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COMPLEMENT ACTIVATION AND INHIBITION IN MYOCARDIAL ISCHEMIA AND REPERFUSION INJURY

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INTRODUCTION

The occurrence and mechanisms of myocardial reperfusion injury have been demonstrated under a diverse array of experimental conditions by many groups of investigators. Observations by Hearse and colleagues (1, 2) demonstrated that the reintroduction of molecular oxygen to the previously hypoxic myocardium results in a sudden increase in irreversible tissue injury, a phenomenon termed the "oxygen paradox." The findings of Jolly et al (3) that the combined administration of superoxide dismutase and catalase results in myocardial tissue salvage implied that toxic oxygen metabolites contribute to in vivo tissue injury beyond that mediated by ischemia alone. The possible sources of toxic oxygen metabolites in myocardial tissue are manifold. The concept has emerged that oxygen toxicity is observed when excessive oxygen radical production overwhelms the normal intracellular antioxidant mechanisms, thereby producing an oxidant stress and leading to tissue damage. Hill & Ward (4) demonstrated the role of the complement system as a mediator of the myocardial inflammatory process, components of which may also contribute to ischemia- and reperfusion-induced tissue injury. Further studies (5–7) revealed that polymorphonuclear leukocyte infiltration into the myocardium after whole blood reperfusion resulted in a significant exacerbation of tissue injury that could be reduced through

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modulation of neutrophil accumulation or activation. Thus, three major components recognized as contributing to the phenomenon of reperfusion injury are molecular oxygen, cellular blood elements (particularly the neutrophil), and components of the activated complement system. The latter two often act in concert, as for example, when C5a activates neutrophils, causing them to release proteases and generate toxic metabolites of oxygen. Timely reperfusion of an ischemic tissue is vital for maintenance of cellular viability. However, reperfusion for the purpose of limiting cell death may contribute to the extension of tissue injury—a phenomenon that has been referred to as "reperfusion injury." Thus, "reperfusion injury" may be viewed as a paradoxical increase in irreversible tissue injury that would not have occurred, at that moment in time, in the absence of reperfusion.

While this discussion concerns myocardial reperfusion injury, the phenomenon is not limited to myocardial tissue. Any tissue or organ subjected to critical ischemia is at risk for increased tissue damage upon reperfusion with oxygenated whole blood. With respect to the myocardium, reperfusion injury has numerous definitions, the most strict referring to irreversible injury to a population of cells as a result of the consequences of reperfusion (8). Reperfusion injury may be encountered in a variety of clinical situations in which blood flow is restored after a critical period of ischemia. These conditions include not only coronary thrombolysis or coronary angioplasty but also coronary artery bypass grafting and myocardial transplant procedures. The factors that determine the extent of tissue injury include the tissue type, its metabolic state, the duration and extent of the ischemic insult, the presence and degree of collateral blood flow, and the manner in which reperfusion is conducted. Given that an ischemic event has occurred, timely reperfusion and the manner in which reperfusion is instituted afford the most significant opportunities for minimizing the loss of viable tissue within the jeopardized tissue. Therefore, the conditions under which reperfusion is performed will be a significant determinant of the extent to which reperfusion injury can be minimized or avoided.

Recent efforts to determine the factors that contribute to reperfusion injury have focused primarily upon the role of toxic oxygen metabolites and the neutrophil-dependent inflammatory response. The topics have been reviewed extensively, exploring the views of both supportive and contrary opinions regarding the biological significance of reperfusion injury (7, 9, 10, 11–14). Evidence also suggests that activation of the complement system can contribute significantly to myocardial tissue damage induced by ischemia and reperfusion. For example, depletion of circulating complement components reduces neutrophil infiltration into the ischemic tissue, and reduces ischemia-induced myocardial tissue injury in experimental animals (4, 15–

17). The extracellular (soluble) domain of a membrane-bound inhibitor of complement, complement receptor type 1 (CR1), inhibits activation of the complement system. Administration of the soluble CR1 (sCR1) to rats limited the infarct size resulting from ischemia and reperfusion to 44% of the control infarct size (18).

The contribution of the complement system to myocardial injury is multifaceted. Generation of the anaphylatoxins is intimately related to the activation of neutrophils and the generation of toxic oxygen metabolites. However, the potential exists for the complement system to produce tissue destruction via formation of the cytolytic membrane attack complex, independent of oxygen metabolite production and neutrophil activation. In fact, the earliest recognized function of the complement system was its ability to induce cell lysis. This potential is relatively unexplored with respect to the myocardial damage that results from ischemia and reperfusion. It is the purpose of this review to focus on the aspects of complement activation that contribute to myocardial ischemic and reperfusion injury. The failure to consider the direct cytotoxic potential resulting from complement activation may, in part, contribute to the diverse views expressed concerning the relevance of reperfusion injury and the efficacy of interventions intended to restrain tissue injury.

COMPLEMENT ACTIVATION, REGULATION, AND FORMATION OF THE MEMBRANE ATTACK COMPLEX

The complement system is an integral part of the body's humoral defense mechanism and also a primary mediator of the inflammatory process. The complement system consists of a number of proteins that circulate in the plasma in an inactive form. Activation of the complement system results in a cascade of interactions between the various protein components of the complement system, leading to the generation of products that possess important biological activities. Certain components of the activated system serve an opsonic function, coating bacteria and immune complexes and thereby facilitating their ingestion by phagocytic cells such as neutrophils. Other products of the complement cascade promote leukocyte activation and chemotaxis through receptor-mediated mechanisms. Finally, the late-acting components of the activated system form a macromolecular complex that is cytotoxic to bacteria, fungi, parasites, virus-infected cells, tumor cells, and, unfortunately, sometimes innocent bystander host cells. A brief overview of the system will highlight the components that may actively contribute to myocardial ischemia and reperfusion-induced tissue injury.

Classical Pathway Activation

The complement system may be activated by two separate pathways, the classical and the alternative pathways (Figure 1). Activation via the classical pathway occurs primarily as a result of immune complex formation. The classical pathway is inactive, therefore, until the body is challenged with antigen to which it was exposed previously. Under normal conditions, antibodies against "self" do not occur, and activation via the classical pathway does not occur. Immune complex interaction with the C1q subunit of the C1 molecule confers enzymatic activity to the C1s subunit of C1. The C1s molecule cleaves C4, releasing C4a (one of three anaphylatoxins), and forms C4b, which binds to the surface of the target cell. C2 in association with C4b also is cleaved by C1q, releasing C2b and leaving the bound

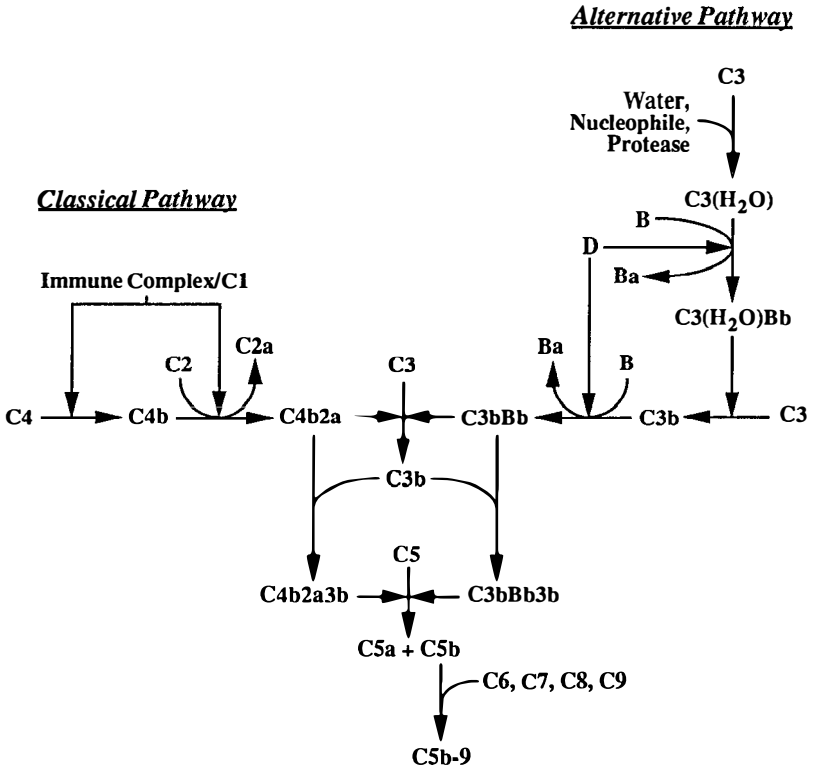

Figure 1 Diagram of the classical and alternative pathways of complement activation. C4b2a and C3bBb are the classical and alternative pathway C3 convertases, respectively. C4b2a3b and C3bBb3b are the classical and alternative pathway C5 convertases, respectively. C5b-9 is the membrane attack complex (MAC).

C4b2a complex that possesses proteolytic activity for C3 and is thus termed the classical pathway C3 convertase. The cleavage of C3 is the point of intersection for the classical and alternative pathways.

Alternative Pathway Activation

Activation of the alternative pathway relies upon nucleophilic chemical structures on the target surface. C3 is cleaved continuously at a slow rate in the fluid phase, forming C3b that binds to nucleophilic groups on target surfaces. This process normally is modulated by the fluid phase regulators of complement activation that include C1 inhibitor, factor H, and factor I, resulting in a balance between activation and inhibition of the alternative pathway. However, if the balance is shifted toward activation by C3b deposition onto a target surface, an explosive positive feedback loop is established, thereby overwhelming the endogenous inhibitors of complement activation. Once factor B combines with bound C3b, it is cleaved by factor D, releasing Ba and leaving the bound C3bBb complex, the alternative pathway C3 convertase. This convertase is stabilized by association with factor P.

Membrane Attack Complex Formation

Both the classical and alternative pathway C3 convertases cleave C3 into C3a and C3b. C3a is released into the fluid phase while C3b may complex with the convertases forming C4b2a3b of the classical pathway, or C3bBbC3b of the alternative pathway. These two complexes are called the C5 convertases and they cleave C5 into C5a and C5b. The cleavage of C5 is the last proteolytic event in the activation of the cascade and begins the formation of the membrane attack complex, the terminal event of complement activation. The C5b, in loose association with the C3b of the convertase, combines with C6 and C7 to form C5b-7, which dissociates from the convertase and has a transient binding site for membrane surfaces. The binding of C7 to the complex also causes a hydrophilic to amphiphilic transition that allows the complex to insert into the target cell membrane. The membrane-bound C5b-7 combines with C8 to form C5b-8, a complex with some slight cytolytic potential. However, the C5b-8 complex is responsible for triggering the polymerization of up to eighteen C9 molecules, which form a ring-like C5b-9 lesion (membrane attack complex, MAC) across the target cell membrane. This results in the formation of a transmembrane channel that allows for unrestricted flow of electrolytes and water between the intra- and extracellular compartments. The inability to regulate water and electrolyte flow leads to cell lysis. It should be noted that nucleated cells can sometimes tolerate low numbers of the MAC on their surface without succumbing to lysis (19). Many cells have the capacity to deplete

their membrane of the MAC by vesiculation before significant numbers accumulate and subsequent lysis ensues. A nonlytic MAC attack results in calcium influx, which activates intracellular processes that are discussed later in this review.

The lytic potential of the complement system and the importance of the membrane-bound regulators of complement activation are exemplified by a genetic deficiency state, paroxysmal nocturnal hemoglobinuria (PNH), in which the hematopoietic cells lack a number of glycan-phosphatidylinositol-linked proteins (20). Decay accelerating factor (DAF), protectin (CD59), and homologous restriction factor (HRF) are three membrane-bound regulators of complement that belong to this class of proteins and are deficient in patients with PNH. In PNH, complement-mediated erythrocyte hemolysis results from the lack of DAF activity, which modulates the C3 and C5 convertases, and the lack of protection against the MAC provided by CD59 and HRF. Continuous low grade activation of the alternative pathway continues unchecked, and leads to lysis of the erythrocytes devoid of the protective components. Paroxysmal nocturnal hemoglobinuria is an example of how complement activation, independent of other mediators of an inflammatory response, is capable of producing cellular injury and death.

Regulators of Complement Activation

Because of its mechanism of activation, the alternative pathway does not discriminate between host or foreign cells. Regulatory mechanisms are present to prevent activation and host tissue damage under normal conditions. A number of regulatory proteins, both soluble and membrane-bound (Table 1), of the complement system have been identified. Complement-mediated cytolysis of host cells is the result of MAC disruption of the cell membrane and therefore is most specifically modulated by membrane-bound proteins capable of restricting the activity of the complement system. The ability of host cells to defend against indiscriminate complement activation and its lytic effects is attributed to the presence of these membrane-bound proteins that regulate the C3 and C5 convertase activity and formation of the membrane attack complex. Since the membrane-bound regulators of complement activation restrict common portions of the cascade, they protect against activation of the complement cascade by either the classical or alternative pathway.

Four of these membrane-bound proteins are decay accelerating factor (DAF, CD55), complement receptor type 1 (CR1, CD35), membrane cofactor protein (MCP, CD46), and homologous restriction factor (HRF). The molecular process by which DAF and CR1 regulate complement activation is termed decay acceleration. This is the process of preventing association of, or causing dissociation of, C3b with factor Bb, and/or C4b with C2a.

Table 1 Regulatory proteins of the human complement system

Fluid phase	Mechanism of regulation
C1 inhibitor (C1-Inh)	Forms covalent 1 : 1 complex with C1r and C1s, removing them from the C1 complex.
C4b binding protein (C4bp)	Accelerates decay of C4b2a; cofactor for C4b cleavage by Factor I.
Anaphylatoxin inactivator	Carboxypeptidase that inactivates C3a, C4a, and C5a by cleaving C-terminal arginine from each.
Factor H	Accelerates decay of C3bBb; cofactor for C3b cleavage by Factor I.
Factor I	Protease that inactivates C3b and C4b in conjunction with cofactors C4bp, H, CR1, or MCP.
S-protein (Vitronectin)	Binds C5b-7, preventing binding of the complex to cell membranes.
Properdin (P)	Positive regulator, stabilizes the C3/C5 convertases.
Membrane bound	
Decay acceleration factor (DAF, CD55)	Accelerates decay of the C4b2a and C3bBb complexes.
Homologous restriction factor (HRF, C8bp)	Binds C8 and C9, regulating assembly of the MAC.
Membrane cofactor protein (MCP, CD46)	Cofactor for the Factor I mediated degradation of C3b and C4b.
Protectin (CD59, HRF20, membrane attack complex inhibition factor [MACIF], membrane inhibitor of reactive lysis [MIRL])	Binds C8 and C9, preventing the unfolding and polymerization of C9 needed for membrane insertion and ring formation.
Complement receptor type 1 (CR1)	Accelerates decay of the C4b2a and C3bBb complexes, cofactor for C3b and C4b cleavage by Factor I.

The result is inactivation of the convertases. As its name implies, MCP possesses cofactor activity in the factor I-mediated degradation of C3b to iC3b in an initial cleavage, and to C3c and C3dg in secondary cleavages. MCP also has cofactor activity for the degradation of C4b. Thus, DAF and MCP together control activation of the complement cascade at the critical level of the C3 convertases. In addition to its decay activity, CR1 possesses cofactor activity for these degradations as well.

Protection against the lytic action of the MAC may occur via regulating activation of the earlier portions of the cascade, but also occurs during assembly of the MAC itself. If the C5b-7 complex does not insert into a target membrane, it is released into solution where it can still complex with C8 and C9. However, the fluid phase complex binds another fluid phase protein, S protein (or vitronectin), forming SC5b-9 which prevents it from

subsequently inserting into a membrane and causing cytolytic damage (21). A second regulator of MAC formation is HRF, also called C8 binding protein (C8bp). This is a 65-kDa, membrane-bound protein that is isolated from solubilized red blood cell membranes and protects against homologous MAC assembly and subsequent lysis (22). It is limited primarily to the surface of red cells, neutrophils, and monocytes.

A third membrane-bound glycoprotein, CD59 (protectin), has as its only known function the restriction of complement-mediated lysis (23). It is widely distributed and found on all peripheral hematopoietic cells, endothelial cells, many types of epithelial cells, and some elements of both the central and peripheral nervous systems (24). CD59 binds to C8 and C9 of the assembling MAC and prevents the unfolding of the first molecule of C9 and insertion of the C9 molecule into the membrane. CD59 also prevents binding of subsequent C9 molecules and their polymerization into the MAC transmembrane ring structure (25). The glycoprotein binds selectively with human C8 and C9, thereby conferring protection against homologous lysis.

Evidently, cell membrane proteins serve an important role both in regulating complement activation and in protecting against complement-mediated host cell injury, especially cell lysis resulting from membrane attack complex formation. An excellent review of these regulatory molecules and their properties has been compiled (26). Soluble forms of CR1 (18) and DAF (27) are available, and a naturally occurring soluble form of HRF has been described (28). These molecules will serve as important tools in elucidating the role of complement-mediated tissue injury in the setting of myocardial ischemia and reperfusion. They represent the forerunners of important therapeutic approaches to modulate the cytotoxic effects attributable to activation of the complement system.

COMPLEMENT ACTIVATION IN MYOCARDIAL ISCHEMIA AND REPERFUSION

Activation and Generation of the Anaphylatoxins

The first suggestion that complement activation was involved in myocardial tissue injury was made in 1971 by Hill & Ward (29), who showed that ischemic myocardial tissue released a protease responsible for the formation of a chemotactic molecule by cleavage of C3. They further demonstrated that depletion of C3, before the induction of ischemia, prevented generation of the chemotactic molecule and reduced neutrophil infiltration into the jeopardized ischemic tissue. Since then, activation of the complement system in the setting of myocardial ischemia and reperfusion has been confirmed

and the mechanism of activation studied in more detail. Pinckard and colleagues (30) demonstrated that human heart mitochondrial membranes bind C1q, in the absence of anti-heart autoantibodies, and thereby cause complement activation. Mitochondrial membrane-mediated complement activation was shown to occur through both the classical and alternative pathways (31, 32). Similarly, Rossen et al (33) demonstrated the presence of C1q in ischemic and reperfused canine myocardium. They indicated that lymphatic fluid from ischemic myocardial tissue contains subcellular constituents rich in mitochondrial membranes that fix C1q and activate complement (34). Rossen et al proposed that myocardial ischemia results in the release of subcellular constituents that bind C1q and activate complement, thereby generating the anaphylatoxins and stimulating infiltration of neutrophils, which may exacerbate tissue injury.

In addition to the pathways described above, studies of the complement system indicate that activation of the cascade can occur as a result of interactions with oxygen metabolites. Shingu & Nobunaga (35) demonstrated that hydrogen peroxide can cause hydrolysis of C5, leading to the generation of a C5a-like molecule that has chemotactic activity. Similarly, hydrogen peroxide and related oxygen species produced by neutrophils mediated complement activation (36). Vogt and colleagues (37, 38) showed that hydroxyl radical is responsible for the conversion of C5 into a product that can complex with the terminal complement components to form the cytolytic membrane attack complex. Subsequently, oxygen metabolite-mediated complement activation in vivo has been demonstrated (39). Complement activation by toxic oxygen metabolites may be particularly relevant in the setting of a myocardial inflammatory response, in which several intracellular and extracellular sources of free radical production may serve to initiate complement activation, thereby localizing and augmenting the response to injury.

Further evidence of complement activation is provided by studies that localized complement components to injured myocardial tissue. C3 was demonstrated on swollen and infarcted baboon myocytes after 4 h of ischemia (16) and was localized to the contractile elements and subcellular membranes of myocytes and vascular smooth muscle cells after 24 h of ischemia (40). Components C4 and C5 were co-localized on myocytes positively stained for C3 (17, 40). Staining for any of these components was not observed in normal myocardial tissue. In addition, plasma concentrations of C4d (41) and the C1rC1s-C1 inhibitor complex (42) were increased after acute myocardial infarction in patients, thus providing further evidence for classical pathway activation. Similarly, plasma concentrations of Bb (41) and the C3bBbP (42) complex were elevated, providing evidence that alternative pathway activation is associated with myocardial infarction. These findings

are consistent with the hypothesis that myocardial injury causes local activation of the complement system. These observations suggest that at least the early portion (through C5) of the complement cascade is activated.

The majority of the complement-derived mediators of an inflammatory response are generated upon activation of the early portion of the cascade. Activation of the classical pathway generates C4a, and activation of either pathway generates C3a and C5a, commonly referred to as the anaphylatoxins. The latter are potent mediators of many of the inflammatory response processes (43–46). Although C5a is the most potent, all three molecules induce smooth muscle contraction, increase vascular permeability, and cause the release of histamine from mast cells and basophils through receptor-mediated mechanisms. C5a is an important mediator of the granulocyte responses inherent to the inflammatory process. These responses include chemotaxis, augmented adherence, toxic oxygen metabolite production, lysosomal enzyme release, and the initiation of cellular metabolic events such as arachidonate metabolism. In addition, C5a interacts directly with endothelial cells to cause the conversion of xanthine dehydrogenase to xanthine oxidase (47), a source of toxic oxygen products. This anaphylatoxin also elicits the release of platelet-activating factor (PAF) from a number of cell types. PAF associated with the surface of endothelial cells activates neutrophils, inducing expression of the β2 integrin receptors, thereby promoting their adhesion (48). Thus, the anaphylatoxins play a primary role in both the cellular and humoral aspects of the inflammatory response that contributes to myocardial tissue injury during ischemia and reperfusion.

The effects of the anaphylatoxin C5a on various parameters that relate to ischemic and reperfusion injury have been well characterized. Infusion of C5a into a coronary artery causes a reduction of regional coronary blood flow that results in myocardial ischemia and a decrease in myocardial contractile function. These changes are associated with intravascular trapping of neutrophils within the myocardium (49). Subsequent studies have demonstrated that the decrease in blood flow and contractile function is mediated by the production and release of thromboxane A_2 and leukotrienes (50, 51), independent of the ability of C5a to induce neutrophil sequestration (52). C5a has been demonstrated to directly affect the myocardium by causing a histamine-mediated increase in contractile tension that is preceded by a slight decrease in tension (53). Therefore, as well as mediating alterations in blood flow and contractile function, C5a participates in the neutrophil sequestration and microvascular obstruction ("plugging") that contributes to the "no-reflow" phenomenon described by Engler (54). Similarly, C3a administered to the guinea pig isolated heart also causes contractile dysfunction and vasoconstriction, as well as tachycardia and arrhythmias (55).

Opsonization and Phagocytosis

Activation of the early portion of the cascade results in the formation of several opsonins. Fixation of complement by cells or other particles leaves C3b and C4b bound to their surfaces. C3b may be degraded to C3c, iC3b, and C3dg. Phagocytic cells possess specific receptors for these particles. These molecules promote a close interaction between the phagocyte and the cell or coated particle, facilitating ingestion, or if the opsonized particle is too large, degranulation that results in the extracellular release of proteases and toxic oxygen metabolites by a process known as "frustrated phagocytosis." CR1 is the principal receptor for C3b and C4b. The principal receptor for iC3b is the CD11b/CD18 glycoprotein (MAC-1, Mol, CR3), a member of the integrin receptor family and an important mediator of neutrophil-endothelial cell interactions. Expression of the receptor is enhanced by the complement activation product C5a (56, 57). Infusion of C5a into porcine coronary arteries in vivo causes a transient leukocyte sequestration (58, 59). Thus, complement fixation on endothelial cells stimulates the rapid induction, mediated by the CD11b/CD18-iC3b interaction (60–62), of neutrophil adhesion to the cells by providing the ligand and stimulating expression of the receptor. An intimate association between neutrophils and the endothelium is the first step in neutrophil extravasation and can lead to further damage of the endothelium by the activated leukocytes (63), thereby exacerbating the inflammatory response.

The Membrane Attack Complex in Ischemia and Reperfusion

Activation of the early portion of the complement system occurs in response to myocardial injury. Therefore, subsequent generation of the membrane attack complex also would occur. Several studies have demonstrated formation of the membrane attack complex in association with myocardial ischemia and necrosis. The plasma concentration of the SC5b-9 complex (noncytolytic form) was increased up to 32-fold in patients 16 h after acute myocardial infarction (42), and subsequently it increased again upon reinfarction during hospitalization. Others (41, 64) reported similar findings and demonstrated a correlation between the SC5b-9 concentration and the peak plasma creatine kinase concentrations (41).

Using antibodies to neoantigens of the C5b-9 complex, investigators have localized the complex (the membrane-bound form) to infarcted human heart (65–67) and rat myocardium (18). Immunocytochemical staining for C5b-9 in the myocardium was very specific and sensitive, allowing for the detection of single cell necrosis. Subsequently, quantitative measurement of SC5b-9 and C5b-9 in myocardial tissue by enzyme-linked immunosorbence assay

documented an increase in these complexes in infarcted tissue that was primarily due to the C5b-9 (the membrane-bound, cytolytic) form (67). These findings document activation of the complete complement system with generation of the cytotoxic membrane attack complex. The formation of the membrane attack complex provides a source of complement-mediated myocardial tissue damage. The deleterious contributions that result from formation of the membrane attack complex in the setting of myocardial ischemia and reperfusion are poorly understood. Whether the membrane attack complex contributes to myocardial injury or merely localizes to previously lethally injured myocytes is unknown.

A number of mechanisms enable the membrane attack complex to mediate tissue injury. Reperfusion of ischemic myocardium results in loss of membrane integrity and electrolyte disturbances, especially a calcium overload of the myocardial tissue. The calcium is accumulated within the mitochondria and causes swelling and disruption of the cristae as well as affecting ATP generation (68). Calcium influx also causes contraction band formation (69, 70). Activation of the complement system and subsequent formation of the membrane attack complex on the myocytes could compromise membrane integrity and provide for easy electrolyte flux. An isolated heart model of MAC-dependent myocardial injury has been used to examine the effects of MAC deposition (71). Studies demonstrate that such deposition results in membrane disruption and alterations of tissue electrolytes that include a loss of potassium and accumulation of sodium. A pronounced accumulation of calcium was measured in the tissue, and hearts developed contracture after a short period of time. A concomitant release of creatine kinase from the myocytes was measured. Ultrastructural studies demonstrated membrane disruption, cellular edema, and swelling of the mitochondria (72). Extrapolation of these findings to an in vivo situation must be performed conservatively because the model utilized a heterologous system of complement activation. Nevertheless, the damage mediated by the MAC is clearly evident, and localization studies have identified the MAC on ischemic and infarcted homologous myocardial tissue.

In addition to the obvious cytolytic potential of the membrane attack complex, the complex can modulate the permeability of cell membranes and effect alterations in various stimulus-response coupling mechanisms and therefore cell function, without causing cytolytic cell death. Sublytic doses of the membrane attack complex function as calcium gates in neutrophil membranes and allow calcium influx to stimulate phospholipase activity and arachidonic acid metabolism (73). Low numbers of the membrane attack complex have a similar effect on glomerular epithelial cells: they cause calcium influx and release from intracellular stores, the activation of phospholipase C, and the generation of prostaglandin $F_{2\alpha}$ and thromboxane A_2

(74). Nonlethal attack of oligodendrocytes caused prostaglandin and leukotriene production by the cells (75). In platelets, insertion of the membrane attack complex initiates secretion in the absence of aggregation (76) and causes calcium-dependent activation of cellular protein kinases (77). The C5b-8 complex has been shown to trigger prostaglandin I_2 formation by cultured porcine pulmonary arterial endothelial cells (78). The membrane attack complex can stimulate the production of toxic oxygen metabolites by mesangial cells (79). The concept that sublytic numbers of the membrane attack complex may act as a stimulatory agent for calcium-dependent cell functions has been well defined by Morgan (80).

These studies suggest that the effects mediated by the membrane attack complex may not be due solely to its cytolytic potential. It is tempting to speculate that the processes observed in other cell types also occur in the endothelium and or myocytes of the heart. Formation of the membrane attack complex causes calcium influx. Before lethal numbers of the membrane attack complex can form, calcium influx could lead to activation of phospholipases, liberation of arachidonic acid, and the formation and release of cyclooxygenase and lipoxygenase products. These processes could contribute to mitochondrial damage, sarcolemmal damage, and the formation and release of vasoactive molecules—consequences that could contribute to tissue injury as a result of complement activation. Continued assault of the endothelial cells or myocytes by further complement activation could eventually lead to cell death.

Experimental studies by Seeger et al (81) have documented the effects of human complement activation in the isolated lung of rabbit. Perfusion of lungs with 6–11% human serum or plasma elicited a pressor response that was dependent upon complement activation, the complement titer, and the presence of C8, but was independent of the anaphylatoxins. The pressor response was associated with the formation and release of thromboxane A_2 and prostaglandin I_2 and was dependent upon the thromboxane generation. Prolonged treatment with human serum led to the formation of marked lung edema. It is likely that similar mechanisms may occur in the heart as a result of complement activation and may contribute to the events that lead to tissue injury as a result of myocardial ischemia and reperfusion.

Complement-mediated tissue damage contributes to both the early and late events of ischemia and reperfusion injury. The anaphylatoxins C3a, C4a, and C5a are generated early by local activation of complement. These compounds initially serve to amplify the local inflammatory response by increasing vascular permeability and attracting polymorphonuclear leukocytes. Neutrophils subsequently contribute to the later events of tissue injury, namely cell necrosis, via their production of reactive oxygen species and the release of lysosomal enzymes. As a result of complement activation,

formation of the MAC may also contribute to the later events of tissue injury by compromising the integrity of endothelial and myocardial cell membranes

Direct Versus Indirect Tissue Injury

Complement-mediated tissue damage can occur in either a direct or an indirect manner. Direct tissue damage occurs as a result of the activated complement components themselves. Indirect damage occurs as the result of an intermediate event triggered by the activated complement components. For example, the anaphylatoxins mediate neutrophil infiltration and activation. As a result, the production of toxic oxygen metabolites and release of proteases lead to tissue damage. Thus, complement activation indirectly mediates tissue damage through activation of inflammatory cells and their recruitment to the affected area. Opsonization of myocytes or endothelial cells leads to site-specific neutrophil-mediated damage. Many of the deleterious aspects of the myocardial inflammatory response are indirectly mediated by complement. Direct tissue damage is mediated by the membrane attack complex, and possibly by direct vasoconstrictor properties of C5a. Insertion of the C5b-9 complex into myocyte or endothelial cell membranes may lead directly to irreversible cell injury and necrosis, whereas the anaphylatoxin-mediated vasoconstriction exacerbates the local ischemic component of injury.

Inhibitors of Complement Activation

An effective inhibitor of complement activation would prove beneficial in reducing tissue injury in a variety of autoimmune and inflammatory disease states. The endogenous regulator of complement activation, complement receptor type 1(CR1; CD35; C3b/C4b receptor), is such a molecule. CR1 is a single-chain, membrane-bound glycoprotein that inhibits complement activation by causing dissociation of the C3 and C5 convertases of either activation pathway. CR1 also serves as a cofactor for the factor I-mediated degradation of C3b and C4b (82, 83). The potential of CR1 to restrict complement activation is limited because it is bound primarily to the membranes of erythrocytes and leukocytes. This limitation has been overcome by the development of the recombinant soluble form of the human molecule, soluble CR1 (sCR1) (18). sCR1 blocks activation of both complement pathways when present in nanomolar concentrations. It has been demonstrated to have complement inhibitory and anti-inflammatory effects in a rat model of myocardial reperfusion injury (18), and additional reports support its cardioprotective role (72, 84, 85). A possible clinical application of sCR1 is suggested by the demonstration that it prevents myocardial tissue

injury resulting from direct activation of human complement and assembly of the MAC (72). The protective effects observed with sCR1 strongly implicate the complement system as a mediator of tissue injury.

DAF is another endogenous complement regulator. It is a glycosylated, single polypeptide chain of 70 kDa that possesses a glycan-phosphatidylinositol membrane anchor. This molecule is found on a wide variety of normal tissues including most circulating blood cells, the endothelium, endocardium, myocardium, and cells of the gastrointestinal and urogenital tracts. Zimmerman et al (86) have demonstrated a loss of DAF in human myocardial tissue damaged by ischemia, while the surrounding unaffected tissue retained the normal DAF distribution. The number of DAF-deficient cells correlated with the extent of ischemic damage and the age of the lesion. They speculated that the DAF loss may be the result of phospholipase activation. Soluble forms of this inhibitor have been produced and demonstrated to inhibit complement activation (27). A form with the glycophosphatidylinositol anchor was capable of reinsertion into membranes and subsequently protected against complement-mediated lysis.

Similar observations have been made with a third inhibitor, CD59. Vakeva et al (87) have demonstrated that the expression of CD59 was lost in infarcted human myocardial tissue of age 1–14 days and that MAC deposition occurred within the CD59 negative lesions. In the border zones of lesions, the CD59 appeared in vesicles, thus suggesting that its removal occurs by shedding. CD59 expression was normal in the unaffected tissue. It is tempting to speculate that the calcium influx resulting from (initially) nonlytic numbers of MAC formation on myocytes mediates activation of a phosphatidylinositol-specfic phospholipase C (86). This would result in cleavage of this class of inhibitors (DAF, CD59, and HRF), and other molecules, from the cell surface and the production of essentially the same defect as in individuals afflicted with PNH. The increased susceptibility to complement owing to the absence of one or more of the membrane-associated regulators of complement activation would result in further tissue injury. A nonlytic number of "hits" upon the cell membrane by the MAC would render the cell susceptible to limited, but nonlethal alterations in intracellular control of water and electrolyte composition from which recovery is possible by shedding of the assembled C5b-9 complexes (reversible injury). On the other hand, a more extensive attack with multiple "hits" by the MAC would leave the cell incapable of repair. The assembly of a lethal number of C5b-9 complexes may contribute to the transition from reversible to irreversible myocardial tissue injury.

The development of recombinant forms of the endogenous regulators of complement such as CR1, DAF, MCP, and HRF have provided much

insight into the mechanisms of complement-mediated tissue injury. In the future, they may provide a viable therapeutic approach to the prevention of tissue injury resulting from myocardial ischemia and reperfusion.

EFFECTS OF COMPLEMENT ON NEUTROPHIL ADHERENCE AND INFILTRATION

The neutrophil constitutively expresses molecules that promote adhesion on its cell surface. However, as part of cell activation, the neutrophils also express certain glycoprotein adhesion receptors that mediate interaction with selective endothelial ligands in response to various stimuli. Review of the numerous adhesion molecules, their ligands, stimuli for expression, and their biological roles is beyond the scope of this work. The reader is referred to reviews on those specific topics (88–91). The present discussion is limited to aspects of these molecules as they relate to the complement system and their participation in regulating the interaction of neutrophils with target tissues.

The selectins (L-selectin, E-selectin, and P-selectin) and the β2 integrins are two important classes of adhesion receptors on the surface of the endothelial cells and neutrophils that mediate cell-cell interactions. Expression of the selectins (especially L-selectin) mediates the initial "loose" interaction of these cell types, and subsequently allows the neutrophil rolling phenomenon that proceeds spreading, attachment, diapedesis, and extravasation (92). The first adhesive interaction involving neutrophil L-selectin brings the phagocytic cell type into close proximity with the endothelium, thereby allowing for the establishment of a firmer adhesion between neutrophil and endothelial cell that is mediated by the β2 integrins and their respective ligands (93). This interaction results in juxtacrine activation of the neutrophil, shedding of L-selectin, and further β2 integrin upregulation (94, 95). Neutrophil migration from the vascular compartment to the interstitium is dependent upon establishment of firm adhesion mediated by the β2 integrins LFA-1 and MAC-1.

Activation of endothelial cells in an area of inflammation has been shown to lead to the subsequent expression of P-selectin and E-selectin. P-selectin, under basal conditions, is found within Weibel-Palade bodies of endothelial cells and the alpha granules of platelets. A number of perturbations, among them platelet activating factor and formation of the membrane attack complex, mediate translocation of P-selectin to the cell surface (96). P-selectin expression occurs rapidly and returns to basal levels by 20 to 60 min. Although the specific receptor for P-selectin has not been identified on the neutrophil, it binds to a sialylated glycoprotein. Thus, complement-mediated activation of the endothelium serves as a first step in neutrophil-endothelial

cell interaction. In addition, low levels of oxidants, as could be derived from the adherent and activated neutrophil, have been shown to induce prolonged expression of P-selectin over several hours (97), possibly contributing to a cycle of neutrophil adherence and activation. E-selectin expression on endothelial cells also promotes adhesion of neutrophils. However, peak adherence does not occur until 4–6 h after stimulation. E-selectin expression on endothelial cells requires synthesis of the protein and is stimulated by the inflammatory cytokines such as IL-1 and TNFα (98).

L-selectin is located primarily on the surface of lymphocytes and neutrophils. It participates in the initial adhesion (rolling) of the neutrophil to stimulated endothelial cells through an interaction with an uncharacterized ligand. The expression of adhesion molecules on the neutrophil surface is dependent upon the activation state of the cell, which in turn is affected by various mediators of inflammation including components of the activated complement system. The anaphylatoxin C5a and other stimuli cause rapid shedding of L-selectin and expression on the neutrophil surface of the adhesion-promoting receptor MAC-1 (57, 95, 99). The selectins and the β2 integrins (especially LFA and MAC-1) act together in targeting neutrophils to areas of inflammation.

MAC-1 is stored in the secondary granules of resting neutrophils, and its expression therefore is independent of new protein synthesis. MAC-1 mediates adhesion-dependent functions such as adherence, chemotaxis, aggregation, and cytotoxicity. Expression of MAC-1 on the cell surface enhances the neutrophil-endothelial cell adhesion via interaction with its ligands, iC3b (60–62, 100, 101) and ICAM-1 (102). iC3b is the factor-I mediated degradation product of complement component C3b. The expression of adhesion molecules and their ligands, and the resulting interactions, occur in a time-dependent manner. The iC3b-MAC-1 interaction occurs in the early phase of the inflammatory response, with peak adhesion occurring within 20 min of activation. The iC3b dependent interaction with MAC-1 is important in establishing a firm attachment of the neutrophil to the endothelial cell as well as inducing neutrophil activation and the associated oxidative burst and release of proteolytic enzymes that participate in the extension of tissue injury.

A second ligand for the MAC-1 receptor, intercellular adhesion molecule-1 (ICAM-1), subsequently is expressed on endothelial cells and is an integral molecule in the extravasation process. Maximal expression of ICAM-1 occurs 4–6 h after activation (103). Antibodies directed against either ICAM-1 or MAC-1 (or the subunit common to these β2 integrins, CD18) have been shown to block neutrophil transmigration. The extravasation of neutrophils into the interstitial space allows for intimate contact of these

cells with cardiac myocytes. Many factors such as C5a, platelet activating factor, tumor necrosis factor, and the interleukins are generated during the inflammatory process. Researchers have demonstrated that the cytokine interleukin-6 is found in post-ischemic cardiac lymph and that IL-6 is capable of stimulating isolated cardiac myocytes to express ICAM-1 (102, 104). The neutrophil-myocyte interaction is subsequently facilitated via binding of the leukocyte MAC-1 receptor. In addition, MAC-1 binding triggers a pronounced respiratory burst in the neutrophil (105, 106). Therefore, this association promotes both the generation and release of toxic oxygen metabolites and lysosomal enzymes. It also facilitates the firm contact between the cells to form a microenvironment into which these products are released.

The use of anti-MAC-1 antibodies has demonstrated the important function of the CD11b/CD18 adhesion-promoting receptor in the attachment of neutrophils to a variety of substrates including vascular endothelium and alveolar endothelium. A monoclonal antibody directed against CD11b prevents neutrophil adhesion and reduces neutrophil-mediated pulmonary tissue injury (107). In a model of regional myocardial ischemia and reperfusion, administration of an anti-MAC-1 monoclonal antibody after 45 min of ischemia protected against reperfusion injury by limiting the size of the infarct at 6 h of reperfusion (108). Infarct size as a percent of the area at risk was reduced without alterations in blood pressure, heart rate, or coronary artery blood flow. Neutrophil accumulation in the ischemic myocardium, assessed by histological analysis, was significantly reduced. These findings suggest that inhibition of neutrophil adhesion can reduce myocardial tissue injury. The activation products (anaphylatoxins) of complement play significant roles in neutrophil activation as well as in mediating adhesion receptor expression on neutrophils (MAC-1) and myocytes (ICAM-1) and receptor formation on endothelial cells (iC3b). The iC3b ligand may also be formed on the surface of myocytes in the face of interstitial complement activation. It is logical that inhibition of complement activation would reduce neutrophil-mediated myocardial tissue injury through a number of related, yet independent, mechanisms such as adherence and activation.

SUMMARY

The myocardial inflammatory response that occurs as a result of ischemia and reperfusion is similar to that which occurs in other tissues. Activation of the complement system is an integral part of the initiation and maintenance of any inflammatory response. It and other immune system mediators participate in the promotion of neutrophil adherence to endothelium by modulating expression of various adhesion molecules. The complement

system also serves an integral role in mediating neutrophil activation, the results of which have been documented in the setting of myocardial ischemia and reperfusion. Another aspect of the complement cascade, which has received little attention with respect to the heart, is the direct effects of complement activation such as endothelial and myocardial cell cytotoxicity mediated by the membrane attack complex. It is likely that this form of tissue injury contributes significantly to myocardial reperfusion injury. Given the numerous contributions of the complement system to the generation of the inflammatory response, and to directly-mediated tissue injury, selective inhibitors of the complement system have great potential to limit reperfusion injury. This has already been demonstrated for the complement inhibitor sCR1. In the future, it is likely that any therapeutic treatment of reperfusion injury will include modulation of the effects of complement activation.

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