane phospholipids, proteins, DNA, and cellular death [70]. ROS will alter the Ca^{2+}-ATPase pump and Na^+-K^+ ATPase function as well as the ability of the sarcoplasmic reticulum (SR) Ca^{2+}-pump, leaving the myocyte unable to remove excess cytoplasmic Ca^{2+} resulting in hypercontracture, myocardial dysfunction, and cell damage [71]. A free radical is an unstable atom or molecule because of the availability of one or more unpaired electrons in its outer electron

shell; this makes it highly reactive and it will try to reach a stable state through giving its excess electrons to other atoms. The cells that will be attacked by the reactive oxygen species (ROS) will undergo oxidative damage and ROS becomes more stable. One extra electron on an O_2 molecule will form the superoxide anion O_2*−; two additional electrons will form hydrogen peroxide (H_2O_2); three more electrons results in a hydroxyl radical (*OH); and at the end, four added electrons leads to the production of water [72]. Sources of ROS during reperfusion include disturbance of mitochondrial electron transport chain, enzymatic reactions catalyzed by the xanthine oxidase (XO), NADPH oxidase, activated neutrophils, and cytochrome P450 oxidases [73]. Mitochondria possess their own ROS scavengers, represented by enzymatic and non-enzymatic molecules. Reduced GSH is one of the important endogenous non-enzymatic free radical scavengers and the severity of the cell damage resulting from ROS is inversely proportional to reduced GSH cellular level [74].

Overproduction of ROS will impair mitochondrial function by altering the electron transport chain. This results in dissipating of mitochondrial membrane potential and leads to the opening of the MPTP and increases calcium ion influx, also by the effect of the pro-apoptotic bcl2 family member protein (BAX), which will cause the formation of MOMP by its binding to the outer membrane of the mitochondria. This then results in an efflux of cytochrome c and other pro-apoptotic factors, such as SMAC/DIABLO, via the opened permeability transition pore into the cytosol, which leads to the activation of caspase cascade and initiates apoptosis [43].

The interaction of O_2*− (super oxide anion) with nitric oxide (NO) will produce peroxynitrite (ONOO−), which is a ROS involved with myocardial reperfusion injury. When peroxynitrite is protonated it will form peroxynitrous acid (ONOOH), which will produce nitrogen dioxide (NO_2) and *OH. After its breakdown, if it reacts with CO_2 the result will be NO_2* and CO_3*—all of which are toxic ROS. The formation of peroxynitrite will cause depletion of the NO level required for its cardio-protective effect. In isolated rat heart models, post-ischemic myocardial dysfunction may be attributed to the formation of peroxynitrite during reperfusion [75].

ROS cause the formation of ROS inside the mitochondria, which further dissipates the membrane potential [76]. Oxygen radicals will stimulate the endothelial cells and other cells to produce the chemo-attractant cytokines IL-8, CXCL1, CXCL2, and complement cleavage products, namely C5a, that mediates the activation and infiltration of neutrophils, macrophages, and, in certain conditions, $CD4^+$ T cells into the ischemic tissue. The binding of IL-8 and CXCL1 to CXCR1 and CXCR2 expressed on neutrophils stimulates them to release ROS, proteases, and cytokines. Further cellular damage can be measured by detecting the level of malondialdehyde (MDA), a product resulting from the damage that occurs to the phospholipid of the cell membrane by the effect of ROS [77].

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