

# Cardiac Surgery and Inflammation: The Inflammatory Response and Strategies to Reduce the Systemic Inflammatory Response Syndrome

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**Abstract:** Despite advances in the techniques of 'off-pump' Cardiac surgery, the vast majority of cardiac operations still involve using cardiopulmonary bypass (CPB) along with some form of myocardial protection. The extracorporeal circuits used in the modern bypass-machine have developed considerably in the last few decades. However contact activation of blood leading to a systemic inflammatory response is to some degree inevitable. Although often remaining sub-clinical and resolving promptly at the end of CPB, in its most extreme form this inflammatory response may be associated with the development of the systemic inflammatory response syndrome (SIRS) that can often lead to major organ dysfunction syndrome (MODs) and death. Here we review the pathophysiology behind the development of this "whole body" inflammatory response and consider the mechanical and pharmacological methods that are currently used to minimise it.

**Key Words:** Inflammation, cardiopulmonary bypass, SIRS.

## I. INTRODUCTION

The response of tissues to a severe or generalised insult is to generate a systemic inflammatory response, the clinical manifestations of which are referred to as the systemic inflammatory response syndrome (or SIRS) [1]. In cardiac surgery, particularly following cardiopulmonary bypass (CPB) generation of a peri-operative SIRS is to some extent inevitable [2]. In the modern era, in the majority of patients activation of SIRS is sub-clinical or mild with no long-term consequences (often described as 'post-pump syndrome'). However, it is the occasional patient in which a gross SIRS occurs who are remembered so vividly by both the cardiac surgeon and intensivist.

CPB contributes to such an inflammatory response through several mechanisms most notably the generation of shear forces from roller pumps driving blood through the bypass circuit, and exposure to the artificial surface within the bypass circuit. Along with systemic hypothermia of circulating blood, CPB activates numerous cascades, including kallikrein and coagulation, as well as the complement systems leading to the generation and release of endogenous inflammatory mediators.

In the article we briefly review the latest evidence regarding activation of the various cascades that make up SIRS following cardiac surgery involving CPB. This includes activation of the coagulation and fibrinolytic systems, activation of the complement system and the role of leukocytes and platelets in the inflammatory response. In addition we review techniques to inhibit the inflammatory response including mechanical methods such as the development 'off pump' cardiac surgery, heparin bonded extracorporeal circuits and leukocyte filters as well as pharmacological methods such as the use of glucocorticoids, complement inhibitors and serine protease inhibitors (Aprotinin). The article concludes with an

overview of current best practice and looks toward future developments particularly as our understanding of the 'genetics' of the inflammatory response is uncovered.

## II. THE INFLAMMATORY RESPONSE: SYSTEMIC ACTIVATION FOLLOWING CPB

### 1. Activation of the Coagulation and Fibrinolytic Systems

Haemostasis is composed of four events that occur in a set order following the loss of vascular integrity. Following vascular constriction, platelets become activated by thrombin and aggregate at the site of injury. To insure stability of the initially loose platelet plug, a fibrin mesh (or clot) forms and entraps the plug forming a thrombus. Finally, the dissolution of the clot occurs through the action of plasmin.

Traditionally, two distinct pathways consisting of a series of enzyme cascades and initiated by distinct mechanisms lead to the formation of thrombus: the intrinsic and extrinsic pathway [3]. The intrinsic pathway begins after contact activation of blood from exposure to collagen in a damaged vascular wall, or exposure of the blood to an artificial surface such as an extracorporeal circuit. This is termed the contact phase. The intrinsic pathway requires the clotting factors VIII, IX, X, XI, and XII. Also required are the proteins prekallikrein and high-molecular-weight kininogen, as well as calcium ions and phospholipids secreted from platelets.

In the extrinsic pathway, the initial stimulus is trauma to the vascular wall, resulting in exposure of blood to non-vascular tissue cells that express an integral membrane protein or cofactor called 'Tissue Factor' (factor III). Factor VII is a circulating plasma protein that then binds to Tissue Factor, creating a complex. In doing so, Factor VII is activated to Factor VIIa. This complex, in the presence of calcium and phospholipids, activates Factor X to Factor Xa.

Activated factor Xa is the site at which the intrinsic and extrinsic coagulation cascades converge into a common pathway. It functions as a protease to convert the inactive molecule prothrombin to the active form thrombin. Throm-

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bin then cleaves fibrinogen to fibrin, which then polymerises to form fibrin strands.

The use of CPB at the time of cardiac surgery leads to extensive activation of both coagulation pathways and thrombus formation and is therefore not possible without first systemically heparinising the patient prior to initiation of CPB. Heparin enhances the action of plasma protease inhibitor antithrombin III (AT-III) that in turn inhibits clotting factor proteases, e.g. FIIa, Xa, Ixa and XIa, by forming stable complexes with them. However despite heparinisation preventing clot formation in the extra corporeal circuit, activation of the coagulation system still occurs as heparin inhibits the coagulation system only at the end of the cascade (by promoting the activity of AT-III) [4,5]. Molecular markers of thrombin generation such as thrombin-antithrombin III complex (TAT) and pro-thrombin fragment (PF1+2) remain elevated peri-operatively in patients undergoing CPB demonstrating that thrombin generation is still occurring [2]. Furthermore, the use of heparin can be associated with heparin induced thrombocytopenia in up to 3% of patients [6] and aldosterone inhibition leading to hyperkalaemia has been described following cardiac surgery [7].

The fibrinolytic system exists as a counterbalance to the coagulation cascade, preventing excessive activation leading to obstruction of normal blood flow as well as localising thrombus formation to the site of vascular insult. Plasminogen is an inactive proenzyme synthesised mainly by the endothelium and can be converted to its active form plasmin by tissue plasminogen activator (t-PA) [8]. Plasmin then has the ability to degrade fibrin strands, preventing the build-up of excess clot.

Plasmin has functions beyond its classical proteolytic and fibrin degrading properties and can induce pro-inflammatory responses. *In vitro*, plasmin was demonstrated to stimulate the release of cytokines and other inflammatory mediators by different cell types [9,10]. Furthermore, plasmin induced cell adhesion and migration *in vitro*, and studies using plasminogen-deficient mice have provided *in vivo* evidence for an essential role of the plasminogen system in cell migration toward inflammatory sites [11-13].

Cardiac surgery with CPB results in increased fibrinolytic activity as demonstrated by increases in D-dimer levels, and t-PA activity [2]. This activation of fibrinolysis is caused by elevated levels of factor XIIa and kallikrein as well as by an increase in t-PA. Elevated D-dimer levels have been correlated with increased blood loss and postoperative bleeding time. Additionally activation of fibrinolysis may also affect other aspects of haemostasis such as reduced platelet adhesion and aggregation capabilities due to redistribution of glycoprotein Ib and IIb/IIIa receptors [14].

## 2. The Haemostatic and Inflammatory Role of Platelets

Platelets are the smallest circulating human cells and lack nuclei, but they play an integral and complex role in the process of thrombosis and are increasingly recognised as an important component in the systemic inflammatory response, particularly after CPB.

Platelet function and number are influenced by mechanical factors as well as activation of various endogenous cas-

ades during CPB. In exposing platelets to bypass circuits, the shear forces generated and the systematic hypothermia that occurs during on-pump cardiac surgery [15], platelet function is disrupted and numbers reduced [15,16]. The use of exogenous drugs such as heparin, the haemodilution affect caused by priming of the CPB circuit with crystalloid fluids and the release of endogenous chemicals will also affect platelet function and cause a thrombocytopenia [17,18].

Platelets interact with other endogenous inflammatory cascades through a range of surface markers whose expression is affected by CPB. These include Glycoprotein Ib and CD31 or PECAM-1 (Platelet Endothelial Cell Adhesion Molecule-1) both of which are down regulated during CPB [17-20]. On the other hand, CD62 (P-selectin) is activated during CPB [20]. P-selectin induces tissue factor expression and fibrin deposition by monocytes leading to the evolution of thrombus [21]. In addition, P-selectin expressed by activated platelets contributes to leukocyte conjugation by binding to P-selectin glycoprotein-1 (PSGL-1) [22]. Activated platelets stimulate conjoined monocytes *via* P-selectin-PSGL-1 interaction leading to the release of pro-inflammatory cytokines (IL-1 $\beta$ , IL-8 and MCP-1 (monocyte chemo attractant protein-1) [23,24].

Endothelial cells express CD40, an adhesion marker that conjugates to a binding ligand on activated platelets CD40L. This induces the endothelium to secrete chemokines and express further adhesion molecules such as IL-8 (chemotactic for neutrophils) and MCP-1 (chemotactic for monocytes). Activated platelets therefore bind to endothelium and are able to initiate recruitment of neutrophils and monocytes [25].

## 3. Activation of the Complement Cascade

The complement system plays an essential role in host defence against infectious agents and in the inflammatory process. As part of the immune system it consists of a complex system of serum and membrane associated proteins that interact in a cascade, to 'complement' an antibody mediated response.

There are two major pathways by which complement activation is initiated. The classical pathway is activated by antibody-antigen (immune) complexes. The alternative pathway is initiated when a previously activated complement component binds to the surface of a pathogen, where it is protected. A third pathway, the lectin pathway, which is activated by a plasma lectin that binds to mannose residues on microbes is recognised [26-28].

The complement system is therefore one of the most important humoral systems mediating many reactions that contribute to host defence and initiating and amplifying inflammation. The biological effects of complement activation are numerous. During CPB activation of the complement cascade occurs through several mechanisms, including exposure to the extracorporeal bypass circuit [29], following protamine administration and formation of protamine-heparin complexes and after reperfusion of the ischaemic myocardium [30].

The resulting complement activation and amplification is mediated by the terminal complement products of C5 frag-

mentation, C5a a potent anaphylatoxin and C5b-9 (Membrane Attack Complex, MAC). Anaphylatoxins are low-molecular weight, biologically active peptides that are defined functionally by their actions on small blood vessels, smooth muscle, mast cells, and peripheral blood leukocytes. MACs can directly lyse cells, including cardiac myocytes. Thus, both C5a and C5b-9 mediate cellular damage, alteration of vascular permeability and tone, leukocyte chemotaxis, initiation of cardiac myocyte apoptosis, initiation of thrombosis and promotion of both cellular activation and adhesion leading to perioperative tissue injury [31].

#### 4. Cytokine Release and Activation of Leukocytes (Monocytes and Neutrophils)

During CPB complement activation along with the generation of a pro-coagulant state are important stages in the development of a systemic inflammatory response. However, the decisive step in tissue injury and its clinical consequences is the cytokine-mediated activation of leukocytes, in particular granulocytes (predominantly neutrophils) and monocytes.

Leukocyte activation occurs in response to thrombin, kallikrein and C5a. Other pro-inflammatory cytokines that appear to be particularly active during CPB include interleukin-1 (IL-1), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6 and IL-8, histamine and heparin. Leukocyte activation is therefore characterised by elevated levels of these pro-inflammatory cytokines as well as neutrophil elastase [32] and the formation of platelet-leukocytes [33].

Neutrophils are granulocytes containing neutral-staining granules and are directly involved in the inflammatory response as well as releasing pro-inflammatory mediators themselves. Neutrophil activation leads to chemotaxis, degranulation of cytotoxic enzymes such as neutrophil elastase, lysozymes and myeloperoxidase and superoxide generation of oxygen free radicals and hydrogen peroxide. Activated neutrophils also directly activate endothelial cells thereby increasing peri-vascular oedema and leukocyte transmigration into extracellular matrix [34].

Monocytes are young cells that already possess migratory, chemotactic, pinocytic and phagocytic activities, as well as receptors for IgG Fc-domains (Fc $\gamma$ R) and iC3b complement. Under migration into tissues, monocytes undergo further differentiation to become multifunctional tissue macrophages. Monocyte activation during CPB plays a major role in thrombin generation *via* expression of tissue factor [35] and release of inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8 [36].

#### 5. Activation of Endothelial Cells and the Importance of Adhesion Molecules

Activated leukocytes bind to endothelium before they transmigrate into sites of inflammation. This involves cell-to-cell interactions mediated by adhesion molecules expressed on activated leukocytes (as well as platelets and endothelial cells) at the time of CPB (see Fig. 1). It is likely to be as a result of contact between leukocytes and the extracorporeal bypass circuit. In addition, activation of endothelial cells leading to expression of adhesion molecules can

occur in response to a variety of agonists including thrombin, C5a, IL-1 $\beta$  and TNF- $\alpha$ . Conditions of endothelial dysfunction, such as after reperfusion injury of ischaemic myocardium are likely to result in up-regulation of adhesion molecules enhancing neutrophil-led tissue damage [37,38].

Adhesion molecules that regulate leukocyte-endothelial cell integration can be divided into three main groups: the selectins, the integrins, and the immunoglobulins [39]. Regional vasoconstriction reduces blood flow rates within local vascular beds to allow neutrophils to play an important role in leukocyte-endothelial interactions. The initial interaction begins with a loose attachment and 'rolling' of the leukocyte on the endothelium by selectins, a group of adhesion molecules divided into L-, E-, and P-selectin [40]. Interleukin-1  $\beta$  and TNF- $\alpha$  induce early expression of P-selectin (CD62P) and the later expression of E-selectin (CD62E) on activated endothelium and mediate 'rolling' of leukocytes under hydrodynamic shear flow by a high affinity interaction and binding of P-selectin glycoprotein ligand (PSGL-1) expressed on leukocytes [41].

L-selectin (CD62L) is expressed on leukocytes and is primarily involved in leukocyte recirculation through lymphoid tissues, binding to counter-receptors GlyCAM-1, CD34 and endoglycan on high endothelial venules. It also plays a role in mediating "secondary rolling" at sites of inflammation *via* adhesion to PSGL-1 expressed on leukocytes previously attached to endothelium [34].

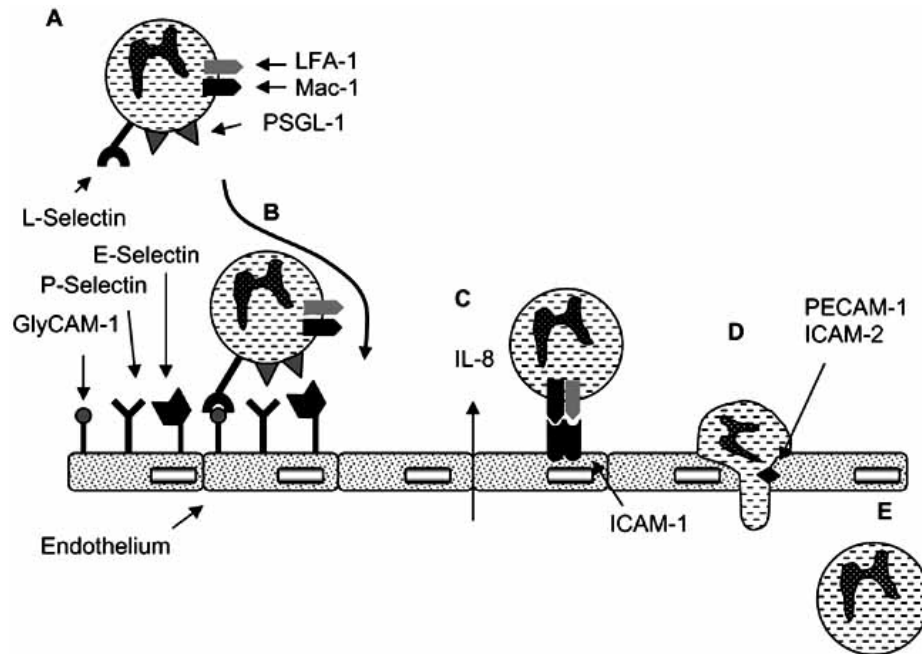
The most abundant group of adhesion molecules found on most cell types including leukocytes are the Integrins, which are sub-classified according to a non-covalently associated  $\beta$ -chain. The binding of integrins to their endothelial ligands regulate many leukocyte responses including firm adhesion to endothelium, migration into tissues, degranulation, and phagocytosis [40].

The immunoglobulin superfamily of adhesion molecules are expressed mainly by endothelial cells and serve as ligands for certain leukocyte integrins. They include Intracellular Adhesion Molecule-1 (ICAM-1) which acts as the primary ligand for  $\beta$ 2-integrins and Vascular Cell Adhesion Molecule 1 (VCAM-1) which binds with  $\alpha$ 4 $\beta$ 1-integrin. IL-1 $\beta$  and TNF- $\alpha$  also induce the synthesis and expression of ICAM-1 and VCAM-1, which firmly bind neutrophils and monocytes to the endothelium and initiate leukocyte trafficking to the extravascular space [41].

During CPB the release of these vasoactive and cytotoxic substances into the circulation and the transmigration of leukocytes across activated endothelium mediate many of the manifestations of the systemic inflammatory response syndrome associated with CPB and clinical cardiac surgery.

#### III. Strategies to Reduce the Systematic Inflammatory Response

In cardiac surgery, strategies to reduce the unwanted effects of SIRS include (1) avoidance of CPB using off-pump surgery (2) mechanical manipulation of CPB circuits (3) haematological 'filters' to remove activated neutrophils and (4) pharmacological modification.



**Fig. (1).** Simplified hypothetical diagram of the multi-step model of leukocyte interaction with inflamed vascular endothelium. Inflamed endothelium expresses P-selectin and E-selectin that binds PSGL-1 expressed on leukocytes, thus mediating. **A:** “attachment” and **B:** “rolling”. L-selectin participates in leukocyte recruitment at sites of inflammation by mediating “secondary rolling” (leukocyte on leukocyte) through its interaction with PSGL-1. **C:** “Activation” of integrins due to chemokines such as IL-8 results in **D:** “firm adhesion” of leukocytes to endothelium *via* binding of LFA-1 and Mac-1 to ICAM-1. Finally **E:** “extravasation” occurs. Adherent leukocytes move towards endothelial cell junctions and transmigrate into the extra cellular matrix with interaction involving PECAM-1 and ICAM-2. Reprinted with permission from Elsevier [42].

## 1. Off-Pump Cardiac Surgery

Much has been written in the literature about the relative merits of off-pump cardiac surgery and in particular off-pump CABG (OPCAB). By avoiding CPB the untoward physiological impact of SIRS should be abolished. Most comparative studies have demonstrated that activation of endogenous cascades and SIRS still occurs in the off-pump patient but is limited and less severe [43,44]. However, despite this obvious advantage there has been wide variation in adoption rates for off-pump cardiac surgery. The quality of distal anastomosis [45] and the potential for incomplete revascularisation [46] has generated persistent concerns.

The clinical outcomes of off-pump cardiac surgery have been studied extensively in retrospective series, but few prospective randomised trials exist. Most of the studies demonstrate the rates of morbidity and mortality compare well with cardiac surgery involving CPB [47], with increasing evidence favouring OPCAB over on-pump CABG in terms of preserving renal function [48]; with less impairment of neurocognitive function [49]; and improved outcomes in patients with pre-existing chronic obstructive airways disease [50].

## 2. Improvements in CPB Circuits

Developments in surface coatings on the lumen of the CPB circuit, most notably heparin-bonded circuits have significantly improved the biocompatibility of extracorporeal circuits. It allows lower doses of systemic heparin to be given prior to initiation of bypass, which may reduce heparin induced platelet function derangement as demonstrated by

activation of glycoprotein IIb/IIIa receptors, expression of P-selectin and enhanced platelet aggregation [51].

Alternative surface coatings for CPB circuits are under evaluation and include surface-modifying additive (SMA), based on polysiloxane co-polymer technology and Poly-2-methoxyethylacrylate (PMEA). Clinical studies demonstrated that SMA treated biomaterial surfaces reduce platelet activation, but not blood loss or transfusion requirements following CPB [52]. PMEA coated material is designed to reduce surface adsorption of plasma proteins, and improved biocompatibility. PMEA-coated circuits appear to cause less platelet activation [53], pro-inflammatory cytokine production [54], and thrombin fibrinogen and bradykinin generation [55].

## 3. Haematological Filters

Activated monocytes and neutrophils play a significant role in the development of SIRS after CPB and this has provoked the introduction of leukocyte-depleting filters being incorporated into the CPB circuit. Reported benefits include reduced circulating activated leucocytes [56], transfusion requirements [57], renal dysfunction [58], neuropsychological outcome [59] and pulmonary inflammation leading to expedited extubation and improved clinical outcomes [60, 61].

## 4. Pharmacological Modification

### a) Corticosteroids

Beyond the numerous widespread influences on physiological systems, the corticosteroids are non-specific inhibi-

tors of inflammation. Given in the context of CPB, corticosteroids have been shown to reduce levels of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, IL-8) [62,63] and to enhance release of anti-inflammatory cytokines (IL-10) [64]. Additionally glucocorticoids attenuate complement activation [65], increases in bronchial epithelial nitric oxide concentration [66], and decreases neutrophil integrin CD11b/CD18 (Mac-1) up regulation [67,68], all of which are beneficial in minimising SIRS. Other clinical benefits include an increased cardiac index (CI) [69], a decreased pulmonary capillary wedge pressure [70], and a decreased incidence of postoperative hyperthermia [71]. Due to the complex interactions of the inflammatory pathways, inhibition of a common upstream target might appear initially attractive. However undesirable effects such as postoperative hyperglycaemia [72], and delayed endotracheal extubation have also been reported [73].

#### **b) Serine Protease Inhibitors (Aprotinin)**

Aprotinin (Trasylol<sup>®</sup>) is a protease inhibitor isolated from bovine lung tissue that was originally used in inflammatory conditions such as acute pancreatitis, before its effect on blood loss after CPB was discovered [74-76]. It inhibits trypsin, chymotrypsin, plasmin, tissue plasminogen activator, kallikrein, elastase, urokinase and thrombin. The use of aprotinin in 'on-pump' CABG has been associated with more than a 40% reduction in the odds of blood transfusion as compared to placebo in many large multicentre studies [77-79]. Its haemostatic action is related to its effects on limiting fibrinolysis *via* inhibition plasmin and kallikrein [80]. In addition to haemostasis it is also reported to preserve platelet function [81-83], reduce the incidence of SIRS [84-86] and even perioperative stroke [87].

The mechanism by which aprotinin is known to preserve platelet function lies in its ability to inhibit platelet activation by preventing proteolysis of the thrombin receptor protease-activated receptor 1 (PAR-1) [88-90], the major thrombin receptor on platelets [91]. This counters the concern that aprotinin by having such potent haemostatic effects might also be prothrombotic and suggests otherwise, that aprotinin may in fact have antithrombotic effects [90]. It is likely that the reported reduction in the incidence of stroke post CPB with aprotinin is also due to PAR1 protection in the central nervous system [87,88,92,93].

Aprotinin has additional inhibitory actions on markers of inflammation and complement activation following CPB, including reducing TNF, IL-6, IL-8 and Mac-1 expression [94-96]. It appears that aprotinin reduces the cell-mediated inflammatory response of platelets indirectly through the effects on plasma proteases and directly through protease-activated receptors on platelets and endothelial cells. The incidence of SIRS following CPB appears to be reduced in patients receiving aprotinin [97,98].

#### **c) Other Anti-Fibrinolytic Agents**

Epsilon aminocaproic acid (EACA) acts as an inhibitor of fibrinolysis through inhibition of plasminogen activator substances. Alternatively, tranexamic acid (Cyklokapron<sup>®</sup>) is a synthetic antifibrinolytic substance, which acts by competitively blocking the conversion of plasminogen to plasmin, thereby preventing binding to fibrin and potentiation of fi-

brinolysis. Although the biological function of cell-bound plasmin has been regarded mainly in terms of fibrinolytic activity, in recent years it has become clear that plasmin can affect various cell functions including pro-inflammatory effects. *In vitro*, tranexamic acid potently inhibited plasmin-induced pro-inflammatory responses [99] but its role in reducing SIRS following CABG is less clear.

#### **d) Targeted Inhibition of Inflammatory Mediators**

Targeted strategies of reducing the inflammatory response associated with CPB include monoclonal antibodies such as Pexelizumab<sup>®</sup>. This agent inhibits is a recombinant antibody fragment that binds to the C5 complement component thereby blocking the generation of C5a and C5b-9. In one clinical study of patients undergoing CPB and CABG those who received Pexelizumab compared with placebo had a significantly reduced risk of myocardial infarction or death 30 days after surgery [38]. Other areas of pharmacological interest such as the CD163 receptor carried by monocytes/macrophages have been linked to anti-inflammatory activity and may offer further targets for targeted inhibition [100].

### **CONCLUSIONS AND FUTURE DEVELOPMENTS**

Despite off-pump coronary surgery gaining in popularity and despite the implementation of some of the counter measures described above, CPB is still employed in more than 75% of coronary artery bypass grafting (CABG) procedures and for most other types of adult cardiac surgery. Although an indispensable part of many adult cardiac operations CPB is paradoxically responsible for a large part of the morbidity associated with cardiac surgery. Due to contact activation of blood in surgical wounds, and synthetic perfusion circuits, to which is often added blood aspirated from the pericardial and pleural cavities, the use of CPB in clinical cardiac surgery provokes an acute inflammatory response that is often unpredictable and carries significant risk of morbidity and mortality. Due to the diversity and intricacy of the multiple pathways involved in manifesting the acute inflammatory response, it appears unlikely that a single drug or intervention will ever be completely effective.

Recently transcriptional gene profiling using high-density microarrays has become the focus of much interest as it has the potential to provide unique data about individual genes and their function during CPB. Analyses of 12,625 genes in skeletal and myocardial tissue showed the use of CPB does not cause an indiscriminate change in gene expression, but rather a distinct pattern of changes in specific pathways such as up-regulation of inflammation/transcription activators, apoptotic genes, and stress genes, as well as down-regulation of immunoglobulin genes. Additionally, multiple genes not previously known to be implicated in the pathophysiology of CPB were identified [101]. By establishing a clearer understanding of the responses to CPB, the evaluation of new treatment modalities potentially even directed at the genome level, may one day become a reality.

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